Agricultural chemicals

[Summary of agricultural chemicals]

Agricultural chemicals are used for insecticide or for herbicide; almost of which have toxicity to human and live stocks. Therefore, if agricultural crops containing residual agricultural chemicals having be used for insecticide or for herbicide are provided to live stocks, they may cause health damage to animals and decreased productivity. Furthermore, if agricultural chemicals stay in animal products, it may cause adverse effects on the person who takes the product.

Along with enforcement of Positive List System (the system essentially prohibiting against distribution of food products containing a certain amount (0.01 ppm) or more of agricultural chemicals of which standard has not been defined) in Food Sanitation Law, in May 2006, regal regulations have been adopted to residual agricultural chemicals in feeds and hay, etc. having been regulated by the "Administrative Guidelines for Hazardous Substances in Feeds (63 Chiku B No. 2050, Notice of Livestock Industry Bureau, MAFF, October 14, 1988) to avoid residual agricultural chemicals in animal products via feeds, based on the maximum residue limits defined in Ordinance of the Standards of Feeds and Feed Additives (MAFF ordinance No. 35, 1976). Presently, the standard values have been set to 60 ingredients.

Furthermore, "Administrative Guidelines for Hazardous Substances in Feeds" was partially amended on August 1, 2009, to promote the utilization of rice straws and rice silage to increase the self-sufficient rate of feeds, and standard guide values were set to 31 agricultural chemicals (60 agricultural chemicals as of April 9, 2012).

The maximum residue limits for agricultural chemicals and analysis methods coming up are summarized in Tables 6-1 and 6-2.

Pay attention to the rule that, when the moisture content in sample exceeds 10 %, the exceeding amount should be deducted from the amount of the sample, for calculating the analysis value of the agricultural chemical when the sample is pasture grass (hay, rice straw, silage, etc.).

$$\text{Analysis value converted } C' = \begin{cases} \dfrac{90}{100-m} C & (m > 10\%) \\ C & (m \le 10\%) \end{cases}$$

Where m % is the moisture content in the sample, and C is analysis value before conversion.

Further, main agricultural chemicals possibly remaining in feeds or feed materials, based on the results of the surveillance of FAMIC and information concerning agricultural chemicals used for feed grains in foreign countries, are listed in Table 6-3.

Table 6-1. Maximum residue limits and analysis methods of agricultural chemicals (as of 2012 for MRLs; 2009 for analysis methods)

Art. # in Sec. 1	Agricultural chemicals	MRL based on Ordinance of the Standard of Feed and Feed additives (ppm)										Regulation value based on administrative guideline (ppm)			Individual analysis method	GC-MS simultaneous method	Other multicomponent analysis method	Remarks
		Oat	Barley	Wheat	Corn	Milo	Rye	Pasture	Cattle feed	Swine feed	Chicken feed	Rice straw	Rice plant silage	Paddy rice				
(α-BHC, β-BHC, γ-BHC and δ-BHC)								0.02	0.005	0.005	0.005							*1
(γ-BHC)								0.4										*2
1	α-BHC														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
1	β-BHC														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
1	γ-BHC														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
1	δ-BHC														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
2	2,4-D	0.5	0.5	0.5	0.05	0.5	0.5	260				1			–	–	Art. 7, Sec. 3	
(DDD, DDE and DDT)								0.1	0.1	0.1	0.1							*1
3	*o*,*p*'-DDT														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
3	*p*,*p*'-DDT														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
4	*p*,*p*'-DDD														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
5	*p*,*p*'-DDE														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
12	Acephate				0.5			3							–	–	Art. 2, Sec. 2	
13	Atrazine	0.02	0.02	0.3	0.2	0.02	0.02	15							–	○	Art. 10, Sec. 3	
16	Alachlor	0.1	0.05		0.2	0.1	0.05	3							–	○	Art. 1,Sec. 2 & Art. 5, Sec. 2	
18	Aldicarb	0.2	0.02	0.02	0.05	0.2	0.02	1							–	–		
(Aldrin and Dieldrin)								0.02	0.02	0.02	0.02							*1
19	Aldrin														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
101	Dieldrin														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
(Isofenphos and Isofenphos-oxon)					0.02													*1
22	Isofenphos														–	○	Art. 2, Sec. 2	
23	Isofenphos-oxon														–	–	Art. 2, Sec. 2	
28	Imidacloprid	0.05	0.05	0.05	0.1	0.05	0.05	6				10	3		○	–	—	
32	Ethion							20							–	○	Art. 2, Sec. 2	
41	Endrin							0.01	0.01	0.01	0.01				–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
(Cartap, Thiocyclam and Bensultap)		0.2	0.2	0.2	0.2	0.2	0.2	0.7										*3
45	Cartap														○	–	–	
98	Thiocyclam														○	–	–	
186	Bensultap														○	–	–	

*1. MRL as a sum

*2. MRL applied in the case being detected γ-BHC only.

*3. The MRL is a total amount equivalent to cartap.

Table 6-1. Maximum residue limits and analysis methods of agricultural chemicals [continued]

Art. # in Sec. 1	Agricultural chemicals	MRL based on Ordinance of the Standard of Feed and Feed additives (ppm)										Regulation value based on administrative guideline (ppm)			Individual analysis method	GC-MS simultaneous method	Other multicomponent analysis method	Remarks
		Oat	Barley	Wheat	Corn	Milo	Rye	Pasture	Cattle feed	Swine feed	Chicken feed	Rice straw	Rice plant silage	Paddy rice				
(Carbendazim, Thiophanate, Thiophanate-methyl and Benomyl)		0.6	0.6	0.6	0.7	0.6	0.6	10				0.3	0.1	10				*4
48	Carbendazim														○	–	–	
99	Thiophanate-methyl														○	–	–	
181	Benomyl														○	–	–	*5
	Thiophanate														–	–	–	
(Carbofuran and 3-OH Carbofuran)		0.1	0.2	0.2	0.05	0.1	0.1	13										*1
50	Carbofuran														–	–	Art. 3, Sec. 3	
51	3-OH Carbofuran														○	–	–	
55	Captan				10										○	–	–	
57	Glyphosate	20	20	5	1	20	0.2	120				0.2	0.2		–	–	Art. 6, Sec. 2	
58	Glufosinate		5	0.2	0.1			15				0.5			–	–	Art. 6, Sec. 2	
64	Chlorpyrifos	0.75	0.2	0.5	0.1	0.75	0.01	13							–	○	Art. 2, Sec. 2	
65	Chlorpyrifos-methyl	10	6	10	7	10	7								–	○	Art. 2, Sec. 2 & Art. 12, Sec. 3	
67	Chlorfenvinphos			0.05	0.50										–	○	Art. 2, Sec. 2	
69	Chlorpropham		0.05	0.05	0.05		0.05								–	○	Art. 5, Sec. 2	
70	Chlorobenzilate				0.02										○	○	Art. 1, Sec. 2	
72	Cyanazine	0.01	0.05	0.1	0.1	0.01	0.01	0.01							–	–	Art. 11, Sec. 3 & Art. 14, Sec. 3	
73	Dicamba	3	0.5	0.5	0.5	3	0.1	200							○	–	–	
76	Dichlorvos	0.2	0.2	0.2	0.2	0.2	0.2	10							○	–	Art. 2, Sec.2 & Art. 3, Sec. 2	*6
77	Diquat	2	5	2	0.05	2	0.03	100				0.05			○	–	–	
80	Cyhalothrin	0.2	0.2	0.05	0.04	0.2	0.02	0.6							–	○	Art. 4, Sec. 2	
83	Cyfluthrin	2	2	2	2	2	2	3							–	–	Art. 4, Sec. 2	
87	Simazine				0.3			9							–	–	Art. 13, Sec. 3	
90	Dimethoate	0.2	0.04	0.05	1	0.2	0.2	2							–	○	Art. 2, Sec. 2	
95	Diazinon	0.1	0.1	0.1	0.02	0.1	0.1	10				2	1		–	○	Art. 2, Sec. 2 & Art. 3, Sec. 2	
97	Thiabendazole	0.05	0.05	0.5	0.05	0.05	0.05	10							○	–	–	
(Deltamethrin and Tralomethrin)		1	1	1	1	1	1	5										*7
110	Deltamethrin														–	○	Art. 4, Sec. 2	
113	Tralomethrin														–	○	–	

*4. The MRL is a total amount equivalent to carbebdazim.

*5. The individual analysis method, for benomyl is available; however, separate estimation to carbendazim is not available.

*6. This is the MRL including naled. The individual analysis method is only available to determine the total amount including naled.

*7. The MRL is a total amount equivalent to deltamethrin. The GC-MS simultaneous analysis method is only available to determine the total amount.

Table 6-1. Maximum residue limits and analysis methods of agricultural chemicals [continued]

Art. # in Sec. 1	Agricultural chemicals	MRL based on Ordinance of the Standard of Feed and Feed additives (ppm)										Regulation value based on administrative guideline (ppm)			Individual analysis method	GC-MS simultaneous method	Other multicomponent analysis method	Remarks
		Oat	Barley	Wheat	Corn	Milo	Rye	Pasture	Cattle feed	Swine feed	Chicken feed	Rice straw	Rice plant silage	Paddy rice				
112	Terbufos	0.05	0.01	0.01	0.01	0.05	0.005	1							–	○	Art. 2, Sec. 2	
118	Tricyclazole	0.02	0.02	0.02	0.02	0.02	0.02	5							○	–	–	
124	Ethylene dibromide	0.01	0.01	0.1	0.01	0.01	0.01								–	–	Art. 8, Sec. 3	
127	Paraquat	0.5	0.05	0.05	0.1	0.5	0.05	5				0.3			○	–	–	
128	Parathion	0.08	0.5	0.3	0.3	0.08	0.05	5							–	○	Art. 2, Sec. 2 & Art. 3, Sec. 2	
133	Piperonyl butoxide	24	24	24	24	24	24								○	–	–	
138	Pirimiphos-methyl	1	1	1	1	1	1								–	○	Art. 2, Sec. 2 & Art. 12, Sec. 3	
140	Fipronil							0.2	0.02	0.02	0.01	0.2	0.1		–	○	–	
142	Fenitrothion	1	5	10	1	1	1	10							–	○	Art. 2, Sec. 2 & Art. 3, Sec. 2	
145	Fenobucarb	0.3	0.3	0.3	0.3	0.3	0.3					5	5	3	–	–	Art. 3, Sec. 3 & Art. 5, Sec. 3	
147	Fenthion				5							2	0.1		–	○	Art. 2, Sec. 2 & Art. 3, Sec. 2	
148	Phenthoate	0.4	0.4	0.4	0.4	0.4	0.4					2	1	0.7	–	○	Art. 2, Sec. 2 & Art. 3, Sec. 2	
149	Fenvalerate							13	8	4	0.5				–	○	Art. 4, Sec. 2 & Art. 17, Sec. 3	
151	Fenpropathrin							20							–	○	Art. 4, Sec. 2	
174	Bromoxynil	0.2	0.2	0.2	0.2	0.2	0.2	0.1							○	–	–	
(Heptachlor and Heptachlor epoxide)								0.02	0.02	0.02	0.02							*1
182	Heptachlor														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
183	Heptachlor epoxide														–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
184	Permethrin	2	2	2	2	2	2	55							–	○	Art. 4, Sec. 2 & Art. 17, Sec. 3	
188	Bentazone	0.2	0.2	0.2	0.2	0.2	0.2	3				0.3	0.1		○	–	–	
189	Pendimethalin	0.1	0.2	0.2	0.2	0.1	0.2	0.1				0.02			–	○	–	
194	Phosmet	0.05	0.05	0.05	0.05	0.05	0.05	40							–	○	Art. 2, Sec. 2	
195	Phorate	0.05	0.05	0.05	0.05	0.05	0.05	1.5							–	○	Art. 2, Sec. 2	
196	Malathion	2	2	8	2	2	2	135				0.2		2	–	○	Art. 2, Sec. 2 & Art. 3, Sec. 2	
202	Methidathion	0.2	0.02	0.02	0.1	0.2	0.02	12							–	○	Art. 2, Sec. 2	
204	Methoprene	5	5	5	5	5	5								○	–	–	
24	Isoprocarb											1	0.1		–	–	Art. 3, Sec. 3 & Art. 5, Sec. 3	
25	Isoprothiolane											40	20	15	–	○	–	
33	Edifenphos											10	1		–	○	Art. 2, Sec. 2	
100	Thiobencarb											0.1			–	○	–	
117	Trichlorfon											2		2	○	–	–	
156	Flutolanil											20	5	5	–	○	–	
175	Bromobutide											2			–	○	–	

Table 6-1. Maximum residue limits and analysis methods of agricultural chemicals [continued]

Art. # in Sec. 1	Agricultural chemicals	MRL based on Ordinance of the Standard of Feed and Feed additives (ppm)										Regulation value based on administrative guideline (ppm)			Hereinafter, listed were compounds of which analysis methods have not been prepared in November, 2009.
		Oat	Barley	Wheat	Corn	Milo	Rye	Pasture	Cattle feed	Swine feed	Chicken feed	Rice straw	Rice plant silage	Paddy rice	
	Azoxystrobin											5	1	2	
	Bensulfuron-methyl											0.1	0.05		
	Benzofenap											0.7			
	Buprofezin											25	15	10	
	Carbosulfan											0.7	1		
	Carpropamid											3	0.7		
	Chlorantraniliprole											0.1			
	Chlorothalonil											0.2	0.1		
	Chromafenozide											5		3	
	Clothianidin											2	1		
	Cyhalofop-butyl											2	0.1	2	
	Dimethametryn											0.2			
	Dinotefuran											5	5		
	Ethiprole											3		1	
	Ferimzone											2		5	
	Fludioxonil											0.05	0.1		
	Fthalide											130	30		
	Furametpyr											5		1	
	Halosulfuron-methyl											0.2	0.1		
	Hydroxyisoxazole											1		0.5	
	MCPA											2			
	Mepronil											25		7	
	Metalaxyl											0.5	0.2		
	Methoxyfenozide											5	2	2	
	Molinate											0.3			
	Orysastrobin											5		1	
	Oxaziclomefone											0.3	0.1		
	Oxolinic acid											10	0.1	3	
	Penoxsulam											0.2	0.1	0.1	
	Probenazole											3	0.7	0.3	
	Prochloraz											0.2	0.1		
	Pyriminobac-methyl											0.2	0.1		
	Pyroquilon											3	0.5		
	Spinosad											0.5	0.2		
	Tebufenozide											20	10		
	Thiacloprid											0.5	0.2		
	Thiamethoxiam											0.2	0.1	3	
	Thiuram											0.04	0.02		

Table 6-2. Analysis methods for agricultural chemicals not set MRL (as of 2012)

Art. # in Sec. 1	Agricultural chemicals	Individual analysis method	GC-MS simultaneous method	Other multicomponent analysis method	Remarks
4	*o*,*p*'-DDD	–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
5	*o*,*p*'-DDE	–	○	Art. 1, Sec. 2 & Art. 2, Sec. 3	
6	EPN	–	○	Art. 2, Sec. 2 & Art. 3, Sec. 2	
7	EPTC	–	–	Art. 8, Sec. 3	
8	2,4,5-T	–	–	Art. 7, Sec. 3	
9	XMC	–	–	Art. 3, Sec. 3 & Art. 5, Sec. 3	
10	Azinphos-methyl	–	–	Art. 9, Sec. 3	
11	Acetochlor	–	○	–	
14	Anilofos	–	○	–	
15	Ametryn	–	○	Art. 11, Sec. 3	
17	Allidochlor	–	○	–	
20	Allethrin	–	○	Art. 4, Sec. 2 & Art. 5, Sec. 2	
21	Isazofos	–	○	–	
26	Iprodione	○	–	–	
27	Iprobenfos	–	○	Art. 2, Sec. 2 & Art. 3, Sec. 2	
29	Indoxacarb	○	–	–	
30	Ethalfluralin	–	○	–	
31	Ethiofencarb	–	–	Art. 4, Sec. 3	
34	Etofenprox	–	○	–	
35	Ethofumesate	–	○	–	
36	Ethoprophos	–	○	Art. 2, Sec. 2	
37	Etridiazole	–	○	–	
38	Etrimfos	–	○	Art. 2, Sec. 2	
39	Endosulfan	–	–	Art. 1, Sec. 2	
40	Endosulfan sulfate	–	–	Art. 1, Sec. 2	
42	Oxadiazon	–	○	–	
43	Oxychlordane	–	○	Art. 1, Sec. 2	
44	Cadusafos	–	○	–	
47	Carfentrazone-ethyl	–	○	–	
49	Carbophenothion	–	–	Art. 2, Sec. 2	
52	Xylycarb	–	–	Art. 3, Sec. 3 & Art. 5, Sec. 3	
53	Quinalphos	–	–	Art. 2, Sec. 2	
54	Chinomethionat	○	–	–	
56	Quintozene	–	○	–	
59	Kresoxim-methyl	–	○	–	
60	Clofentezine	○	–	–	
61	Chlorthal-dimethyl	–	○	–	
62	Chlordane	–	○	Art. 1, Sec. 2	
63	Chloropicrin	○	–	–	
66	Chlorfenapyr	–	○	–	
68	Chlorfluazuron	○	–	–	
71	Fenbutatin oxide	–	–	Art. 13, Sec. 3	
74	Diclofop-methyl	–	○	–	
75	Dichloran	–	○	Art. 1, Sec. 2 & Art. 5, Sec. 2	
78	Dicofol	–	–	Art. 15, Sec. 3	
79	Zineb	○	–	–	*1
81	Diphenamid	–	○	–	
82	Difenoconazole	–	○	–	
84	Diflubenzuron	○	–	–	
85	Cyhexatin	–	–	Art. 13, Sec. 3	

*1 The analysis methods for zineb is same as that for mancozeb, and they can not be analyzed separately.

Table 6-2. Analysis methods for agricultural chemicals not set MRL [continued]

Art. # in Sec. 1	Agricultural chemicals	Individual analysis method	GC-MS simultaneous method	Other multicomponent analysis method	Remarks
86	Cypermethrin	○	–	Art. 4, Sec. 2	
88	Dimethipin	○	–	–	
89	Dimethenamid	–	○	–	
91	Dimepiperate	–	○	–	
92	Methyl bromide	○	–	–	
93	Silafluofen	–	○	–	
94	Triphenyltin hydroxide	○	–	–	
96	Terbacil	–	○	–	
102	Tecnazene	–	○	–	
103	Tetrachlorvinphos	–	○	–	
104	Tetraconazole	–	○	–	
105	Tetradifon	–	○	–	
106	Tetramethrin	–	–	Art. 4, Sec. 2	
107	Tebuconazole	○	○	Art. 16, Sec. 3	
108	Tebufenpyrad	–	○	–	
109	Tefluthrin	–	○	Art. 4, Sec. 2	
111	Terbutryn	–	○	–	
114	Triadimenol	–	–	Art. 6, Sec. 3	
115	Triadimefon	–	○	Art. 6, Sec. 3	
116	Tri-allate	–	○	–	
119	Trifluralin	–	○	Art. 15, Sec. 3	
120	Trifloxystrobin	–	○	–	
121	Tolylfluanid	–	○	–	
122	Tolclofos-methyl	–	–	Art. 2, Sec. 2	
123	Napropamide	–	○	–	
125	Nitrofen	–	–	Art. 1, Sec. 2	
126	Nonachlor	–	–	Art. 1, Sec. 2	
129	Parathion-methyl	–	○	Art. 2, Sec. 2	
130	Halfenprox	–	○	–	
131	Picolinafen	○	–	–	
132	Bifenthrin	–	○	Art. 4, Sec. 2	
134	Piperophos	–	○	–	
135	Pyridaphenthion	–	○	–	
136	Pyridaben	–	○	–	
137	Pyriproxyfen	–	○	–	
139	Vinclozolin	○	○	–	
141	Fenarimol	–	○	Art. 16, Sec. 3	
143	Fenothiocarb	–	○	–	
144	Phenothrin	–	○	–	
146	Fensulfothion	–	—	Art. 2, Sec. 2	
150	Fenbuconazole	–	○	–	
152	Butachlor	–	—	Art. 1, Sec. 2	
153	Butamifos	–	○	–	
154	Flamprop-methyl	–	○	–	
155	Flucythrinate	–	○	Art. 4, Sec. 2	
157	Flutriafol	–	○	–	
158	Fluvalinate	–	○	Art. 4, Sec. 2	
159	Flumioxazin	–	○	–	
160	Flumichlorac-pentyl	–	○	–	
161	Pretilachlor	–	–	Art. 1, Sec. 2	
162	Procymidone	–	○	–	

Table 6-2. Analysis methods for agricultural chemicals not set MRL [continued]

Art. # in Sec. 1	Agricultural chemicals	Individual analysis method	GC-MS simultaneous method	Other multicomponent analysis method	Remarks
163	Prothiofos	–	–	Art. 2, Sec. 2	
164	Propachlor	–	○	—	
165	Propazine	–	○	—	
166	Propanil	–	○	—	
167	Propargite	–	○	—	
168	Propiconazole	–	○	Art. 6, Sec. 3	
169	Propham	–	○	—	
170	Profenofos	–	○	Art. 9, Sec. 3	
171	Propetamphos	–	○	–	
172	Propoxur	–	–	Art. 3, Sec. 3 & Art. 5, Sec. 3	
173	Prometryn	–	–	Art. 11, Sec. 3	
176	Bromopropylate	–	○	–	
177	Bromophos	–	○	–	
178	Hexachlorobenzene	–	–	Art. 1, Sec. 2	
179	Hexaconazole	–	○	–	
180	Benoxacor	–	○	–	
185	Penconazole	–	○	–	
187	Bendiocarb	–	–	Art. 3, Sec. 3 & Art. 4, Sec. 3	
190	Benfluralin	–	○	–	
191	Phoxim	○	–	–	
192	Phosalone	–	○	Art. 2, Sec. 2 & Art. 3, Sec. 2	
193	Fosthiazate	–	○	–	
197	Mancozeb	○	–	–	*1
198	Myclobutanil	–	–	Art. 14, Sec. 3	
199	Mecarbam	–	–	Art. 2, Sec. 2	
200	Methacrifos	–	○	–	
201	Methiocarb	–	–	Art. 4, Sec. 3	
203	Methoxychlor	–	○	Art. 1, Sec. 2 & Art. 5, Sec. 2	
205	Metominostrobin (*E* -isomer)	–	○	–	
206	Metolachlor	–	○	Art. 1, Sec. 2	
207	Metolcarb	–	–	Art.3, Sec.3 &Art.5, Sec.3	
208	Mevinphos	–	○	–	
209	Monocrotophos	–	–	Art. 2, Sec. 2	
210	Linuron	○	–	–	
211	Phosphorus hydride	○	–	–	

Table 6-3. Agricultural chemicals to be noted for risk control

	Agricultural chemicals with MRL	Agricultural chemicals without MRL
To be specially noted	BHC (lindane), DDT, aldicarb, aldrin (including dieldrin), glyphasate, glufosinate, chlorpyrifos, chlorpyrifos-methyl, pirimiphos-methyl, fenitrothion, malathion	
To be noted	Dichlorvos, diazinon, terbufos, parathion, bromoxynil, heptachlor, permethrin	Chlordane
Infrequently detected	EPN, acephate, alachlor, isofenphos, carbaryl, carbofuran, chlorfenvinphos, cyhalothrin, cyfluthrin, dimethoate, deltamethrin, paraquat, piperonyl butoxide, fenthion, phenthoate, fenvalerate, fenpropathrin, phorate	Edifenphos, cypermethrin, methyl bromide, tebuconazole, tefluthrin, triadimefon, parathion-methyl, bifenthrin, flucythrinate, fluvalinate, propiconazole, phosalone, metolachlor, metolcarb

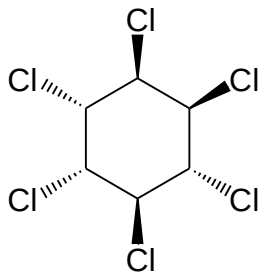
Section 1. Monographs

Contents

Halfenprox	HCB	HEOD	Heptachlor
Heptachlor epoxide	Hexachlorobenzene	Hexaconazole	HHDN
Hydrogen phosphide	IBP	Imidacloprid	Indoxacarb
IPC	Iprobenfos	Iprodione	Isazofos
Isofenphos	Isofenphos-oxon	Isoprocarb	Isoprothiolane
Kresoxim-methyl	Lindane	Linuron	Malathion
Mancozeb	MBC	MDBA	Mecarbam
MEP	Methacrifos	Methidathion	Methiocarb
Methoprene	Methoxychlor	Methyl bromide	Metolachlor
Metolcarb	(*E*)-Metominostrobin	Mevinphos	MIPC
Monocrotophos	MPMC	MPP	MTMC
Myclobutanil	NAC	Naled	Napropamide
NIP	Nitrofen	Nonachlor	Oxadiazon
Oxychlordane	2,4-PA	PAP	Paraquat
Parathion	Parathion-methyl	PCNB	Penconazole
Pendimethalin	Permethrin	PHC	Phenothrin
Phenthoate	Phorate	Phosalone	Phosmet
Phosphine	Phosphorus hydride	Phoxim	Picolinafen
Piperonyl butoxide	Piperophos	Pirimiphos-methyl	PMP
Pretilachlor	Procymidone	Profenofos	Prometryn
Propachlor	Propanil	Propargite	Propazine
Propetamphos	Propham	Propiconazole	Propoxur
Prothiofos	Pyridaben	Pyridaphenthion	Pyriproxyfen
Quinalphos	Quintozene	Silafluofen	Simazine
2,4,5-T	TBZ	TCTP	Tebuconazole
Tebufenpyrad	Tecnazene	Tefluthrin	Terbacil
Terbufos	Terbutryn	Tetrachlorvinphos	Tetraconazole
Tetradifon	Tetramethrin	Thiabendazole	Thiobencarb
Thiocyclam	Thiophanate	Thiophanate-methyl	Tolclofos-methyl
Tolylfluanid	TPTH	Tralomethrin	Triadimefon
Triadimenol	Tri-allate	Trichlorfon	Tricyclazole
Trifloxystrobin	Trifluralin	Triphenyltin hydroxide	Vinclozolin
XMC	Xylycarb	Zineb	

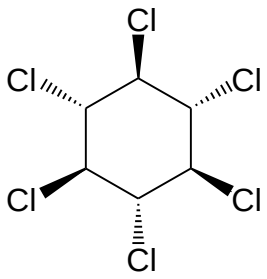
1 BHC (*α*-BHC, *β*-BHC, *γ*-BHC (Lindane) and *δ*-BHC)

α-BHC



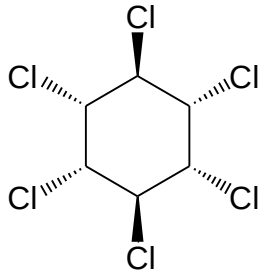
1a,2a,3b,4a,5b,6b-hexachlorocyclohexane
$C_6H_6Cl_6$ MW: 290.8
CAS No.: 319-84-6

β-BHC



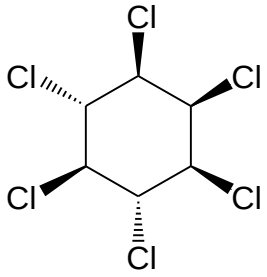
1a,2b,3a,4b,5a,6b-hexachlorocyclohexane
$C_6H_6Cl_6$ MW: 290.8
CAS No.: 319-85-7

γ-BHC (Lindane)



1a,2a,3b,4a,5a,6b-hexachlorocyclohexane
$C_6H_6Cl_6$ MW: 290.8
CAS No.: 58-89-9

δ-BHC



1a,2a,3a,4b,5a,6b-hexachlorocyclohexane
$C_6H_6Cl_6$ MW: 290.8
CAS No.: 319-86-8

[Summary of BHC]

BHC is the abbreviation of benzene hexachloride. It has been defined to be noted formally as HCH (abbreviation of hexachlorocyclohexane) to discriminate from hexachlorobenzene. However, "BHC" is commonly used even now.

Organic chlorine insecticides including BHC are chemically stable and liposoluble, and therefore, highly persist in agricultural crops, which had been a social problem. As a result, they were become to be banned to use from 1960's in advanced nations. In Japan, BHC and DDT were banned to be used in 1971, and the other organic chlorine agricultural chemicals were limited to be used. However, these agricultural chemicals have high persistence, and are detected even now, in spite of not being used.

BHC includes stereoisomer *α*, *β*, *γ*, *δ*, *ε*, *η* and *θ*, and the raw material is a mixture consisted of 65-70 % of *α*, 6-14 % of *β*, 10-13 % of *γ* and 5-8 % of *δ*, gray-brown solid with special odor.

The insecticidal potency is derived from *γ*-BHC (lindane) out of these isomers, and therefore, *γ*-BHC had been used in Europe and USA; however, the raw material had been used in Japan. Further, BHC had been more used than DDT in Japan, and chemically stable *β*-BHC had persisted in human milk and bovine milk.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of

Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(Sum of *α*-BHC, *β*-BHC, *γ*-BHC and *δ*-BHC (applied when any of *α*-BHC, *β*-BHC and *δ*-BHC was detected))

Pasture grass: 0.02 ppm

Swine, chicken/quail, cattle, sheep, goat and deer feeds: 0.005 ppm

(*γ*-BHC (lindane))

Pasture grass: 0.4 ppm

Swine and chicken/quail feeds: 0.05 ppm, cattle, sheep, goat and deer feeds: 0.4 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

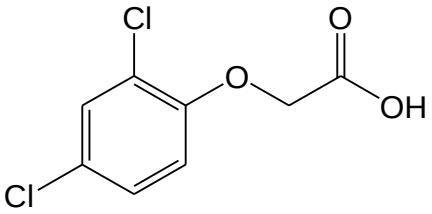
Refer to Article 1, Section 2 in this chapter.

3. Simultaneous analysis method of organochlorine agricultural chemicals by gas chromatography

Target Analytes: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, endrin, dieldrin, heptachlor and heptachlor epoxide (15 compounds)

Refer to Article 2, Section 3 in this chapter.

2　2,4-D (2,4-PA)



(2,4-dichlorophenoxy)acetic acid

$C_8H_6Cl_2O_3$　MW: 221.04　CAS No.: 94-75-7

[Summary of 2,4-D]

Agricultural chemical 2,4-D is a hormone type selective herbicide, colorless crystal, developed in USA in 1940's. This agricultural chemical was generally used as a paddy herbicide in Japan.

This chemical was registered as an agricultural chemical in 1950, in Japan (as 2,4-D sodium salt). Registered name is 2,4-PA. It was also registered as 2,4-D dimethylamine salt, 2,4-D isopropylamine salt and 2,4-D ethyl.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

(2,4-D, 2,4-D sodium salt, 2,4-D dimethylamine salt, 2,4-D ethyl, 2,4-D isopropyl, 2,4-D butoxyethyl and 2,4-D alkanolamine salt are included)

Corn: 0.05 ppm, oat, barley, wheat, milo and rye: 0.5 ppm, pasture grass: 260 ppm, rice straw: 1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for 2,4-D and 2,4,5-T by gas chromatography

Target Analytes: 2,4-D and 2,4,5-T (2 compounds)

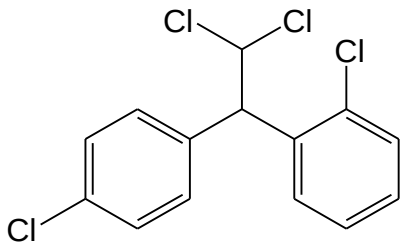
Refer to Article 7, Section 3 in this chapter.

3 DDD (*o*,*p*′-DDD and *p*,*p*′-DDD)

4 DDE (*o*,*p*′-DDE and *p*,*p*′-DDE)

5 DDT (*o*,*p*′-DDT and *p*,*p*′-DDT)

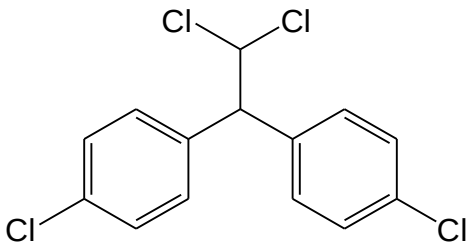
o,*p*′-DDD



1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane

$C_{14}H_{10}Cl_4$ MW: 320.0 CAS No.: 53-19-0

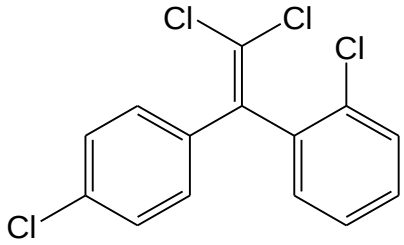
p,*p*′-DDD



1,1-dichloro-2,2-bis(4-chlorophenyl)ethane

$C_{14}H_{10}Cl_4$ MW: 320.0 CAS No.: 72-54-8

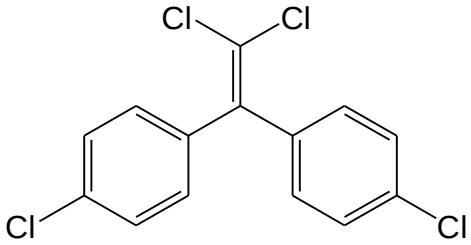
o,*p*′-DDE



1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl) ethylene

$C_{14}H_8Cl_4$ MW: 318.0 CAS No.: 3424-82-6

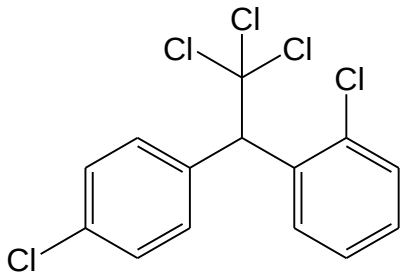
p,*p*′-DDE



1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene

$C_{14}H_8Cl_4$ MW: 318.0 CAS No.: 72-55-9

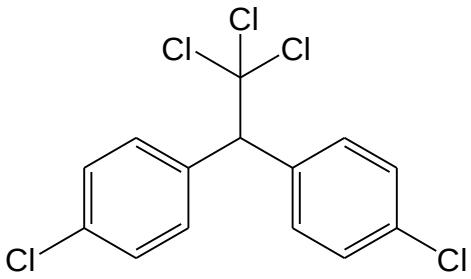
o,*p*′-DDT



1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane

$C_{14}H_9Cl_5$ MW: 354.5 CAS No.: 789-02-6

p,*p*′-DDT



1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane

$C_{14}H_9Cl_5$ MW: 354.5 CAS No.: 50-29-3

[Summary of DDD, DDE and DDT]

DDT, DDE and DDD are homologous compounds of DDT, white crystalline organic chlorine insecticides. These agricultural chemicals registration for an agricultural chemical were expired in 1971, in Japan.

They were absolutely banned to manufacture, distribute and use in 1981, in Japan.

Pure *p*,*p*′-DDT is white needle-shaped crystal, and is degraded over time by ultraviolet rays. DDT is very stable, said to require 10 years or more for being degraded in soil. On the other hand, DDT is metabolized to DDE in vivo, which is accumulated in adipose tissue more highly than DDT; therefore, DDE is detected more than DDT in vivo.

The standard value of DDT is defined as the sum of 4 compounds, i.e., *p*,*p*′-DDT, *o*,*p*′-DDT, *p*,*p*′-DDE and *p*,*p*′-DDD by Feed Safety Act and Food Sanitation Law.

DDT, DDD and DDE are listed separately in Analytical Standards of Feeds.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(Sum of *p*,*p*′-DDD, *p*,*p*′-DDE, *p*,*p*′-DDT and *o*,*p*′-DDT)

Pasture grass: 0.1 ppm

Swine, chicken/ quail, cattle, sheep, goat and deer feeds: 0.1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

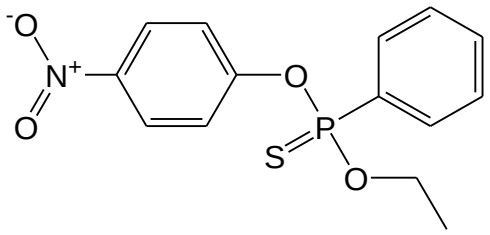
Refer to Article 1, Section 2 in this chapter.

3. Simultaneous analysis method of organochlorine agricultural chemicals by gas chromatography

Target Analytes: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, endrin, dieldrin, heptachlor and heptachlor epoxide (15 compounds)

Refer to Article 2, Section 3 in this chapter.

6 EPN



(*RS*)-(*O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate)

$C_{14}H_{14}NO_4PS$ MW: 323.3 CAS No.: 2104-64-5

[Summary of EPN]

EPN, launched in 1950 by DuPont (USA), is an organic phosphorus insecticide having insecticidal action via inhibiting cholinesterase. EPN has contact toxicity and dietary toxicity, having permeability into plant bodies, and effects on soil pests.

EPN is light yellow crystal, insoluble in water, soluble in organic solvents, and degraded in alkalinity.

EPN was registered as an agricultural chemical in 1951, in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat: 0.2 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

3. Systematic analysis methods for organophosphorus agricultural chemicals by gas

chromatography (2)

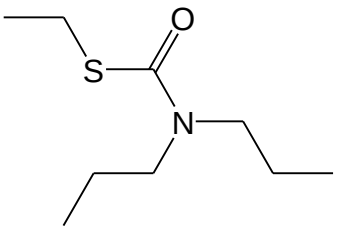
Target Analytes:

Group A: EPN, diazinon, parathion, fenitrothion, fenthion, phenthoate, phosalone and malathion (8 compounds).

Group B: Dichlorvos (1 compound)

Refer to Article 3, Section 2 in this chapter.

7　EPTC



S-ethyl dipropyl (thiocarbamate)

$C_9H_{19}NOS$　　MW: 189.3　　CAS No.: 759-94-4

[Summary of EPTC]

EPTC is a thiocarbamate herbicide developed by Stauffer Chemical (USA), in 1995, having global effects on annual bent grasses and broad leaf weeds.

EPTC was registered as agricultural chemical in 1968, in Japan. However, it was expirede in 1979.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.1 ppm

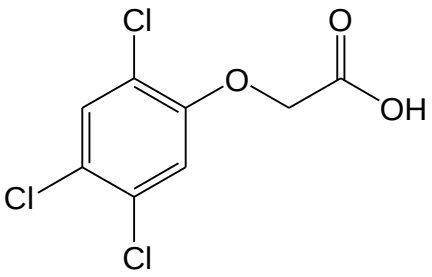
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for EPTC and ethylene dibromide by gas chromatograph-mass spectrometer

Target Analytes: EPTC and ethylene dibromide (2 compounds)

Refer to Article 8, Section 3 in this chapter.

8　2,4,5-T



(2,4,5-trichlorophenoxy)acetic acid

$C_8H_5Cl_3O_3$　MW: 255.48　CAS No.: 93-76-5

[Summary of 2,4,5-T]

The agricultural chemical 2,4,5-T is a hormone type translocated selective herbicide, colorless crystal. This agricultural chemical has higher effects on trees and bushes as compared to grasses, and very effective for foliage treatment as well as soil treatment at high temperature. This agricultural chemical has been formulated as sodium salt, potassium salt, ammonium salt, amine salt and alkyl salt.

2,4,5-T was registered as an agricultural chemical in 1964, in Japan. However, it was expired in 1975.

«Maximum Residue Limits in grains in the Food Sanitation Law»

To be not detectable (detection lower limit: 0.05 ppm).

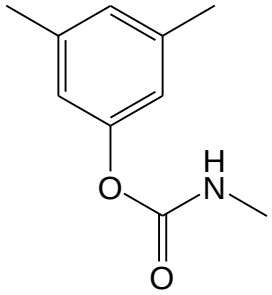
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for 2,4-D and 2,4,5-T by gas chromatography

Target Analytes: 2,4-D and 2,4,5-T (2 compounds)

Refer to Article 7, Section 3 in this chapter.

9 XMC



3,5-xylyl methylcarbamate

$C_{10}H_{13}NO_2$ MW: 179.22 CAS No.: 2655-14-3

[Summary of XMC]

XMC is a carbamate insecticide developed by Hodogaya Chemical and Hokko Chemical, an agricultural chemical being used for protecting plant hopper and green rice leafhopper, etc. in rice plant. Its chemical structure is similar to that of xylylcarb.

XMC was registered as an agricultural chemical in 1968, in Japan. However, it was expired in 2008.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.2 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)

Target Analytes: XMC, aldicarb (including aldicarb sulfoxide and aldicarb sulfone), isoprocarb, carbaryl, carbofuran, xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb (12 compounds)

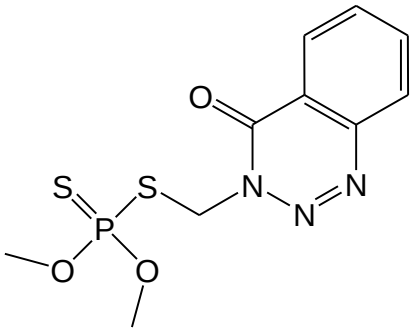
Refer to Article 3, Section 3 in this chapter.

2. Simultaneous analysis method of carbamate agricultural chemicals by gas chromatography

Target Analytes: XMC, isoprocarb, carbaryl, xylylcarb, fenobucarb, propoxur and metolcarb (7 compounds)

Refer to Article 5, Section 3 in this chapter.

10　Azinphos-methyl



S-3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl *O*,*O*-dimethyl phosphorodithioate

$C_{10}H_{12}N_3O_3PS_2$　MW: 317.3　CAS No.: 86-50-0

[Summary of azinphos-methyl]

Azinphos-methyl is an organic phosphorus insecticide developed by Bayer (Germany), yellow crystalline powder.

Azinphos-methyl has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn: 2 ppm

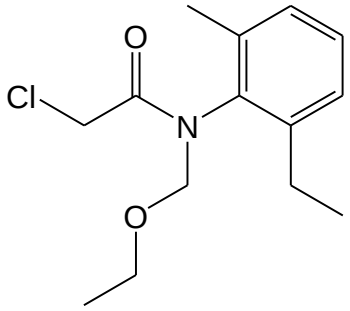
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for azinphos-methyl and profenofos by gas chromatography

Target Analytes: Azinphos-methyl and profenofos (2 compounds)

Refer to Article 9, Section 3 in this chapter.

11 Acetochlor



2-chloro-*N*-ethoxymethyl-6'-ethylacet-*o*-toluidide

$C_{14}H_{20}ClNO_2$ MW: 269.8 CAS No.: 34256-82-1

[Summary of acetochlor]

Acetochlor is an acid amide herbicide developed by Monsanto (USA), having herbicidal action via mainly inhibiting the growth of radicles in weeds by inhibiting biosynthesis enzymes in long-chain fatty acids with carbon number of 20 or more in plants.

Acetochlor has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat: 0.02 ppm, corn: 0.05 ppm, other grains: 0.02 ppm

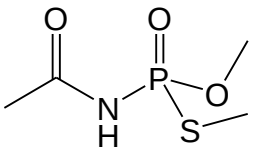
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

12 Acephate



(*RS*)-(*O*,*S*-dimethyl acetylphosphoramidothioate)
$C_4H_{10}NO_3PS$ MW: 183.17 CAS No.: 30560-19-1

[Summary of acephate]

Acephate is a permeable organic phosphorus insecticide developed by Chevron Chemical (USA), colorless crystal. Acephate is effective mainly to pests to vegetables. Main metabolite includes methamidophos.

Acephate was registered as an agricultural chemical in 1974, in Japan. The trade name is "Ortolan".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Corn: 0.5 ppm, pasture grass: 3 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

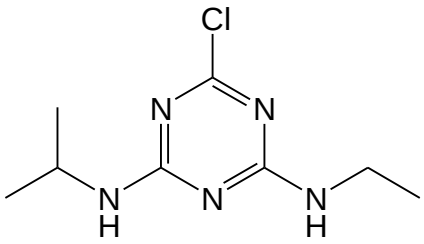
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

13 Atrazine



6-chloro-*N*^{2}-ethyl-*N*^{4}-isopropyl-1,3,5-triazine-2,4-diamine

$C_8H_{14}ClN_5$ MW: 215.7 CAS No.: 1912-24-9

[Summary of atrazine]

Atrazine is a non-hormone type triazine translocated herbicide developed by Ciba-Geigy (Switzerland), and sprayed on fields or to stems and leafs as a herbicide to annual weeds in fields such as cornfields.

Atrazine was registered as an agricultural chemical in 1965, in Japan. The trade name is “Gesaprim”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Oat, barley, milo and rye: 0.02 ppm, corn: 0.2 ppm, wheat: 0.3 ppm, pasture grass: 15 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

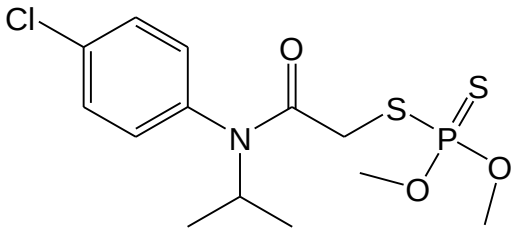
Refer to Article 1, Section 3 in this chapter.

2. Simultaneous analysis method for atrazine and simazine by gas chromatography

Target Analytes: Atrazine and simazine (2 compounds)

Refer to Article 10, Section 3 in this chapter.

14 Anilofos



S-4-chloro-*N*-isopropylcarbaniloylmethyl *O*,*O*-dimethyl phosphorodithioate

$C_{13}H_{19}ClNO_3PS_2$ MW: 367.8 CAS No.: 64249-01-0

[Summary of anilofos]

Anilofos is an organic phosphorus herbicide.

Anilofos was registered as an agricultural chemical as a paddy herbicide in 2006, in Japan. Anilofos is commercially supplied as a mixed preparation with other agricultural chemicals.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

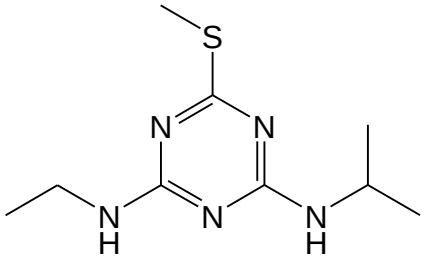
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

15 Ametryn



N^2-ethyl-*N*^{4}-isopropyl-6-methylthio-1,3,5-triazine-2,4-diamine

$C_9H_{17}N_5S$ MW: 227.3 CAS No.: 834-12-8

[Summary of ametryn]

Ametryn is a triazine herbicide, initially registered to be used to sugarcanes in 1964, in USA. It is now used for corns, pineapples as well as sugarcanes.

In Japan, ametryn had been registered as an agricultural chemical in 1965 However, it was expired in 2005.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn：0.3 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

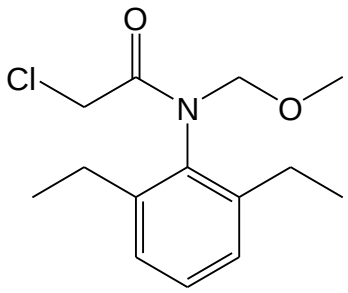
Refer to Article 1, Section 3 in this chapter.

2. Simultaneous analysis method for ametryn, cyanazine and prometryn by liquid chromatograph-mass spectrometer

Target Analytes: Ametryn, cyanazine and prometryn (3 compounds)

Refer to Article 11, Section 3 in this chapter.

16 Alachlor



(*S*)-*α*-cyano-3-phenoxybenzyl (*Z*)-(1*R*)-*cis*-2,2-dimethyl-3-[2-(2,2,2-trifluoro-1-trifluoromethylethoxycarbonyl)vinyl] cyclopropanecarboxylate

$C_{14}H_{20}ClNO_2$ MW: 269.8 CAS No.: 15972-60-8

[Summary of alachlor]

Alachlor is a non-hormone type translocated chloroacetanilide soil-applied herbicide developed by Monsanto (USA), having high inhibiting effects to weed's germination. It has no effect on weeds having developed. Alachlor is absorbed from plumlets and radicles of weeds, and suppresses the germination by inhibiting the protein biosynthesis.

Alachlor is solid crystal, poorly soluble in water, soluble in normal organic solvents, and hydrolyzed by strong acid or strong alkali.

Alachlor was registered as an agricultural chemical in 1970, in Japan. The trade name is "Lasso".

«**Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds**» **[Ordinance of the Standard of Feed and Feed Additives]**

Barley and rye: 0.05 ppm, oat and milo: 0.1 ppm, corn: 0.2 ppm, pasture grass: 3 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

3. Systematic analysis methods for alachlor, allethrin, chlorpropham, dichloran and methoxychlor by gas chromatography

Target Analytes:

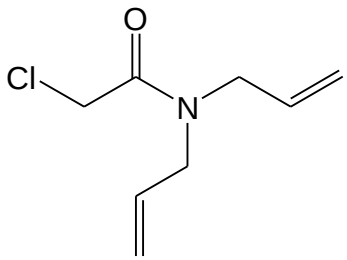
Group A: Alachlor, allethrin and dichloran (3 compounds)

Group B: Methoxychlor (1 compound)

Group C: Chlorpropham (1 compound)

Refer to Article 5, Section 2 in this chapter.

17 Allidochlor



N,*N*-diallyl-2-chloroacetamide

$C_8H_{12}ClNO$ MW: 173.6 CAS No.: 93-71-0

[Summary of allidochlor]

Allidochlor is an acetamide herbicide.

Allidochlor has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn: 0.05 ppm

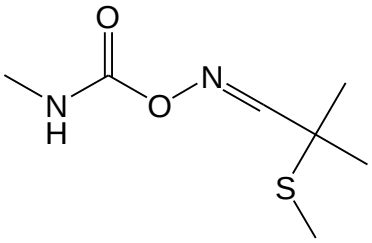
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

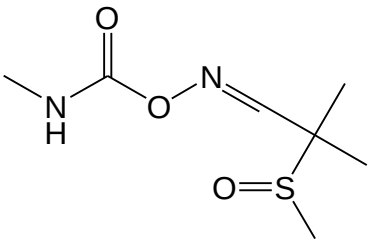
18　Aldicarb
(including aldicarb sulfoxide and aldicarb sulfone）



(*EZ*)-2-methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxime

$C_7H_{14}N_2O_2S$　MW: 190.26　CAS No.: 116-06-3

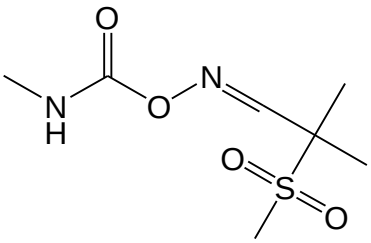
Aldicarb sulfoxide



$C_7H_{14}N_2O_3S$　MW: 206.26

CAS No.: 1646-87-3

Aldicarb sulfone



$C_7H_{14}N_2O_4S$　MW: 222.26

CAS No.: 1646-88-4

[Summary of aldicarb]

Aldicarb is an oximecarbamate insecticide, used for treating soil to exterminate sucking pests, etc. by being absorbed from the roots and translocated to bodies of the plant. Aldicarb is oxidized in plant and soil to metabolites such as sulfoxide and sulfone, which are also bioactive. The sulfoxide body has 10 to 20-fold cholinesterase activity as compared with aldicarb.

Aldicarb has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(As aldicarb single body)

Barley, wheat and rye: 0.02 ppm, corn: 0.05 ppm, oat and milo: 0.2 ppm, pasture grass: 1 ppm

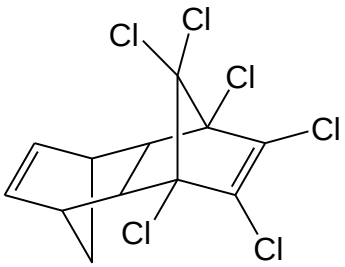
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)

Target Analytes: XMC, aldicarb (including aldicarb sulfoxide and aldicarb sulfone), isoprocarb, carbaryl, carbofuran, xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb (12 compounds)

Refer to Article 3, Section 3 in this chapter.

19 Aldrin (aldrin and dieldrin) (HHDN)



(1*R*,4*S*,4a*S*,5*S*,8*R*,8a*R*)-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene

$C_{12}H_8Cl_6$ MW: 364.9 CAS No.: 309-00-2

[Summary of aldrin]

Aldrin is an organic chlorine insecticide, white crystal with the melting point of 104 °C. The industry product contains the main component (HHDN) at 95 %, and other compounds at 5 %.

Aldrin is effective to controlling soil pests; however, being resistant to decomposition in soil, it caused pollution of soil and agricultural crops. Aldrin is oxidized to dieldrin.

Aldrin had been registered as an agricultural chemical in 1954, in Japan. However, it was expired in 1975.

Aldrin and dieldrin (p.846) are listed separately in Analytical Standards of Feeds; however, in Ordinance Concerning Ingredient Specification, the standard value is defined as a sum of both amounts.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(A sum of amounts of aldrin and dieldrin)

Pasture grass: 0.02 ppm

Swine, chicken/quail, cattle, sheep, goat and deer feeds: 0.02 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, β-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

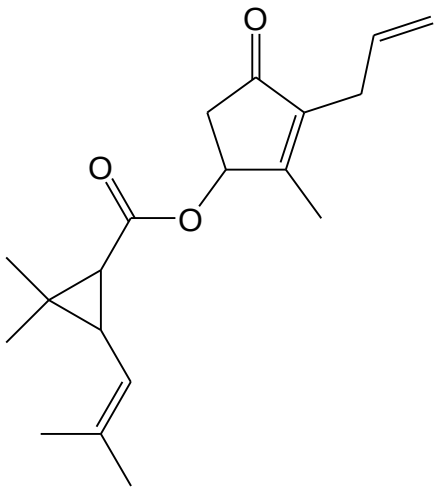
Refer to Article 1, Section 2 in this chapter.

3. Simultaneous analysis method of organochlorine agricultural chemicals by gas chromatography

Target Analytes: α-BHC, β-BHC, γ-BHC, δ-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, endrin, dieldrin, heptachlor and heptachlor epoxide (15 compounds)

Refer to Article 2, Section 3 in this chapter.

20 Allethrin



(*RS*)-3-allyl-2-methyl-4-oxocyclopent-2-enyl (1*RS*)-*cis-trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate

$C_{19}H_{26}O_3$ MW: 302.4 CAS No.: 584-79-2

[Summary of allethrin]

Allethrin is a synthetic pyrethroid insecticide, synthesized by changing the molecular structure of cinerin, a pyrethrin homolog. Allethrin is light yellow oily liquid, insoluble in water, and soluble in normal organic solvents.

Allethrin was registered as an agricultural chemical in 1967, in Japan. Allethrin is mainly used as an insecticide for home gardening. The trade name is "Kadan A".

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

Refer to Article 4, Section 2 in this chapter.

3. Systematic analysis methods for alachlor, allethrin, chlorpropham, dichloran and methoxychlor by gas chromatography

Target Analytes:

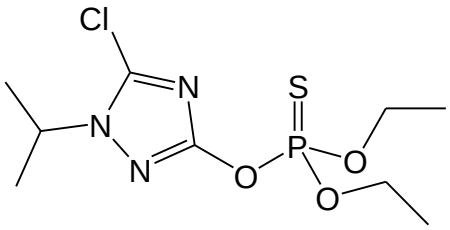
Group A: Alachlor, allethrin and dichloran (3 compounds)

Group B: Methoxychlor (1 compound)

Group C: Chlorpropham (1 compound)

Refer to Article 5, Section 2 in this chapter.

21 Isazofos



O-5-chloro-1-isopropyl-1*H*-1,2,4-triazol-3-yl *O*,*O*-diethyl phosphorothioate

$C_9H_{17}ClN_3O_3PS$ MW: 313.7 CAS No.: 42509-80-8

[Summary of isazofos]

Isazofos is an organic phosphorus insecticide.

Isazofos has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

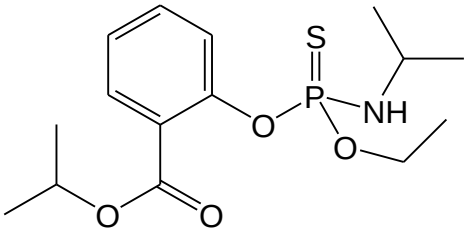
1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

22 Isofenphos
(Isofenphos and isofenphos oxon)
23 Isofenphos oxon

Isofenphos

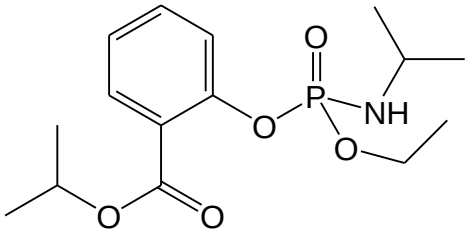


isopropyl (*RS*)-*O*-[ethoxy(isopropylamino)phosphinothioyl]salicylate

$C_{15}H_{24}NO_4PS$ MW: 345.4

CAS No.: 25311-71-1

Isofenphos oxon



isopropyl (*RS*)-*O*-[ethoxy(isopropylamino)phosphinyl]salicylate

$C_{15}H_{24}NO_5P$ MW: 329.3

CAS No.: 31120-85-1

[Summary of isofenphos]

Isofenphos is an organic phosphorus insecticide developed by Bayer (Germany), and mainly used to control soil pests of sugarcane, peanut, etc.

Isofenphos had been registered as an agricultural chemical in 1986, in Japan. However, it was expired in 2004.

Isofenphos oxon is a metabolite of isofenphos.

Isofenphos and isofenphos oxon are listed separately in Analytical Standards of Feeds; however, the standard value is defined as the sum of both amounts in Ordinance of the Standard of Feed and Feed Additives.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(The sum of amounts of isofenphos and isofenphos oxon converted to the content equivalent to isofenphos)

Corn: 0.02 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer),

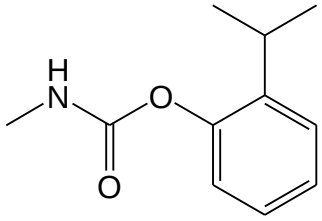
chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

24 Isoprocarb (MIPC)



2-isopropylphenyl methylcarbamate

$C_{11}H_{15}NO_2$ MW: 193.24 CAS No.: 2631-40-5

[Summary of isoprocarb]

Isoprocarb is a white crystalline carbamate insecticide developed by Mitsubishi Chemical. Isoprocarb is effective to plant hoppers and dodgers in rice plants, generally used as granular formulation by spreading on soil (or on water surface). It has residual efficacy, as well.

Isoprocarb was registered as an agricultural chemical in 1967, in Japan. Registered name is MIPC. The trade name is “Mipcin”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Administrative Guidelines for Hazardous Substances in Feeds]

Rice straw: 1 ppm / Rice plant silage: 0.1 ppm

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)

Target Analytes: XMC, aldicarb (including aldicarb sulfoxide and aldicarb sulfone), isoprocarb, carbaryl, carbofuran, xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb (12 compounds)

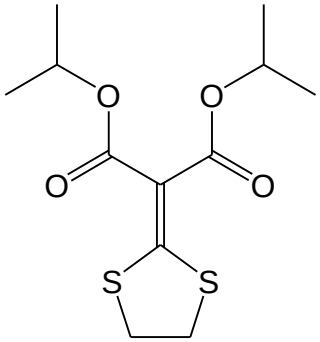
Refer to Article 3, Section 3 in this chapter.

2. Simultaneous analysis method of carbamate agricultural chemicals by gas chromatography

Target Analytes: XMC, isoprocarb, carbaryl, xylylcarb, fenobucarb, propoxur and metolcarb (7 compounds)

Refer to Article 5, Section 3 in this chapter.

25 Isoprothiolane



Diisopropyl 1,3-dithiolan-2-ylidenemalonate

$C_{12}H_{18}O_4S_2$ MW: 290.4 CAS No.: 50512-35-1

[Summary of isoprothiolane]

Isoprothiolane is a bactericide with a dithiolan nucleus, developed by Nihon Nohyaku in 1968. Isoprothiolane has strongly inhibiting activity to fungal filament growth of Magnaporthe oryzae, *Helminthosporium sigmoideum* Ca vara, *Helminthosporium sigmoideum* Var. irregular-ra Cralley et Tullis, *Plectosphaera cryptomeriae* and *Rosellinia necatrix* Prill.

Isoprothiolane was registered as an agricultural chemical targeting rice plants in 1974, in Japan. The trade name is “Fujiwan”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Administrative Guidelines for Hazardous Substances in Feeds]

Rice straw: 40 ppm / Rice plant silage: 20 ppm / Paddy rice: 15 ppm

«Standard values defined to grains defined by Food Sanitary Act»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

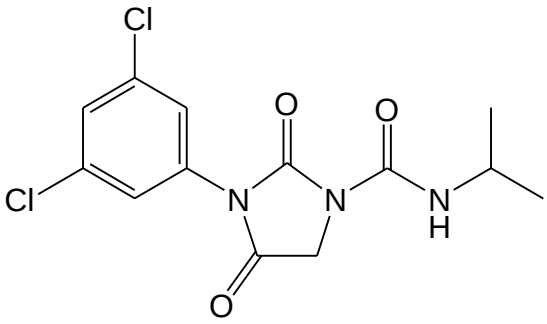
1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

26 Iprodione (including iprodione metabolite)

Iprodione

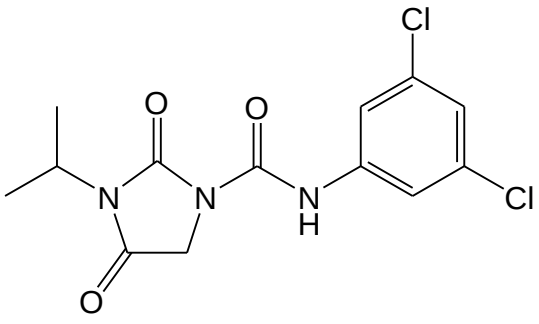


3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide

$C_{13}H_{13}Cl_2N_3O_3$ MW: 330.17

CAS No.: 36734-19-7

Iprodione metabolite



N-(3,5-dichlorophenyl)-3-isopropyl-2,4-dioxoimidazolidine-1-carboxamide

$C_{13}H_{13}Cl_2N_3O_3$ MW: 330.17

CAS No.: 63637-89-8

[Summary of iprodione]

Iprodione is a dicarboximide bactericide developed by Rhône-Poulenc (France), effective to various diseases including botrytis rot and crown rot in fruits and vegetables. The residual standard value was defined as the total amount of iprodione and its metabolite in Japan.

Iprodione was registered as an agricultural chemical in 1980, in Japan. The trade name is “Rovral”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(The sum of iprodione and *N*-(3,5-dichlorophenyl)-3-isopropyl-2,4-dioxoimidazolidine1-carboxamide)

Wheat, barley, rye, corn and other grains: 10 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Gas chromatography [Analytical Standards of Feeds Chapter 6, Section 1, Article 26.1]

Target analytes: Iprodione and iprodione metabolite (2 compounds)

A. Reagent preparation

1) Iprodione standard stock solution. Weigh accurately 50 mg of iprodione [$C_{13}H_{13}Cl_2N_3O_3$], place in a 100 mL volumetric flask, add acetone to dissolve, and further add acetone up to the marked line to prepare the iprodione standard stock solution (1 mL of this solution contains an amount equivalent to 0.5 mg of iprodione).

2) Iprodione metabolite standard stock solution. Weigh accurately 50 mg of iprodione metabolite [$C_{13}H_{13}Cl_2N_3O_3$], place in a 100 mL volumetric flask, add acetone to dissolve, and further add acetone up to the marked line to prepare the iprodione metabolite standard stock solution. (1 mL of this solution contains an amount equivalent to 0.5 mg of iprodione metabolite).

3) Iprodione mixed standard solution. Mix a definite amount of iprodione standard stock solution and iprodione metabolite standard stock solution, and dilute accurately with acetone to prepare several

standard solutions containing amounts equivalent to 0.1 – 5.0 μg/mL of iprodione and iprodione metabolite, respectively.

B. Quantification

Extraction. Weigh 10.0 g of the analysis sample, place in a stoppered 200 mL Erlenmeyer flask, add 15 mL of water, allow to stand for 30 minutes, further add 100 mL of acetone, and shake for 60 minutes to extract. Place a 300 mL recovery flask under a Büchner funnel, filter the extract by suction through filter paper (No. 5B), wash the said Erlenmeyer flask and the residue serially with 50 mL of acetone, and filter by suction in a similar way. Condense the filtrate to approximately 15 mL in a water bath at 40 °C or lower under reduced pressure[1], and add 5 g of sodium chloride to prepare the sample solution subject to the column treatment I.

Column treatment I. Place the sample solution in a porous diatom earth column (for 20 mL retention), and allow to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask having contained the sample solution 3 times with 10 mL each of hexane, add the washings serially to the column, and flow down until the liquid surface reaches the top of the column packing material to elute iprodione and its metabolite. Further add 70 mL of hexane to the column to elute in a similar way, condense the eluate almost into dryness in a water bath at 40 °C or lower under reduced pressure, further dry up by the flow of nitrogen gas. Add accurately 10 mL of cyclohexane – acetone (4 : 1) to dissolve the residue, and filter through membrane filter (pore size: 0.5 μm or less) to obtain the sample solution subject to gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into a gel permeation chromatograph, take the fraction containing eluted iprodione and iprodione in a 100 mL recovery flask, condense almost into dryness in a water bath at 40 °C or lower under reduced pressure, further dry up by the flow of nitrogen gas. Add accurately 5 mL of hexane to dissolve the residue to prepare the sample solution subject to column treatment II.

Example of operating conditions

Column: Styrene divinylbenzene copolymer column[2] (internal diameter: 20 mm, length: 300 mm, particle size: 15 μm)

Guard column: Styrene divinylbenzene copolymer column[2] (internal diameter: 20 mm, length: 100 mm, particle size: 15 μm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Sampling fraction: 90-115 mL

Column treatment II. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of diethyl ether and 5 mL of hexane.

Place the sample solution in the minicolumn, flow down until the liquid surface reaches the top of the column packing material, wash the recovery flask having contained the sample solution 3 times with 5 mL each of hexane, and add the washings serially to the minicolumn. Further add 20 mL of hexane-diethyl ether (17 : 3) and wash the minicolumn. Place a 50 mL recovery flask under the minicolumn, and add 15 mL of hexane – ethyl acetate (17 : 3) to the minicolumn to elute iprodione and its metabolite.

Condense the eluate almost into dryness in a water bath at 40 °C or lower under reduced pressure, further dry up by the flow of nitrogen gas.

Add accurately 1 mL of acetone to dissolve the residue to prepare the sample solution subject to gas chromatography.

Gas chromatography. Inject 2 µL each of the sample solution and respective mixed standard solution into a gas chromatograph to obtain the chromatogram.

Example of measurement conditions

Detector: Flame thermionic detector

Column: Fused silica capillary column (100 % dimethylpolysiloxane coating, internal diameter: 0.32 mm, length: 7 m, film thickness: 0.1 µm)[3][4]

Carrier gas: He (1 mL/min)

Makeup gas: He (5 mL/min)

Hydrogen: 3 mL/min

Drying air: 60 mL/min

Sample injection: Splitless (60 s)

Injection port[5] temperature: 250 °C

Column oven temperature: initial temperature 100 °C (hold 1 min) → ramp 20 °C/min → 280 °C (hold 2 min)

Detector temperature: 280 °C

Calculation. Calculate the peak height or peak area from the obtained chromatogram[6] to prepare a calibration curve, and determine the total amount of iprodione and iprodione metabolite in the sample as the amount of iprodione.

«Summary of analysis methods»

This method is intended to determine the amount of iprodione and iprodione metabolite in feed by extracting with hydrous acetone, purifying with a porous diatom earth column, GPC and a Florisil minicolum, and quantifying using a gas chromatograph attached with an alkali flame ionization detector (or nitrogen-phosphorus detector).

The flow sheet of analysis methods is shown in Figure 6.1.26-1.

Sample (10 g)
- Add 15 mL of water, and allow to stand for 30 min.
- Add 100 mL of acetone, and stir for 60 min.
- Filter by suction (No. 5B).
- Wash with 50 mL of acetone.
- Condence (to approximately 15 mL) under reduced pressure.
- Add 5 g of NaCl.

Chem Elut cartridge (for 20 mL retention)
- Load the sample solution, and allow to stand for 5 min.
- Elute with 100 mL of hexane.
- Condense under reduced pressure, and introduce nitrogen gas to evaporate into dryness.
- Add 10 mL of cyclohexane-acetone (4 : 1).

GPC (inject 5 mL of the sample solution)
- Collect 90-115 mL of the fraction.
- Condense under reduced pressure, and introduce nitrogen gas to evaplrate into dryness.
- Add 5 mL of hexane.

Sep-Pak Plus Florisil cartridge
(having been washed with 5 mL of ethylether and 5 mL of hexane)
- Load with the sample solution.
- Wash with 5 mL each of hexane (3 times)
- Wash with 20 mL of hexane-diethylether (17 : 3).
- Elute with 15 mL of hexane-ethyl acetate (17 : 3).
- Condense under reduced pressure, and introduce nitrogen gas to evaplrate into dryness.
- Add 1 mL of acetone.

GC-FTD or GC-NPD

Figure 6.1.26-1. Flow sheet of gas chromatography of iprodione in feed

References: Manabu Matsuzaki, Daisaku Makino: Research Report of Animal Feed, 28, 42 (2003)

«Method validation»

• Spike recovery and repeatability

Spiked ingredient	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Iprodione	Chicken formula feed	100-500	3	92.3-96.8	7.5
	Swine formula feed	100-500	3	93.6-96.5	5.3
	Fescue	100-500	3	85.6-94.2	7.6
	Timothy	100-500	3	83.2-86.8	5.8
Iprodione metabolite	Chicken formula feed	100-500	3	100.5-106.2	16.2
	Swine formula feed	100-500	3	99.1-116.2	3.9
	Fescue	100-500	3	92.7-101.1	18.2
	Timothy	100-500	3	94.6-98.3	10.4

• Collaborative study

Target	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Iprodione	Adult chicken formula feed	6	500	92.6	5.4	9.7	1.53
	Soymeal	6	500	93.8	6.1	12.2	1.92
Iprodione metabolite	Adult chicken formula feed	6	500	101.6	8.6	8.6	1.37
	Soymeal	6	500	97.8	4.2	5.0	0.79

・Quantification lower limit: iprodione and iprodione metabolite in sample: 20 μg/kg, respectively

«Notes and precautions»

[1] Take care of sudden boiling during extraction procedure, because it possibly occurs during condensing under reduced pressure. Since remaining acetone, if any, makes following procedures complex, completely volatilize acetone.

[2] A column filled with styrene divinilbenzene copolymer hard gel using the eluent.

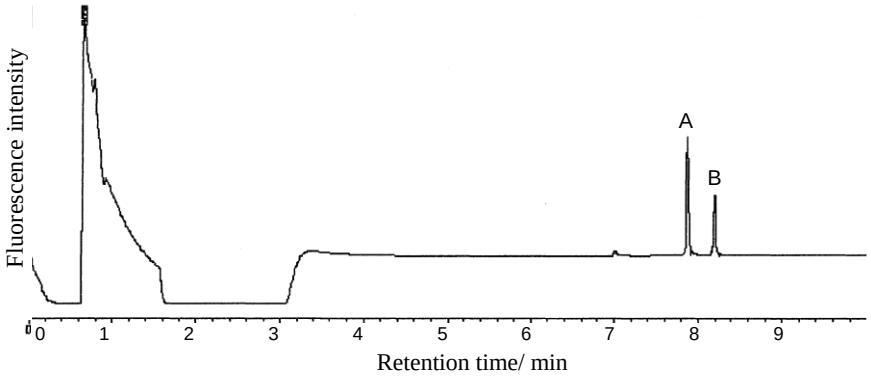
[3] It is known that iprodione and its metabolites are easily adsorbed to columns or degraded by heat, and the metabolite strongly shows this tendency. Therefore, it is required to make thin (0.1 μm) the film thickness of the column liquid phase and make short (7 m) the column length.

[4] DB-1 (Agilent Technologies) and others are available.

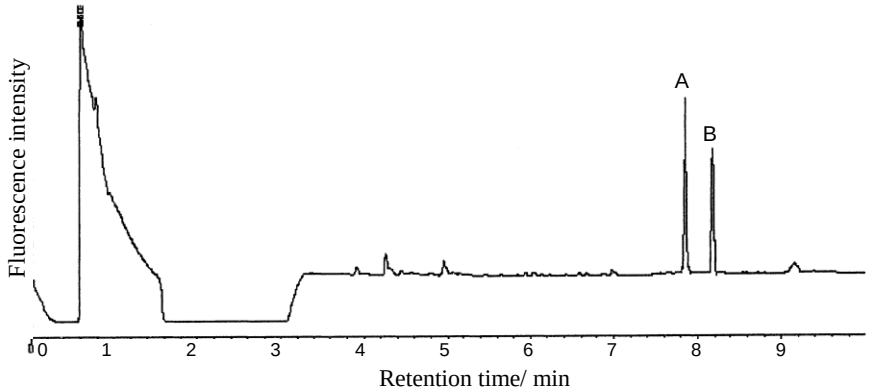
[5] When an inlet insert containing glass wool is used, sensitivity may become insufficient due to adsorbing to the glass wool. In such case, you may change the glass wool or use an inlet insert without glass wool.

[6] An example of chromatogram is shown in Figure 6.1.26-2.

(1)



(2)



(3)

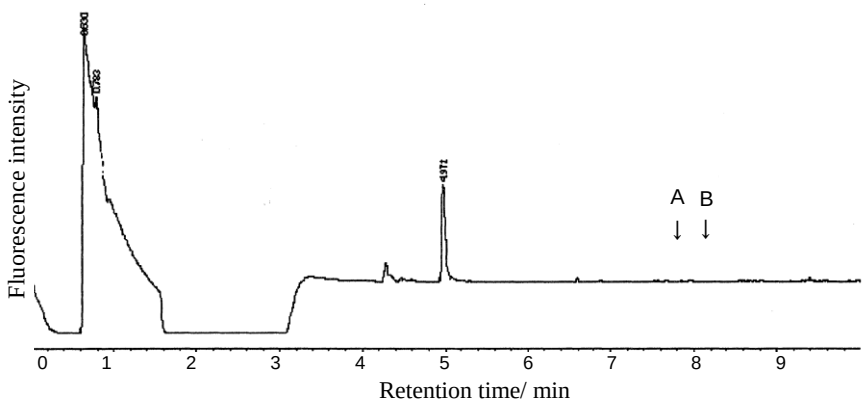


Figure 6.1.26-2. Gas chromatograms of the mixed standard solution and sample solution (A and B indicate the peak of iprodione and iprodione metabolite, respectively)

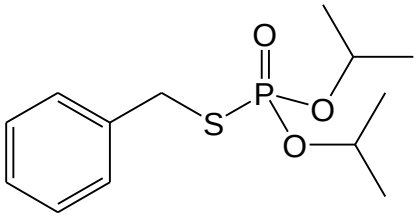
Gas chromatograph conditions: Refer to operating conditions (example).

(1) Mixed standard solution (5 ng each of iprodione and iprodione metabolite)

(2) Chicken formula feed added with amounts equivalent to 500 μg/kg each of iprodione and iprodione metabolite.

(3) Blank (timothy)

27 Iprobenfos (IBP)



S-benzyl *O*,*O*-diisopropyl phosphorothioate

$C_{13}H_{21}O_3PS$ MW: 288.34 CAS No.: 26087-47-8

[Summary of iprobenfos]

Iprobenfos is an organic phosphorus bactericide, yellow liquid, developed by Kumiai Chemical Industry. Iprobenfos is used to control rice blast.

Iprobenfos was registered as an agricultural chemical in 1967, in Japan. Registered name is IBP. The trade name is "Kitajin P".

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

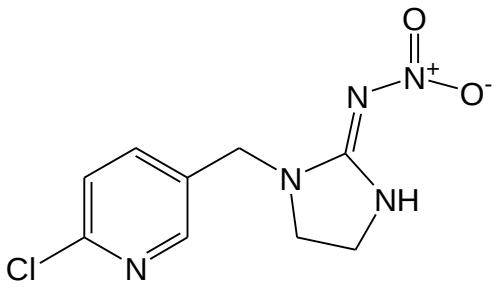
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

28 Imidacloprid



(*E*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine

$C_9H_{10}ClN_5O_2$ MW: 255.7 CAS No.: 138261-41-3

[Summary of imidacloprid]

Iimidacloprid is a chloronicotinyl insecticide used to control many kinds of pests to rice plants, fruit trees, vegetables, etc.

Iimidacloprid was registered as an agricultural chemical in 1992, in Japan. The trade name is "Admire".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

Oat, barley, wheat, miloa and rye: 0.05 ppm, corn: 0.1 ppm, pasture grass: 6 ppm

Rice plant silage: 3 ppm, rice straw: 10 ppm (Percentage of rice straw etc. should not exceed 70 % of total amounts of feed)

[Methods listed in the Analytical Standards of Feeds]

1. Analysis method for imidacloprid by liquid chromatograph-mass spectrometer [Analytical Standards of Feeds Chapter 6, Section 1, Article 28.1]

A. Reagent preparation

1) Imidacloprid standard solution. Weigh accurately 10 mg of imidacloprid [$C_9H_{10}ClN_5O_2$][1], place in a 100 mL volumetric flask, add methanol to dissolve, and further add methanol to the marked line to prepare the imidacloprid standard stock solution (1 mL of this solution contains an amount equivalent to 0.10 mg of imidacloprid.).

At the time of use, accurately dilute a definite quantity of standard stock solution with methanol to prepare several imidacloprid standard solutions containing amounts equivalent to 0.002-0.2 µg/mL of imidacloprid.

2) 0.5 mol/L phosphoric acid buffer solution. Weigh 52.7 g of dipotassium hydrogen phosphate and 30.2 g of potassium dihydorogen phosphate, dissolve in 500 mL of water, adjust the pH to 7.0 with sodium hydroxide solution (1 mol/L) or hydrochloric acid (1 mol/L), and further add water to make 1 L.

B. Quantification

Extraction. Weigh 10.0 g of analysis sample, place in a stoppered 200 mL Erlenmeyer flask, add 50 mL

(100 mL for hay) of acetonitrile – water (13 : 7), and shake for 30 minutes to extract. Place a 200 mL tall beaker under a Büchner funnel, filter the extract by suction through filter paper (No. 5B), wash the said Erlenmeyer flask and the residue serially with 25 mL of acetonitrile (50 mL for hay), and filter by suction in a similar way. Place the filtrate in a 100 mL (200 mL for hay) volumetric flask, wash the said tall beaker with a small amount of acetonitrile, and add the washings to the volumetric flask. Further add acetonitrile to the volumetric flask up to the marked line to prepare the sample solution subject to liquid-liquid extraction[*1].

Liquid-liquid extraction. Exactly place 20 mL (10 mL for hay) of the sample solution in a 100 mL separating funnel. Add 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphoric acid buffer solution to the separating funnel, shake for 10 minutes, allow to stand, and remove the water layer (lower layer) to obtain the acetonitrile layer (upper layer) as the sample solution subject to column treatment I.

Column treatment I. Wash an octadecylsilylated silica gel minicolumn (500 mg)[*2] with 10 mL of acetonitrile.

Place a 100 mL Erlenmeyer flask under the minicolumn, place the sample solution in the minicolumn, and naturally flow down until the liquid surface reaches the top of the column packing material to flow out imidacloprid. Further add 2 mL of acetonitrile to the minicolumn, and flow out in a similar way. Dehydrate the effluent with an appropriate quantity of sodium sulfate (anhydrous), filter with filter paper (No. 5B) into a 100 mL recovery flask, wash the said Erlenmeyer flask with a small amount of acetonitrile, and add the washings to the filtrate through the said filter paper.

Condense the filtrate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas. Add 2 mL of acetonitrile – toluene (3 : 1) to dissolve the residue to prepare the sample solution subject to column treatment II.

Column treatment II. Wash a graphite carbon/aminopropyl silylated silica gel minicolumn (500 mg/500 mg)[*3] with 10 mL of acetonitrile – toluene (3 : 1).

Place a 50 mL recovery flask under the minicolumn, place the sample solution in the minicolumn, flow down until the liquid surface reaches the top of the column packing material to flow out imidacloprid[*4]. Wash the recovery flask having contained the sample solution twice with 2 mL each of acetonitrile – toluene (3 : 1), and add the washings serially to the minicolumn to flow out in a similar way[*4]. Further add 16 mL of acetonitrile – toluene (3 : 1) to the minicolumn to flow out in a similar way[*4].

Condense the effluent almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas. Add 2 mL of methanol accurately to dissolve the residue, and centrifuge at 5,000×*g* for 5 minutes to obtain the supernatant as the sample solution subject to measurement by liquid chromatograph-mass spectrometer.

Measurement by liquid chromatograph-mass spectrometer. Inject 5 µL each of the sample solution and respective imidacloprid standard solutions into a liquid chromatograph mass spectrometer to obtain selected ion monitoring chromatograms

Example of measurement conditions

Column: Octadecylsilylated silica gel column (internal diameter: 3.0 mm, length: 250 mm, particle size: 5 µm)[*5]

Eluent: 5 mmol/L ammonium acetate solution[*6] – 5 mmol/L ammonium acetate methanol solution[*7]
(17 : 3) → 1 min → (3 : 2) (hold 2.5 min) → 2.5 min → (1 : 1) → 2 min → (9 : 11) → 9.5 min → (1 :19) (hold 12.5 min) → (17 : 3) (hold 17 min)

Flow rate: 0.2 mL/min

Column oven temperature: 40 °C

Detector: Quadrupole mass spectrometer[*8]

Ionization method: Electrosplay ionization (ESI) method (positive ion mode)

Nebulizer gas: N_2 (2.5 L/min)

Drying gas: N_2 (10 L/min)

Heat block temperature: 200 °C

CDL temperature: 250 °C

Monitor ion: *m*/*z* 256

Calculation. Calculate the peak height or peak area from the obtained selected ion monitoring chromatogram[2] to prepare a calibration curve to determine the amount of imidacloprid in the sample.

* 1. When the amount of imidacloprid contained in the sample is large, dilute the extract with acetonitrile before starting the following procedures.

2. Supelclean LC-18 (syringe volume: 6 mL, Supelco) or an equivalent.

3. ENVI-Carb/LC-NH_2 (Supelco) or an equivalent.

4. Flow rate is 2-3 mL/min. Use a vacuum manifold if necessary.

5. ZORBAX Eclipse XDB-C18 (Agilent Technologies) or an equivalent.

6. Dissolve 7.7 g of ammonium acetate in water to make 1 L, and further dilute 50 mL of the solution with water to make 1 L.

7. Dissolve 7.7 g of ammonium acetate in methanol to make 1 L, and further dilute 50 mL of the solution with methanol to make 1 L.

8. Conditions for LCMS-2010EV (Shimadzu Corporation)

«Summary of analysis methods»

This method is intended to determine the amount of imidacloprid in feed by extracting with hydrous acetonitrile, purifying with the liquid-liquid distribution using phosphoric acid buffer solution, a C_{18} minicolumn and a graphite carbon/NH_2 minicolumn, and quantifying with a liquid chromatograph mass spectrometer.

The flow sheet of analysis methods is shown in Figure 6.1.28-1.

Sample (10.0 g)
- Add 50 mL (100 mL for hay) of acetonitrile-water (13 : 7).
- Stir for 30 min.
- Filter by suction (No. 5B).
- Wash with 25 mL (50 mL for hay) of acetonitrile.
- Determine the volume to 100 mL (200 mL for hay) with acetonitrile.

Sample solution (20 mL) (10 mL for hay)

100 mL separating funnel
- Add 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer solution.
- Stir for 10 min.

Acetonitrile layer (upper layer) | Water layer (lower layer)

Supelclean LC-18 cartridge (previously wash with 10 mL of acetonitrile)
- Load the sample solution
- Add 2 mL of acetonitrile.
- Dehydrate with an appropriate amount of sodium sulphate (anhydrous).
- Filter (filter paper: No. 5B)
- Condense under reduced pressure (40 °C or lower), and evaporate into dryness with nitrogen g
- Add 2 mL of acetonitrile-toluene (3 : 1).

ENVI-Carb/LC-NH_2 Cartridge

(Previously wash with 10 mL of acetonitrile-toluene (3: 1))
- Load the sample solution
- Wash twice with 2 mL each of acetonitrile-toluene (3 : 1).
- Add 16 mL of acetonitrile-toluene (3 : 1).
- Condense under reduced pressure (40 °C or lower), and evaporate into dryness with nitrogen g
- Add 2 mL of methanol.

LC-MS

Figure 6.1.28-1. Flow sheet of analysis method for imidacloprid

References: Tomoharu Nozaki, Toshiaki Yamata: Research Report of Animal Feed, 32, 23 (2007)

Japan Food Research Laboratories: Development of analysis methods for establishing the fiscal 2006 residue standards for hazardous substances, etc. in feeds, contract projects for researching transitional hazardous substances to live stocks, and development of analysis methods for hazardous substances in feeds, 42 (2007)

«Method validations»

• Spike recovery and repeatability

Sample type	Spike concentratior (μg/kg)	Repeat	Spike recovery (%)	Repeatability RSD (% or less)
Corn	5-100	3	83.4-92.6	3.1
Ryegrass	200-6,000	3	89.9-92.2	1.6

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Corn	8	100	83.0	3.0	14.6	0.66
Alfalfa	8	5,000	84.3	3.5	12.1	0.96

• Quantification lower limit: 5 μg/kg (spike recovery and relative standard deviation)

• Detection lower limit: 2 µg/kg (*SN* ratio)

«Notes and precautions»

[1] The standard preparation is commercially available from Kanto Chemical, Wako Pure Chemical, etc.

[2] An example of selected ion monitoring (SIM) chromatogram is shown in Figure 6.1.28-2.

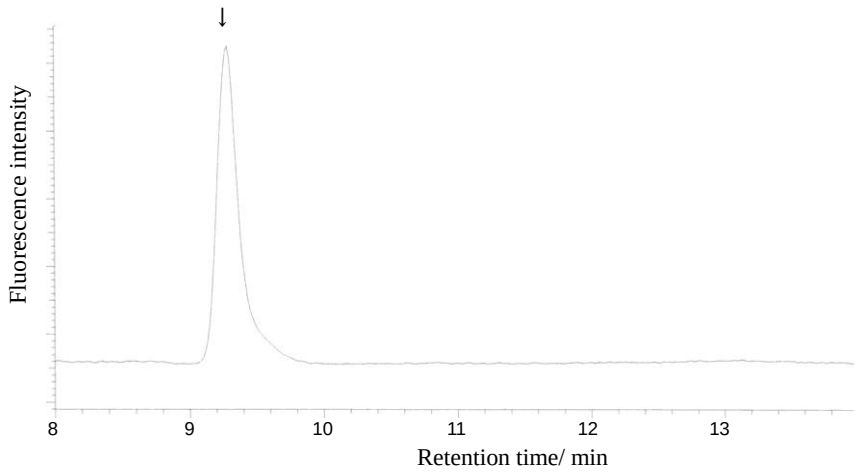
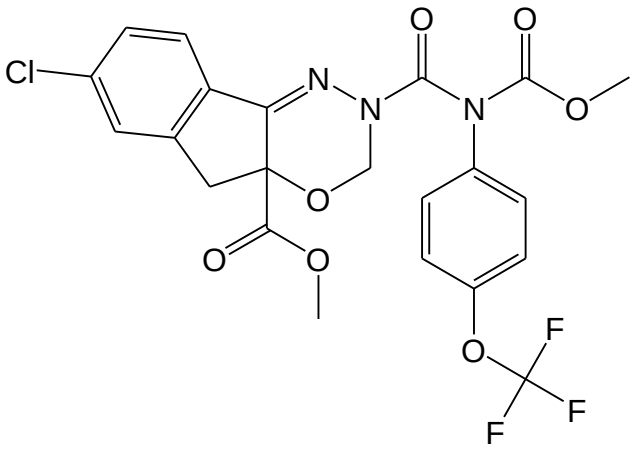


Figure 6.1.28-2. SIM chromatogram for imidacloprid standard solution (500 µg/mL)
(The arrow indicates the peak of imidacloprid)

29 Indoxacarb



(*S*)-methyl 7-chloro-2,5-dihydro-2-[[(methoxycarbonyl) [4-(trifluoromethoxy)phenyl]amino]carbonyl] indeno[1,2-*e*][1,3,4] oxadiazine-4*a*(3*H*)-carboxylate

$C_{22}H_{17}ClF_3N_3O_7$ MW: 527.9 CAS No.: 144171-61-9 (MP), 173584-44-6 (*S*-isomer)

[Summary of indoxacarb]

Indoxacarb is an oxadiazine insecticide developed by DuPont (USA). Indoxacarb has an action mechanism other than that of existing insecticides that interrupts the signal transduction via nervous system by blocking sodium channels in the nerve axon.

Indoxacarb was registered as an agricultural chemical in 2001, in Japan (as indoxacarb MP (racemic body)). The trade name is “Tornado”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn: 0.02 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Analysis method for indoxacarb by liquid chromatograph-mass spectrometer [Analytical Standards of Feeds Chapter 6, Section 1, Article 29.1]

A. Reagent preparation

Indoxacarb standard solution. Weigh accurately 25 mg of indoxacarb MP[1] [$C_{22}H_{17}ClF_3N_3O_7$], place in a 50 mL volumetric flask, add acetonitrile to dissolve, and further add the solvent up to the marked line to prepare the indoxacarb standard stock solution (1 mL of this solution contains an amount equivalent to 0.5 mg of indoxacarb).

At the time of use, dilute accurately a definite quantity of standard stock solution with acetonitrile to prepare several indoxacarb standard solutions containing amounts equivalent to 0.001-1 μg/mL of indoxacarb.

B. Quantification

Extraction. Weigh accurately 10.0 g of the analysis sample, place in a stoppered 200 mL Erlenmeyer

flask, add 20 mL (30 mL for hay) of water, allow to stand for 30 minutes, further add 100 mL of methanol, and shake for 30 minutes to extract. Place a 200 mL volumetric flask under a Büchner funnel, filter the extract through filter paper (No. 5B) by suction, wash the said Erlenmeyer flask and residue serially with 50 mL of methanol, filter by suction in a similar way, and further add methanol up to the marked line of the volumetric flask. Place accurately 20 mL of this solution (20 mL of solution further diluted 100-fold with methanol for hay) in a 100 mL recovery flask, and condense to approximately 2 mL (almost into dryness for hay) under reduced pressure in a water bath at 40 °C or lower to prepare the sample solution subject to column treatment I.

Column treatment I. Add 15 mL of water to the sample solution, place in a porous diatom earth column (for 20 mL retention), and allow to stand for 5 minutes. Place a 200 mL recovery flask under the column, wash the recovery flask having contained the sample solution 3 times with 20 mL each of ethyl acetate − hexane (1 : 1), add the washings serially to the column, and flow down until the liquid surface reaches the top of the column packing material to elute indoxacarb. Further add 40 mL of ethyl acetate − hexane (1 : 1) to the column, elute in a similar way, condense the eluate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add 5 mL of hexane − diethyl ether (9 : 1) to dissolve the residue to prepare the sample solution subject to column treatment II.

Column treatment II. Wash a silica gel minicolumn (690 mg) with 5 mL of hexane-diethyl ether (9 : 1). Place the sample solution in the minicolumn, and flow out until the liquid surface reaches the top of the column packing material. Wash the recovery flask having contained the sample solution 3 times with 5 mL each of hexane − diethyl ether (9 : 1), add the washings serially to the minicolumn, and flow out in a similar way. Further add 10 mL of hexane − diethyl ether (17 : 3) to the minicolumn and wash.

Connect a synthetic magnesium silicate minicolumn (910 mg) having been washed with 5 mL of hexane − diethyl ether (7 : 3) under the sillica gel minicolumn. Add 20 mL of hexane − diethyl ether (7 : 3) to the minicolumn, and flow down until the liquid surface reaches the top of the column packing material[*1] to transfer indoxacarb to the synthetic magnesium silicate minicolumn.

Then, remove the silica gel minicolumn, place a 50 mL recovery flask under the synthetic magnesium silicate minicolumn, and add 20 mL of hexane − acetone (17 : 3) to the synthetic magnesium silicate minicolumn to elute indoxacarb.

Condense the eluate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas. Add 2 mL of acetonitrile accurately to dissolve the residue, and centrifuge at 5,000×*g* for 5 minutes to obtain the supernatant as the sample solution subject to measurement by liquid chromatograph-mass spectrometer.

Measurement by liquid chromatograph-mass spectrometer. Inject 5 µL each of the sample solution and respective indoxacarb standard solutions into a liquid chromatograph mass spectrometer to obtain selected ion monitoring chromatograms.

Example of measurement conditions

Column: Octadecylsilylated silica gel column (internal diameter: 3.0 mm, length: 250 mm, particle size: 5 µm)[*2]

Eluent: Methanol − 5 mmol/L ammonium acetate solution (4 : 1)
Flow rate: 0.5 mL/min
Column oven temperature: 40 °C
Detector: Quadrupolar type mass spectrometer*3
Ionization method: Atmospheric pressure chemical ionization (APCI) method [2] (positive ion mode)
Nebulizer gas: N_2 (2.5 L/min)
Interface temperature: 400 °C
Heat block temperature: 200 °C
CDL temperature: 250 °C
Monitor ion: *m*/*z* 528[3]

Calculation. Calculate the peak area or height from the obtained selected ion monitoring chromatogram[4] to prepare a calibration curve, and determine the amount of indoxacarb*4 in the sample.

* 1. The flow rate is approximately 1 mL/min. Use a vacuum manifold if necessary.
2. ZORBAX Eclipse XDB-C18 (Agilent Technologies, the retention time of indoxacarb is approximately 6 minutes under the operating conditions) or an equivalent.
3. Conditions with LCMS-2010EV (Shimadzu Corporation)
4. The *S*-isomer and *R*-isomer of indoxacarb are not separated, and quantified as a total amount.

«Summary of analysis methods»

This method is intended to determine the amount of indoxacarb in feeds by extracting with hydrous methanol, purifying with a porous diatom earth column, silica gel minicolumn and Florisil minicolumn, and quantifying with a liquid chromatograph mass spectrometer (ionization method: APCI method).

The flow sheet of analysis methods is shown in Figure6.1.29-1.

10 g of sample (200 mL stoppered Erlenmeyer flask)
- Add 20 mL of water (30 mL for hay), and allow to stand for 30 min.
- Add 100 mL of methanol, and stir for 30 min.
- Filter by suction (No. 5B).
- Wash with 50 mL of methanol.
- Determine the volume with 200 mL of methanol.

20 mL of sample solution (20 mL of the solution diluted 100-fold with methanol for hay)
(receive with a 100 mL recovery flask)
- Condense to 2 mL or less (40 °C or lower)

Chem Elut cartridge (for 20 mL retention)
- Load the sample solution with adding 15 mL of water, and allow to stand for 5 min.
- Wash 3 times with 20 mL each of ethyl acetate-hexane (1 : 1).
(receive with a 200 mL recovery flask)
- Elute with 40 mL of ethyl acetate-hexane (1 : 1).
- Condense under reduced pressure (40 °C or lower), and introduce nitrogen gas to evaporate into dryr
- Add 5 mL of hexane-diethylether (9 : 1).

Followed with the next page

Sep-Pak Plus Silica cartridge (having been washed with 5 mL of hexane-diethyl ether (9 : 1))
- Load the sample solution
- Wash 3 times with 5 mL each of hexane-diethylether (9 : 1).
- Wash with 10 mL of hexane-diethylether (17 : 3).
- Connect a Sep-Pak Plus Florisil cartridge under a Sep-Pak Plus Silica cartridge (having been washed with 5 mL of hexane-diethylether (7 : 3))
- Transfer indoxacarb to a Sep-Pak Plus Florisil cartridge with 20 mL of hexane-diethylether (7 : 3).
- Remove the Sep-Pak Plus Silica cartridge.
- Elute with 20 mL of hexane-acetone (17 : 3) (receive with a 50 mL recovery flask)
- Condense under reduced pressure (40 ℃ or lower), and introduce nitrogen gas to evapolate into dr
- Add 2 mL of actonitrile (exactly)
- Centrifuge at 5,000×g for 5 min

LC-MS

Figure 6.1.29-1. Flow sheet of the analysis method for indoxacarb

References: Daisaku Makino, Rie Matsuno, Miho Yamada: Research Report of Animal Feed, 34, 1 (2009)

«Method validations»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Adult chicken formula feed	5-500	3	81.5-89.1	9.3
Beef cattle formula feed	5-500	3	83.5-91.7	4.3
Corn	5-500	3	77.2-85.0	6.3
Alfalfa hay	0.5-50 mg/kg	3	77.7-91.8	17

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Beef cattle formula feed	6	50 μg/kg	93.8	5.1	8.1	0.37
Alfalfa hay	6	5 mg/kg	87.6	4.9	14	1.1

• Quantification lower limit: 5 μg/kg (0.5 mg/kg for hay) (*SN* ratio)

• Detection lower limit: 2 μg/kg (0.2 mg/kg for hay) (*SN* ratio)

«Notes and precautions»

[1] Indoxacarb is consists of 2 optical isomers, i.e. *S*-isomer and *R*-isomer, and the former only has insecticidal effect. However, *S*-isomer and *R*-isomer can not be separated under usual liquid chromatograph conditions, and the standard preparation of indoxacarb MP and the bulk of the current agricultural chemical is a mixture of *S*-isomer and *R*-isomer. Therefore, indoxacarb MP, the racemic mixture, is employed as the standard preparation in this method.

[2] For model used at the time of validation of this method, the recovery rate sometimes decreased by using the electrospray ionization (ESI) method; therefore, APCI method was employed in this method. When ESI method is used for quantification, you must preliminarily confirm that the model to be used

can achieve a precision equivalent to APCI method.

[3] A protonated molecule $[M+H]^+$ of indoxacarb is used as ions to be monitored. The mass spectrum of indoxacarb is shown in Figure 6.1.29-2.

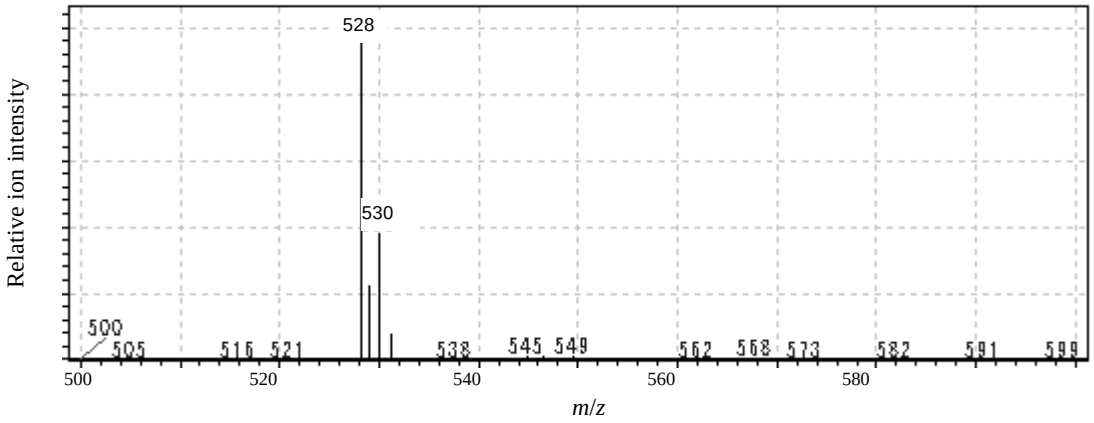
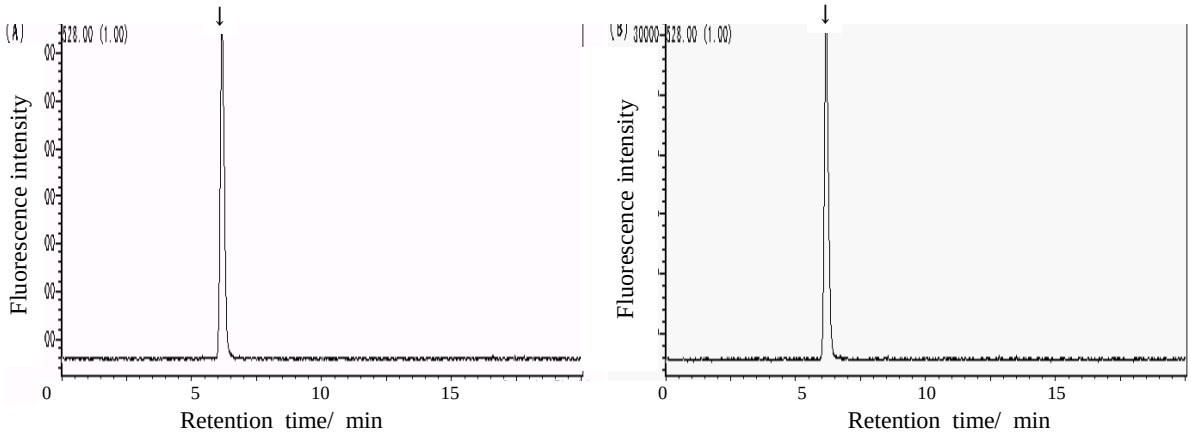


Figure 6.1.29-2. Mass spectrum of indoxacarb standard solution

[4] Examples of SIM chromatograms obtained for the standard solution and sample solution are shown in Figure 6.1.29-3.

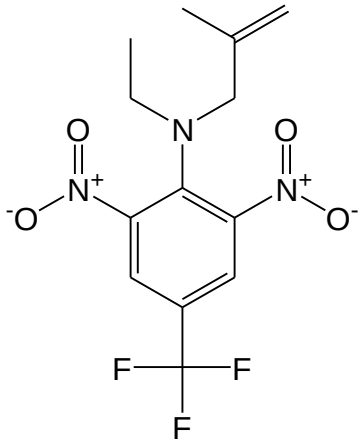


Standard solution (equivalent to 0.125 ng) Adult chicken formula feed (equivalent to 50 µg/kg)

Figure 6.1.29-3. SIM chromatogram for the standard solution and sample solution (The arrow indicates the peak of indoxacarb.)

Refer to the "Operating conditions (example)" for operating conditions.

30 Ethalfluralin



N-ethyl-*α*,*α*,*α*-trifluoro-*N*-(2-methylallyl)-2,6-dinitro-*p*-toluidine

$C_{13}H_{14}F_3N_3O_4$ MW: 333.3 CAS No.: 55283-68-6

[Summary of ethalfluralin]

Ethalfluralin is a dinitroaniline herbicide.

Ethalfluralin has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

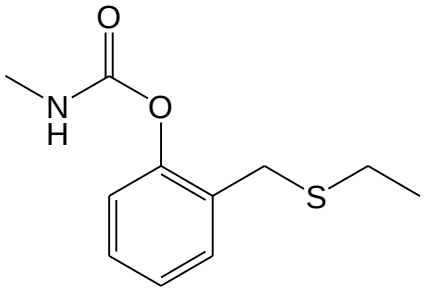
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

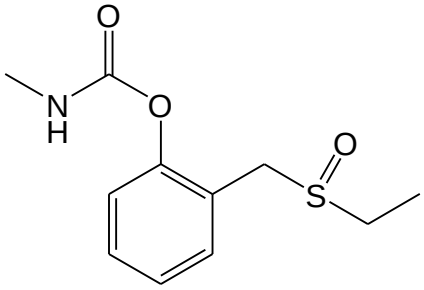
31 Ethiofencarb
(including ethiofencarb sulfoxide and ethiofencarb sulfone)



α-ethylthio-*o*-tolyl methylcarbamate

$C_{11}H_{15}NO_2S$ MW: 225.31 CAS No.: 29973-13-5

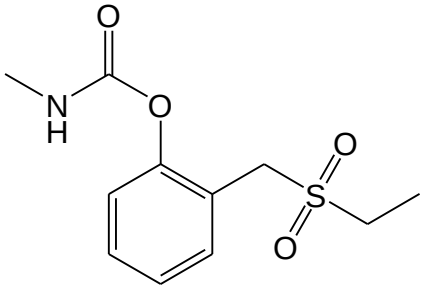
Ethiofencarb sulfoxide



$C_{11}H_{15}NO_3S$ MW: 241.31

CAS No.: 53380-22-6

Ethiofencarb sulfone



$C_{11}H_{15}NO_4S$ MW: 257.31

CAS No.: 53380-23-7

[Summary of ethiofencarb]

Ethiofencarb is a carbamate insecticide developed by Bayer (Germany). Ethiofencarb has insecticidal effect on aphids by inhibiting cholinesterase, giving instant results with its contact effect and permeable effect.

Ethiofencarb is rapidly acidized to metabolites, the sulfoxide-form and sulfone-form, within the plant, and these acidized metabolites also have physiological activity.

Ethiofencarb had been registered as an agricultural chemical in 1982, in Japan. However, it was expired in 2007.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye and corn: 1.0 ppm, other grains: 0.05 ppm

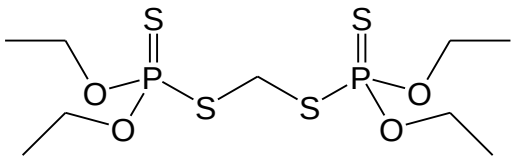
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (2)

Target Analytes: Ethiofencarb (including ethiofencarb sulfoxide and ethiofencarb sulfone), bendiocarb and methiocarb (including methiocarb sulfoxide and methiocarb sulfone) (3 compounds)

Refer to Article 4, Section 3 in this chapter.

32 Ethion



O,*O*,*O*',*O*'-tetraethyl *S*,*S*'-methylene bis(phosphorodithioate)

$C_9H_{22}O_4P_2S_4$ MW: 384.5 CAS No.: 563-12-2

[Summary of ethion]

Ethion is an organic phosphorus insecticide, miticide developed by FMC (UK). Ethion has a special effect on sucking type pests, giving instant results, and has residual efficacy.

Ethion had been registered as an agricultural chemical in 1963, in Japan. However, it was expired in 2005.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Pasture grass: 20 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

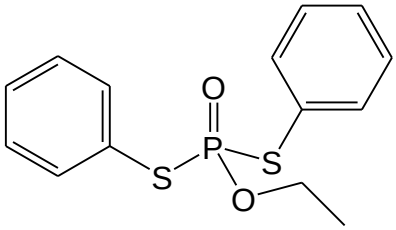
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

33 Edifenphos (EDDP)



O-ethyl *S*,*S*-diphenyl phosphorodithioate

$C_{14}H_{15}O_2PS_2$ MW: 310.4 CAS No.: 17109-49-8

[Summary of edifenphos]

Edifenphos is an organic phosphorus bactericide, yellowish brown liquid, developed by Bayer (Germany). Edifenphos is effective to crown rot, *Cochliobolus miyabeanus* as well as to blast.

Edifenphos was registered as an agricultural chemical in 1967, in Japan. Registered name is EDDP. The trade name is "Hinosan".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Administrative Guidelines for Hazardous Substances in Feeds]

Rice plant silage: 1 ppm, rice straw: 10 ppm

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

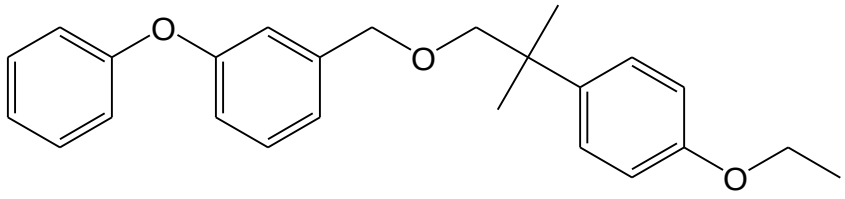
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

34 Etofenprox



2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether

$C_{25}H_{28}O_3$ MW: 376.5 CAS No.: 80844-07-1

[Summary of etofenprox]

Etofenprox is a pyrethroid-like insecticide with a specific chemical structure with ether linkage, developed by Mitsui Chemical.

Etofenprox was registered as an agricultural chemical targeting grains, vegetables, fruits, etc. in 1987, in Japan. The trade name is “Trebon”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye and corn: 0.5 ppm

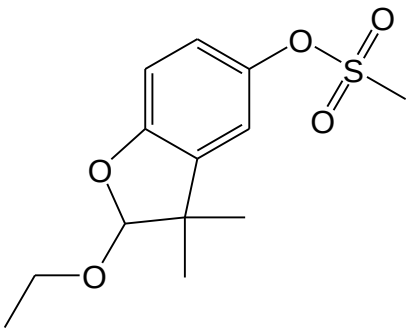
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

35 Ethofumesate



(*RS*)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate

$C_{13}H_{18}O_5S$ MW: 286.3 CAS No.: 26225-79-6

[Summary of ethofumesate]

Ethofumesate is a benzofuran herbicide with an action mechanism inhibiting photosynthesis and respiration as well as mitosis.

Ethofumesate has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye and corn: 1.0 ppm

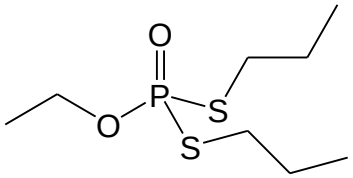
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

36 Ethoprophos



O-ethyl *S*,*S*-dipropyl phosphorodithioate

$C_8H_{19}O_2PS_2$ MW: 242.3 CAS No.: 13194-48-4

[Summary of ethoprophos]

Ethoprophos is an organic phosphorus insecticide, light yellow liquid. Ethoprophos is used as a nematicidal agent, may be used for soil treatment.

Ethoprophos had been registered as an agricultural chemical in 1994, in Japan. However, it was expired in 2002.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

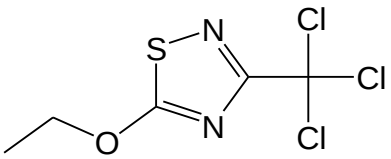
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

37 Etridiazole



Ethyl 3-trichloromethyl-1,2,4-thiadiazol-5-yl ether

$C_5H_5Cl_3N_2OS$ MW: 247.5 CAS No.: 2593-15-9

[Summary of etridiazole]

Etridiazole is a thiazole bactericide developed by Olin (USA) in1964.

Etridiazole was registered as an agricultural chemical in 1973, in Japan. Registered name is echlomezole. The trade names are “Sun Yard” or “Pansoil”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat: 0.05 ppm, corn: 0.1 ppm

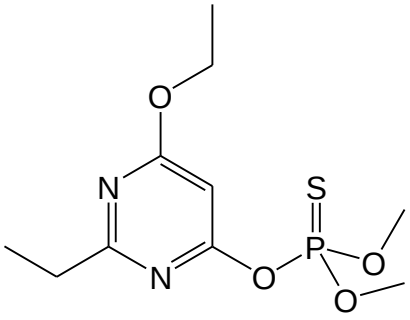
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

38 Etrimfos



O-6-ethoxy-2-ethylpyrimidin-4-yl *O*,*O*-dimethyl phosphorothioate

$C_{10}H_{17}N_2O_4PS$ MW: 292.3 CAS No.: 38260-54-7

[Summary of etrimfos]

Etrimfos is an organic phosphorus insecticide, colorless liquid, developed by Sandoz (Switzerland). Etrimfos has effects mainly on vegetable pests such as cutworm and chafer.

Etrimfos had been registered as an agricultural chemical in 1984, in Japan. However, it was expired in1993.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

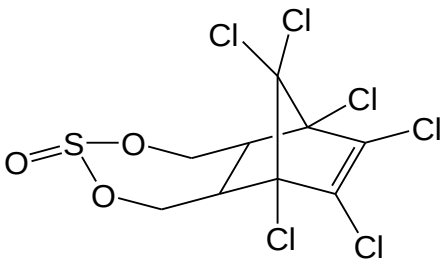
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

39 Endosulfan
(*α*-endosulfan and *β*-endosulfan)



6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide

$C_9H_6Cl_6O_3S$ MW: 406.93

CAS No.: 115-29-7 (*α*-Endosulfan: 959-98-8; *β*-Endosulfan: 33213-65-9)

[Summary of endosulfan]

Endosulfan is an organic chlorine insecticide giving instant results with residual efficacy to various pests to fruit plants, vegetables, etc. The bulk is a mixture of *α*-body (64-67 %) and *β*-body (29-32 %). Endosulfan sulfate is its metabolite. The residual standard value is defined as a total amount of *α*-endosulfan and *β*-endosulfan.

Endosulfan was registered as an agricultural chemical in 1962, in Japan. Registered name is benzoepin. The trade names are “Marix” or “Thiodan”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(a total amount of *α*-endosulfan and *β*-endosulfan)

Barley, rye and corn: 0.1 ppm, wheat: 0.2 ppm, other grains: 0.1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

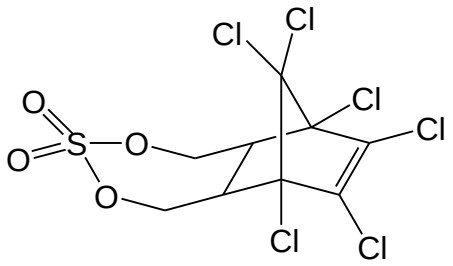
Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

40 Endosulfan sulfate



6,7,8,9,10,10-hexachloro01,5,5a,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3,3-dioxide

$C_9H_6Cl_6O_4S$ MW: 422.92 CAS No.: 1031-07-8

[Summary of endosulfan sulfate]

Endosulfan sulfate is a metabolite of endosulfan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

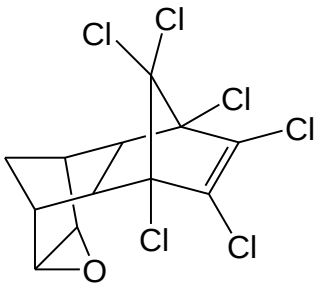
Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

41 Endrin



(1a*R*,2*S*,2a*S*,3*S*,6*R*,6a*R*,7*R*,7a*S*)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphtho[2,3-*b*]oxirene

$C_{12}H_8Cl_6O$ MW: 380.9 CAS No.: 72-20-8

[Summary of endrin]

Endrin is one of organic chlorine insecticides, white crystal, which is an *endo-endo* type isomer of dieldrin.

Endrin has strong acute toxicity to humans and animals, and designated to a poisonous substance.

Endrin is an agricultural chemical easily staying behind soil and accumulates in the human body via agricultural crops.

Endrin had been registered as an agricultural chemical in 1954, in Japan. However, it was expired in1993. The production, distribution and use of this agricultural chemical were banned for all intended purpose in 1981.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Pasture grass: 0.01 ppm

Swine, chicken and quail feeds, cattle, sheep, goat and deer feed: 0.01 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, β-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

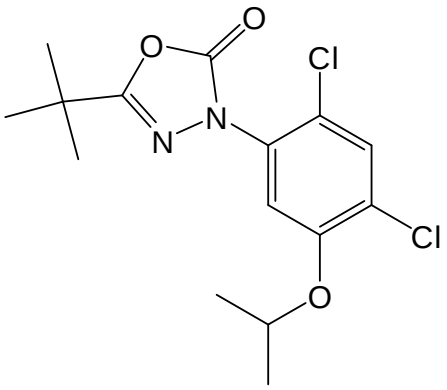
Refer to Article 1, Section 2 in this chapter.

3. Simultaneous analysis method of organochlorine agricultural chemicals by gas chromatography

Target Analytes: α-BHC, β-BHC, γ-BHC, δ-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, endrin, dieldrin, heptachlor and heptachlor epoxide (15 compounds)

Refer to Article 2, Section 3 in this chapter.

42 Oxadiazon



5-*tert*-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2(3*H*)-one
$C_{15}H_{18}Cl_2N_2O_3$ MW: 345.2 CAS No.: 19666-30-9

[Summary of oxadiazon]

Oxadiazon is an oxadiazolone herbicide developed by Rhône-Poulenc (France) in 1963.

Oxadiazon had been registered as an agricultural chemical initially in 1972, in Japan; after then, however, fell into abeyance, and registered again as an agricultural chemical to rice plants in 2003. The trade name is "Ronstar".

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

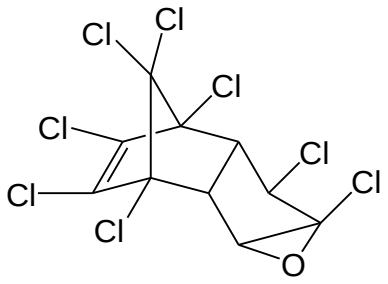
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

43 Oxychlordane



(1a*α*,1b*β* 2*α*,5*α*,5a*β*,6*β*,6a*α*)-2,3,4,5,6,6a,7,7-octachloro-1a,1b,5,5a,6,6a-hexahydro-2,5-methano-2*H*-indeno[1,2-*b*]oxirene

$C_{10}H_4Cl_8O$ MW: 423.8 CAS No.: 27304-13-8

[Summary of oxychlordane]

Oxychlordane is a metabolite of chlordane (62 in this chapter). The residual standard values in animal products and aquatic products are defined as the total amount of *cis*-chlordane, *trans*-chlordane and oxychlordane by Food Sanitation Law.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

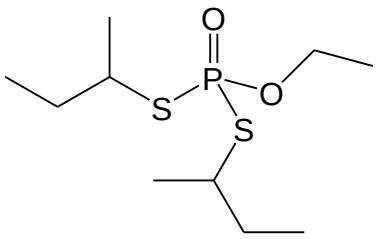
Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

44 Cadusafos



S,*S*-di-*sec*-butyl *O*-ethyl phosphorodithioate

$C_{10}H_{23}O_2PS_2$ MW: 270.4 CAS No.: 95465-99-9

[Summary of cadusafos]

Cadusafos is an organic phosphorus insecticide developed by FMC (UK) in 1982, having insecticidal effects by inhibiting the acetylcholine esterase activity.

Cadusafos has been registered as an agricultural chemical for fruits, vegetables, etc. in USA (import tolerance only), Australia, Spain, Korea and other countries.

Cadusafos was registered as an agricultural chemical for vegetables, soy, etc., in 2000, in Japan. The trade name is “Rugby”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

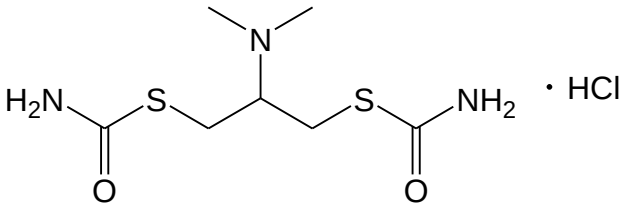
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

45 Cartap hydrochloride (cartap, thiocyclam and bensultap)



S,*S*′-(2-dimethylaminotrimethylene) bis(thiocarbamate) hydrochloride

Cartap	$C_7H_{15}N_3O_2S_2$	MW: 237.3	CAS No.: 15263-53-3
Cartap hydrochloride	$C_7H_{16}ClN_3O_2S_2$	MW: 273.8	CAS No.: 15263-52-2

[Summary of cartap]

Cartap is a nereistoxin insecticide having action inhibiting impulse transmission by acetylcholine in insects.

Cartap was registered as an agricultural chemical (as cartap hydrochloride) for rice, vegetables, etc. in 1967, in Japan. The trade name is “Badan”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(The sum of the amount of cartap and bensultap converted to that of cartap and the amount of thiocyclam converted to that of cartap)

Oat, barley, wheat, corn, milo and rye: 0.2 ppm, pasture grass: 0.7 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Analysis method for cartap, thiocyclam and bensultap by liquid chromatograph-mass spectrometer[*1] [Analytical Standards of Feeds Chapter 6, Section 1, Article 45.1]

A. Reagent preparation

1) Nereistoxin standard solution[1]. Weigh 64.1 mg of nereis toxin oxalate [$C_5H_{11}NS_2 \cdot C_2H_2O_4$], place in a 100 mL volumetric flask, add methanol to dissolve, and further add methanol up to the marked line to prepare the nereis toxin standard stock solution (1 mL of this solution contains an amount equivalent to 0.40 mg of nereistoxin).

At the time of use, dilute a definite quantity of standard stock solution accurately with heptafluorobutyric acid solution – methanol (4 : 1) to prepare several nereis toxin standard solutions containing amounts equivalent to 0.002-0.2 μg/mL of nereistoxin.

2) Heptafluorobutyric acid solution. Dilute 10 mL of heptafluorobutyric acid solution (0.5 mol/L in water)[2] with water to make 1 L.

3) Extraction solvent. Dissolve 10 g of L-cysteine hydrochloride monohydrate in hydrochloric acid (1 : 100) to make 1 L (Prepare at the time of use).

4) Nickel chloride solution. Dissolve 2 g of nickel chloride (II) (anhydrous) in water to make 100 mL.

B. Quantification

Extraction. Weigh 10.0 g of the analysis sample, place in a stoppered 200 mL Erlenmeyer flask, add 100 mL of the extraction solvent (150 mL for hay), and shake for 30 minutes to extract. Place the extract in a 50 mL centrifuge tube[3], centrifuge at 650×*g* for 5 minutes, collect accurately 20 mL (15 mL for hay) of the supernatant in a stoppered 200 mL Erlenmeyer flask as the sample solution subject to alkaline hydrolysis.

Alkaline hydrolysis. Add 2 mL of nickel chloride solution and 5 mL of ammonia solution to the sample solution, shake for 15 minutes, and hydrolyze cartap, thiocyclam and bensultap to nereistoxin to prepare the sample solution subject to column treatment.

Column treatment. Place the sample solution in a porous diatom earth column (for 50 mL retention), and allow to stand for 10 minutes. Place a 300 mL recovery flask under the column, wash the Erlenmeyer flask having contained the sample solution 3 times with 10 mL each of hexane, and add the washings serially to the column. Naturally flow down until the liquid surface reaches the top of the column packing material to elute nereistoxin, further add 120 mL of hexane to the column, elute in a similar way, and add 0.5 mL of acetone – diethylene glycol (49 : 1) to the eluate.

Condense the eluate to approximately 2 mL under reduced pressure in a water bath at 37 °C or lower, and allow to stand until evaporated into dryness[*2] [4]. Add accurately 4 mL of heptafluorobutyrate solution – methanol (4 : 1) to dissolve the residue, and centrifuge at 5,000×*g* for 5 minutes to obtain the supernatant as the sample solution subject to measurement with a liquid chromatograph mass spectrometer.

Measurement with a liquid chromatograph mass spectrometer. Inject 2 µL each of the sample solution and respective nereistoxin standard solutions into a liquid chromatograph mass spectrometer to obtain the selected ion monitoring chromatogram.

Example of measurement conditions

Column: Octadecylsilylated silica gel column (internal diameter: 3.0 mm, length: 250 mm, particle size: 5 µm)[*3]

Eluent: Heptafluoro butyrate solution-methanol (4 : 1)

Flow rate: 0.2 mL/min

Column oven temperature: 40 °C

Detector: Quadrupolar type mass spectrometer[*4]

Ionization method: Electrosplay ionization (ESI) method (positive ion mode)

Nebulizer gas: N_2 (2.5 L/min)

Drying gas: N_2 (10 L/min)

Heat block temperature: 200 °C

CDL temperature: 250 °C

Monitor ion: *m/z* 150

Calculation. Calculate the peak height or peak area from the obtained selected ion monitoring chromatogram[5] to prepare a calibration curve, and calculate the amount of nereistoxin in the sample, to which multiply 1.83 to obtain the total amount of cartap, thiocyclam converted to cartap, and

bensultap converted to cartap.

* 1. In this method, the amount of cartap [$C_7H_{15}N_3O_2S_2$·HCl], thiocyclam [$C_5H_{11}NS_3$·$C_2H_2O_4$] and bensultap [$C_{17}H_{21}NO_2S_2$] in the sample are converted to that of nereistoxin to quantify the total amount of cartap, thiocyclam converted to cartap and bensultap converted to cartap in the sample.

2. Mildly volatilize the solvent to avoid the loss of nereistoxin caused by introducing nitrogen gas.

3. ZORBAX Eclipse XDB-C18 (Agilent Technologies) or an equivalent.

4. Conditions with LCMS-2010EV (Shimadzu Corporation).

«Summary of analysis methods»

This method is intended to determine the total amount of cartap, bensultap and thiocyclam in feeds by extracting with diluted hydrochloric acid containing L-cysteine, hydrolyzing into nereistoxin under ammonia-basic condition, purifying with a porous diatom earth column, and quantifying with a liquid chromatograph mass spectrometer.

The flow chart of analysis method is shown in Figure 6.1.45-1.

Sample (10 g)
- Add 100 mL (150 mL for hay) of L-cysteine-hydrochloric acid solution, and stir for 30 min.
- Centrifuge (at 650×*g* for 5 min).
- Collect 20 mL (15 mL for hay) of the supernatant.

Hydrolysis
- Add 2 mL of nickel chloride solution and 5 mL of ammonia solution.
- Stir for 15 min.

Chem Elut cartridge (for 50 mL retention)
- Load the sample solution, and allow to stand for 10 min.
- Wash with 10 mL of hexane (3 times).
- Elute nereistoxin with 120 mL of hexane.
- Add 0.5 mL of acetone-diethylene glycol (49 : 1).
- Evaporate into dryness (37 °C or lower)
- Add 4 mL of heptafluorobutyrate solution-methanol (4 : 1).

LC-MS (inject 2 µL)

Figure 6.1.45-1 Flow sheet of the analysis method for cartap, thiocyclam and bensultap by liquid chromatograph-mass spectrometer

References: Japan Food Research Laboratories: contract projects for the fiscal 2005 residue standards for hazardous substances, etc. in feeds (development of analysis methods), and development of analysis methods for hazardous substances, etc in feeds, 3-134 (2006).
Daisaku Makino, Masahisa Yoshimura: Research Report of Animal Feed, 32, 140 (2007)
Toshiaki Yamata: Research Report of Animal Feed, 32,144 (2007)

«Method validations»

• Spike recovery* and repeatability

Spiked ingredient	Sample type	Repeat	Spike concentration (μg/kg)	Spike recovery (%)	Repeatability RSD (% or less)
Cartap	Corn	3	20-200	84.6-90.4	4.3
	Ryegrass	3	40-600	67.7-86.5	5.1
Thiocyclam	Corn	3	20-200	80.1-88.2	5.8
	Ryegrass	3	40-600	85.1-92.4	9.4
Bensultap	Corn	3	60-300	61.2-64.8	6.5
	Ryegras	3	120-900	57.5-57.5	3.1

• Collaborative study

Ingredient	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Cartap	Corn	7	200	74.3	8.1	25.8	1.27
	Timothy	7	700	72.3	3.7	21.0	1.25

• Quantification lower limit: cartap and thiocyclam: 20 μg/kg (40 μg/kg for hay), bensultap: 60 μg/kg (120 μg/kg for hay) (spike recovery and relative standard deviation)

• Detection lower limit: cartap and thiocyclam: 6 μg/kg (10 μg/kg for hay), bensultap: 20 μg/kg (40 μg/kg for hay) (*SN* ratio)

* In this recovery study, the standard solution was added before adding the extraction solvent; however, bensultap may be degraded, causing decreased recovery rate, in this manner.

«Notes and precautions»

[1] The standard preparation of nereistoxin is commercially available from Kanto Chemical, Wako Pure Chemical, etc. as nereis toxin oxalate.

[2] IPC-PFFA-4 (Tokyo Chemical Industry) and others are available.

[3] Collect an amount obtainable 20 mL of the supernatant via centrifuge; if insufficient by 50 mL, you may collect a larger amount.

[4] You can save the time by volatilizing the solvent warmed with your body temperature, etc.

[5] An example of chromatogram is shown in Figure6.1.45-2.

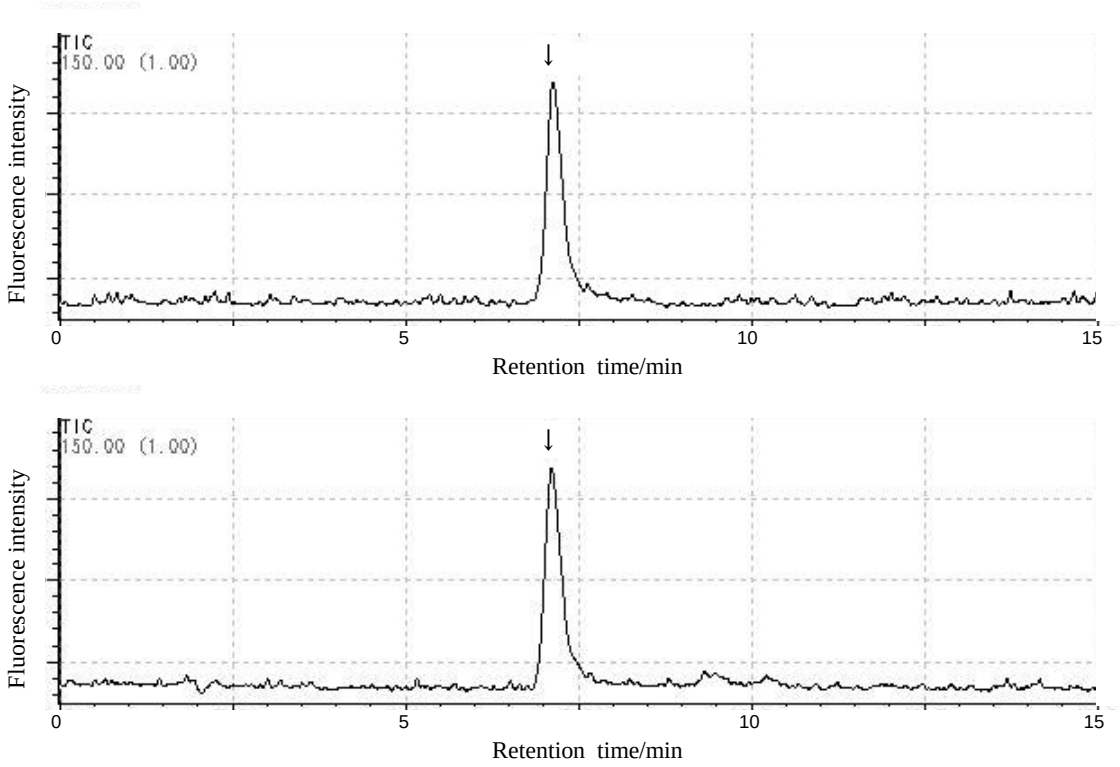
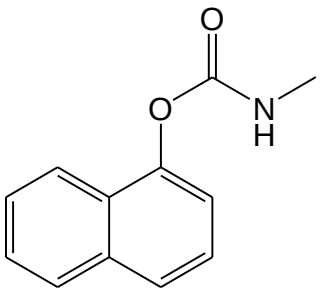


Figure 6.1.45-2. SIM chromatograms for the standard solution and sample solution (The arrow indicates the peak of nereistoxin)

(A) Nereistoxin standard solution (50 ng/mL)

(B) Corn (added an amount equivalent to 200 μg/kg of cartap)

46 Carbaryl (NAC)



1-naphthyl methylcarbamate

$C_{12}H_{11}NO_2$ MW: 201.22 CAS No.: 63-25-2

[Summary of carbaryl]

Carbaryl is a carbamate insecticide, white crystal. Carbaryl is one of the most used carbamate agricultural chemicals. This agricultural chemical is effective to rice plant hopper and sucking pests of dodgers. It is also used as a preventive agent for weevils. Carbaryl is effective as a permeable systemic agent having insecticidal effects by being absorbed from roots and culms as well as a contact agent. Further, this agricultural chemical is used as an apple thinning agent controlling the growth.

Carbaryl was registered as an agricultural chemical in 1959, in Japan. Registered name is NAC. The trade names are “Denapon”, “Sevin” or “NAC”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Corn: 0.1 ppm, wheat: 2 ppm, barley and rye: 5 ppm, oat and milo: 10 ppm, pasture grass: 250 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)

Target Analytes: XMC, aldicarb (including aldicarb sulfoxide and aldicarb sulfone), isoprocarb, carbaryl, carbofuran, xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb (12 compounds)

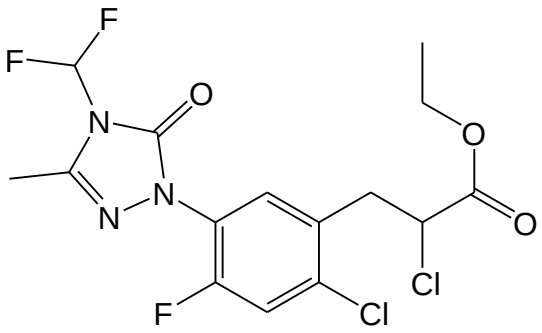
Refer to Article 3, Section 3 in this chapter.

2. Simultaneous analysis method of carbamate agricultural chemicals by gas chromatography

Target Analytes: XMC, isoprocarb, carbaryl, xylylcarb, fenobucarb, propoxur and metolcarb (7 compounds)

Refer to Article 5, Section 3 in this chapter.

47 Carfentrazone-ethyl



Ethyl (*RS*)-2-chloro-3-{2-chloro-5-[4-(difluoromethyl)-4, 5-dihydro-3-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl]-4-fluorophenyl}propanoate

$C_{15}H_{14}Cl_2F_3N_3O_3$ MW: 412.2 CAS No.: 128639-02-1

[Summary of carfentrazone-ethyl]

Carfentrazone-ethyl is a photobleaching triazolone herbicide generating cytotoxic substances by inhibiting protoporphyrinogen oxidase involving plant photosynthesis.

Carfentrazone-ethyl was registered as an agricultural chemical targeting lawn grasses, wheat, etc. in 2002, in Japan. The trade name is "Task DF".

«Maximum Residue Limits in grains in the Food Sanitation Law»

Barley, rye and corn: 0.08 ppm, wheat: 0.1 ppm, other grains: 0.08 ppm

[Methods listed in the Analytical Standards of Feeds]

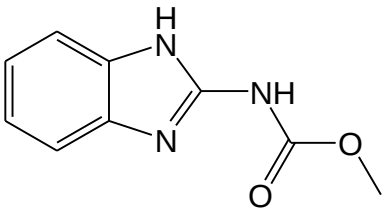
1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

48 Carbendazim
(Carbendazim (MBC), thiophanate-methyl and benomyl)

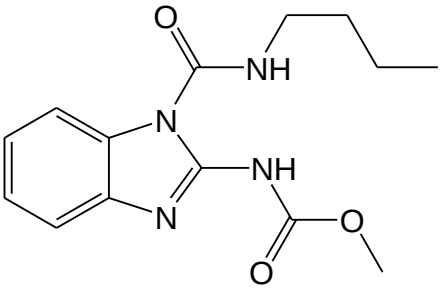
Carbendazim



methyl benzimidazol-2-ylcarbamate

$C_9H_9N_3O_2$ MW: 191.2
CAS No.: 10605-21-7

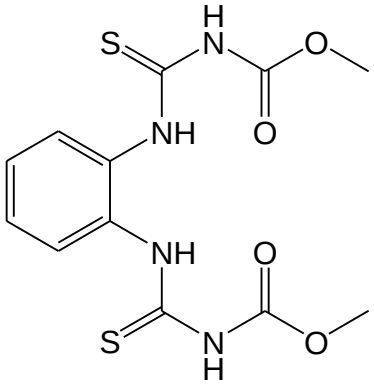
Benomyl



methyl 1-[(butylamino)carbonyl]-1*H*-benzimidazol-2-yl carbamate

$C_{14}H_{18}N_4O_3$ MW: 290.3
CAS No.: 17804-35-2

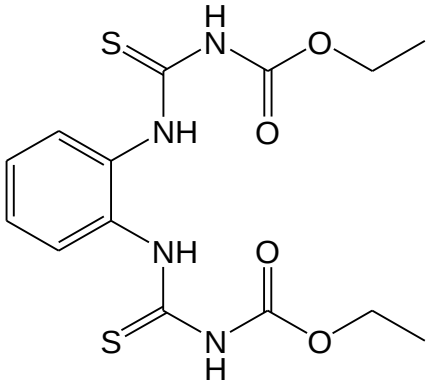
Thiophanate-methyl



dimethyl 4,4′-(*o*-phenylene)bis(3-thioallophanate)

$C_{12}H_{14}N_4O_4S_2$ MW: 342.39
CAS No.: 23564-05-8

Thiophanate-ethyl (reference)



diethyl 4,4′-(*o*-phenylene)bis(3-thioallophanate)

$C_{14}H_{18}N_4O_4S_2$ MW: 370.45
CAS No.: 23564-06-9

[Summary of carbendazim]

Carbendazim is a metabolite of benomyl or thiophanate-methyl. Benomyl and thiophanate-methyl are described as benzimidazole bactericides.

Carbendazim was registered as an agricultural chemical in 1973, in Japan. Registered name is carbendazole.

It is known that benomyl and thiophanate-methyl generate stable carbendazim via hydrolysis, which combines to microtube proteins in bacteria, inhibiting the mitosis; this is considered to be the action mechanism.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

(A total amount of carbendazim and benomyl converted to carbendazim, thiophanate converted to carbendazim and thiophanate-methyl converted to carbendazim)

Oat, barley, wheat, milo and rye: 0.6 ppm, corn: 0.7 ppm, pasture grass: 10 ppm

Rice plant silage: 0.1 ppm, rice straw: 0.3 ppm, paddy rice: 10 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Analysis method for carbendazim, thiophanate-methyl and benomyl by liquid chromatograph-mass spectrometer [Analytical Standards of Feeds Chapter 6, Section 1, Article 48.1][*1,2] [1]

A. Reagent preparation[2][3]

1) Carbendazim standard solution. Weigh accurately 10 mg of carbendazim [$C_9H_9N_3O_2$], place in a 100 mL volumetric flask, add methanol to dissolve, and further add methanol up to the marked line to prepare the carbendazim standard stock solution (1 mL of this solution contains an amount equivalent to 0.10 mg of carbendazim).

At the time of use, dilute accurately a definite quantity of standard stock solution with methanol to prepare the carbendazim standard solution, to be subjected to ring-closing reaction, containing an amount equivalent to 20 μg/mL of carbendazim.

2) Thiophanate-methyl standard solution (to be used when residual thiophanate-methyl is suspected in the analysis sample). Weigh accurately 10 mg of thiophanate-methyl [$C_{12}H_{14}N_4O_4S_2$], place in a 100 mL volumetric flask, add methanol to dissolve, and further add methanol up to the marked line to prepare the thiophanate-methyl standard stock solution (1 mL of this solution contains an amount equivalent to 0.10 mg of thiophanate-methyl).

At the time of use, dilute accurately a definite quantity of standard stock solution with methanol to prepare the carbendazim standard solution, to be subjected to ring-closing reaction, containing an amount equivalent to 20 μg/mL of thiophanate-methyl.

B. Quantification

Extraction. Weigh accurately 10.0 g of analysis sample, place in a stoppered 200 mL Erlenmeyer flask, add 0.4 g of L-ascorbic acid and 15 mL of water (30 mL for hay), and allow to stand for 30 minutes. Further add 100 mL (120 mL for hay) of methanol, and shake 30 minutes to extract[*3]. Place a 200 mL volumetric flask under a Büchner funnel, and filter the extract through filter paper (No. 5B) by suction. Wash the said Erlenmeyer flask and the residue serially with 50 mL of methanol, and filter by suction in a similar way. Further add methanol up to the marked line of the volumetric flask to prepare the sample solution subject to the liquid-liquid distribution I[*4].

Liquid-liquid distribution I. Weigh accurately 10 mL (20 mL for hay) of the sample solution, place in the 500 mL separating funnel A, add 3 g of L-ascorbic acid, 150 mL of sodium chloride solution (10 w/v%) and 100 mL of hexane, shake for 5 minutes, and allow to stand.

Collect the water layer (lower layer) in a 200 mL tall beaker, adjust the pH to 6.7-7.1 with sodium

hydrate solution (4 mol/L and 0.4 mol/L)[*5, 6].

Then, place 500 mL of the adjusted water layer in the 500 mL separating funnel B, add 100 mL of dichloromethane, shake for 5 minutes, allow to stand, and transfer the dichloromethane layer (lower layer) into a 300 mL Erlenmeyer flask. Add 100 mL of dichloromethane to the separating funnel B, process in a similar way, and add the dichloromethane layer to the Erlenmeyer flask. Dehydrate the dichloromethane layer with an appropriate quantity of sodium sulfate (anhydrous)[*7], and filter through filter paper (No. 5B) into a 300 mL recovery flask. Wash the Erlenmeyer flask with a small amount of dichloromethane, and add the washings to the filtrate through the said filter.

Add 0.5 mL of acetic acid, condense to approximately 0.5 mL under reduced pressure in a water bath at 40 °C or lower[*8], further dry up by the flow of nitrogen gas. Add 2 mL of methanol to dissolve the residue to prepare the sample solution subject to ring-closing reaction.

Ring-closing reaction. Add 10 mL of acetic acid (1+1) solution, 0.2 g of copper acetate and 2-3 pieces of boiling stone to the recovery flask containing the sample solution, connect to a reflux condenser, heat on an oil bath at 120 °C for 30 minutes to transform thiophanate-methyl to carbendazim, and allow to be cool.

Add 10 mL of hydrochloric acid (1 mol/L) to the reflux condenser downward to wash the tube wall, add to the sample solution to prepare the sample solution subject to the liquid-liquid distribution II.

Liquid-liquid distribution II. Place the sample solution in the 100 mL separating funnel C, wash the recovery flask having contained the sample solution with 20 mL of hydrochloric acid (1 mol/L), and add the washings to the sample solution. Further add 5 g of sodium chloride and 20 mL of hexane to the separating funnel C, shake for 5 minutes, allow to stand, and transfer the water layer (lower layer) to the 100 mL separating funnel D.

Add 20 mL of hexane to the separating funnel D, shake for 5 minutes, and allow to stand. Place the water layer in a 100 mL tall beaker, adjust the pH to 6.8-6.9 with sodium hydrate solution (10 mol/L and 1 mol/L)[*6, 9], and place in the 300 mL separating funnel E.

Add 50 mL of ethyl acetate to the separating funnel E, shake for 5 minutes, allow to stand, and transfer the water layer (lower layer) and ethyl acetate layer (upper layer) in the 300 mL separating funnel F and in a 200 mL Erlenmeyer flask, respectively. Add 50 mL of ethyl acetate to the separating funnel F, shake for 5 minutes, allow to stand, remove the water layer, and add the ethyl acetate layer to the said Erlenmeyer flask. Dehydrate the ethyl acetate layer with an appropriate quantity of sodium sulfate (anhydrous), and filter through filter paper (No. 5B) into a 200 mL recovery flask. Wash the said Erlenmeyer flask with a small amount of ethyl acetate, and add the washings to the filtrate through the filter paper.

Condense the filtrate under reduced pressure to approximately 1 mL in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas. Add 5 mL of ethyl acetate – methanol (19 : 1) to dissolve the residue to prepare the sample solution subject to the column treatment.

Column treatment. Wash an ethylene diamine-*N*-propylsilylated silica gel minicolumn (500 mg)[*10] with 5 mL of ethyl acetate.

Place a 50 mL recovery flask under the minicolumn, place the sample solution in the minicolumn, and

flow down until the liquid surface reaches the top of the column packing material[*11] to flow out carbendazim. Wash the recovery flask having contained the sample solution twice with 5 mL each of ethyl acetate – methanol (19 : 1), add the washings serially to the minicolumn, and flow out in a similar way. Further add 10 mL of ethyl acetate – methanol (19 : 1) to the minicolumn, and flow out in a similar way.

Condense the effluent to approximately 1 mL under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas. Add 2 mL of water – methanol (1 : 1) accurately to dissolve the residue, and centrifuge at 5,000×*g* for 5 minutes to obtain a supernatant as the sample solution subject to measurement by liquid chromatograph-mass spectrometer.

Ring-closing reaction of the standard solution. Place accurately 1 mL of carbendazim standard solution or thiophanate-methyl standard solution (thiophanate-methyl standard solution is used only in the case that thiophanate-methyl is suspected to be remaining in the sample) in a 200 mL recovery flask. Then, carry out the ring-closing reaction, liquid-liquid distribution II and column treatment in a way similar to those for the sample solution, condense the effluent from the minicolumn under reduced pressure to approximately 1 mL in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add accurately 20 mL of water – methanol (1 : 1) to dissolve the residue, and further dilute a definite quantity of this solution with the solvent to prepare several standard solutions containing amounts equivalent to 5-200 ng/mL of carbendazim or thiophanate-methyl.

Measurement by liquid chromatograph-mass spectrometer. Inject 2 µL each of the sample solution and respective standard solutions into a liquid chromatograph mass spectrometer to obtain the selected ion monitoring chromatogram.

Example of measurement conditions

Column: Octadecylsilylated silica gel column (internal diameter: 3.0 mm, length: 250 mm, particle size: 5 µm)[*12]

Eluent: 2 mmol/L-ammonium acetate solution[*13] – methanol (3 : 1) → 15 min → (2 : 3) → 0.1 min → (1 : 9) (hold 7 min) → 0.1 min → (3 : 1) (hold 8 min)

Flow rate: 0.2 mL/min

Column oven temperature: 40 °C

Detector: Quadrupole mass spectrometer[*14]

Ionization method: Electrosplay ionization (ESI) method (positive ion mode)

Fragmenter voltage: 100 V

Nebulizer gas: N_2 (340 kPa)

Drying gas: N_2 (10 L/min, 350 °C)

Capillary voltage: 4,000 V

Monitor ion: *m/z* 192

Calculation. Calculate the peak height or peak area from the obtained selected ion monitoring chromatogram[4] to prepare a calibration curve, and determine the amount of carbendazim (including thiophanate-methyl and benomyl converted to carbendazim) in the sample[*15].

* 1. In this method, thiophanate-methyl [$C_{12}H_{14}N_4O_4S_2$] and benomyl [$C_{14}H_{18}N_4O_3$] in the sample are

converted to carbendazim, and a total amount of carbendazim, thiophanate-methyl converted to carbendazim and benomyl converted to carbendazim in the sample is determined.

Furthermore, presence or absence of thiophanate [$C_{14}H_{18}N_4O_4S_2$] in the sample can be simultaneously confirmed by this method. In this case, the monitor ion in measurement with a liquid chromatograph mass spectrometer is *m*/*z* 206.

2. Since thiophanate-methyl and carbendazim are easily disappeared in the analyzing process, it is preferable to do the procedure rapidly to finish the process including the ring-closing reaction within 24 hours.
3. Benomyl extracted at this time is converted to carbendazim.
4. Conduct the following processes after diluting the extract with methanol, when the amounts of carbendazim, thiophanate-methyl and benomyl in the sample are large.
5. After adding approximately 4 mL of sodium hydrate solution (4 mol/L), finely adjust with the said solution (0.4 mol/L).
6. After adjusting the pH, quickly perform the first shaking.
7. When a large amount of sodium sulfate (anhydrous) is used for dehydrating the dichloromethane layer, the layer may be lost by adsorption: therefore, use a minimum necessary amount.
8. After condensing to approximately 0.5 mL, gently introduce nitrogen gas to evaporate dichloromethane, because overdrying possibly cause degradation of thiophanate-methyl.
9. After adding approximately 14 mL of sodium hydrate solution (10 mol/L), finely adjust with the said solution (1 mol/L).
10. Bond Elut Jr. PSA (Varian) connected with a reservoir with a suitable volume, or an equivalent.

11 The flow rate is 2-3 mL/min. Use a suction manifold if necessary.

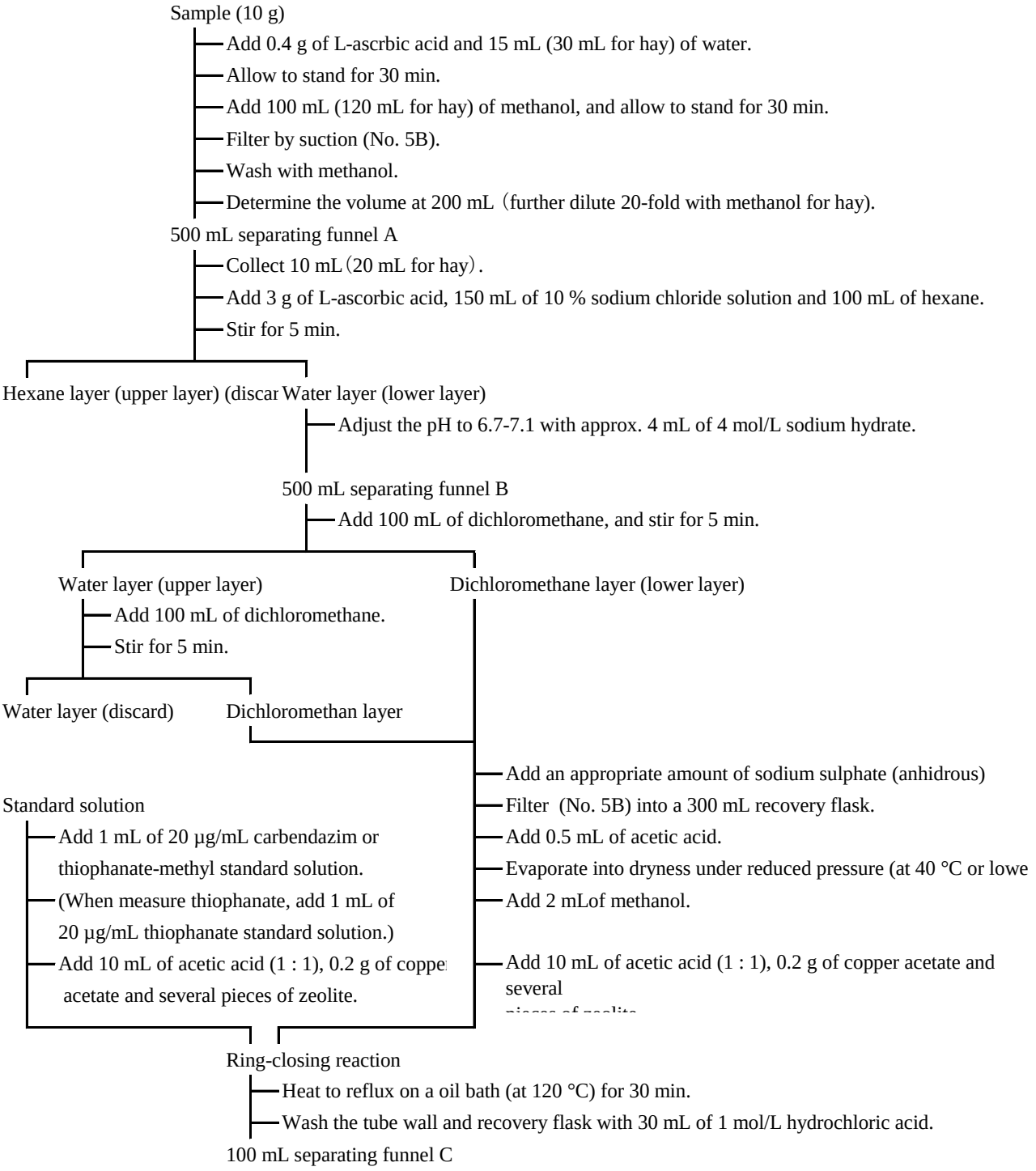
12 ZORBAX Eclipse XDB-C18 (Agilent Technologies, the retention time of carbendazim under this operating conditions is approximately 13 minutes) or an equivalent.

13. Dissolve 7.7 g of ammonium acetate in water to make 1 L, and further dilute 20 mL of this solution with water to make 1 L.
14. An example condition for Agilent 1100 Series MSD SL (Agilent Technologies).
15. Since thiophanate-methyl standard solution is used for thiophanate-methyl, the amount of carbendazim in the sample is obtained by converting from the amount of thiophanate-methyl in the sample obtained from the calibration curve by multiplying 0.56.

«Summary of analysis methods»

This method is intended to determine the amount of carbendazim by extracting with hydrous methanol (benomyl is converted to carbendazim), washings with hexane, replacing solvent with dichloromethane under neutral pH, being subjected to ring-closing reaction by heating to reflux under the presence of acetic acid and copper ion (converting thiophanate-methyl to carbendazim), allowing to be cool, washings with hexane under acidity, replacing solvent with ethyl acetate under neutral pH, purifying with a PSA cartridge, and quantifying with a liquid chromatograph mass spectrometer. Carbendazim obtained contains carbendazim from benomyl and thiophanate-methyl.

The flow sheet of analysis methods is shown in Figure 6.1.48-1.



Followed with the next page

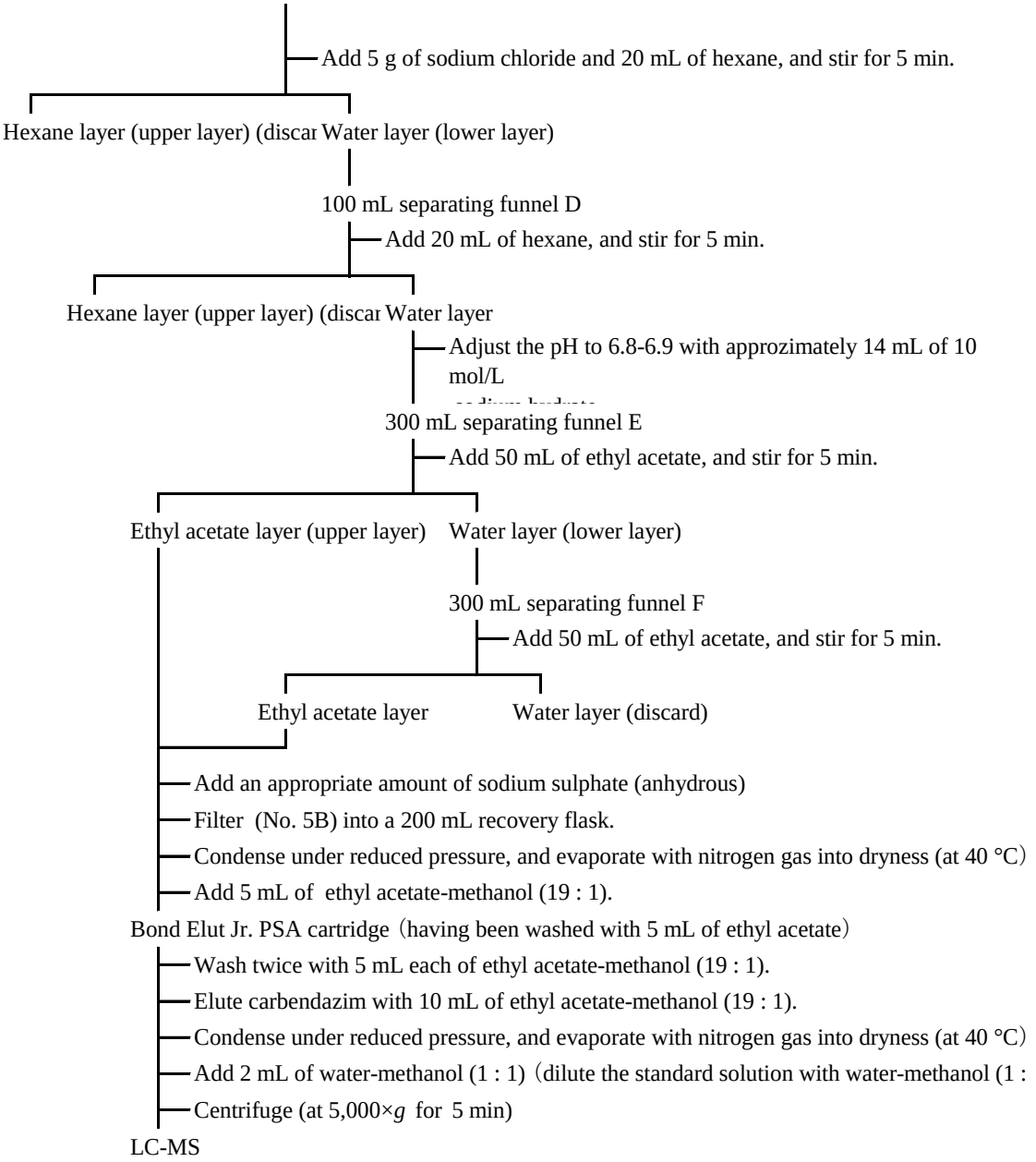


Figure 6.1.48-1. Flow sheet of analysis method for carbendazim, thiophanate-methyl and benomyl by liquid chromatograph-mass spectrometer

References: Asuka Horigome, Tomoharu Nozaki: Research Report of Animal Feed, 32, 30 (2007)

«Method validations»

• Spike recovery and repeatability

Spiked ingredient	Sample type	Repeat	Spike concentration (μg/kg)	Spike recovery (%)	Repeatability RSD (% or less)
Carbendazim	Corn	0.7	3	84.4	6.8
	Timothy	10	3	98.3	0.8
Thyophanate-methyl	Corn	0.7	3	84.4	9.5
	Timothy	10	3	82.1	6.1
Benomyl	Corn	1.0	3	101.4	3.2
	Timothy	15	3	105.8	5.0
Thyophanate	Corn	0.7	3	57.9	4.4
	Timothy	10	3	64.2	8.1

• Collaborative study

Ingredient	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Thyophanate-methyl	Corn	6	1.3	87.8	3.2	15.0	0.95
	Timothy	6	20	83.1	4.6	18.5	1.77
Thyophanate	Corn	6	1.3	53.5	4.6	14.3	0.85
	Timothy	6	20	41.6	4.3	26.1	2.25

• Quantification lower limit: carbendazim: 50 μg/kg, thiophanate-methyl: 40 μg/kg, benomyl: 60 μg/kg (spike recovery and relative standard deviation)

• Detection lower limit: carbendazim: 20 μg/kg, thiophanate-methyl: 10 μg/kg, benomyl: 20 μg/kg (*SN* ratio)

«Notes and precautions»

[1] This method can confirm the presence or absence of thiophanate, where prepare the standard solution of thiophanate to subject to ring-closing reaction (refer to the flow sheet in Figure 6.1.48-1). Pay attention to that adequate recovery rate has not been obtained as shown in the validation results.

[2] Standard solutions used for measuring respective compounds are as follows.

Target compounds	Standard solution
Carbendazim and benomyl	Carbendazim
Thiophanate-methyl	Thiophanate-methyl
Thiophanate	Thiophanate

[3] Employ carbendazim or thiophanate-methyl standard solution based on the actual status of the usage of the agricultural chemical. When residual carbendazim and/or benomyl are suspected, use the carbendazim standard solution, while, when residual thiophanate-methyl is suspected, use thiophanate-methyl standard solution.

It is desirable for the sample solution and standard solution to be prepared for a series of procedures. Therefore, when the actual status of the usage of the agricultural chemical is unclear, it is preferable to prepare calibration curves from both standard solutions of carbendazim and thiophanate-methyl at the time of measuring the sample, and employ either calibration curve for calculation when the actual status of usage of the agricultural chemical becomes clear.

[4] An example of chromatogram is shown in Figure 6.1.48-2.

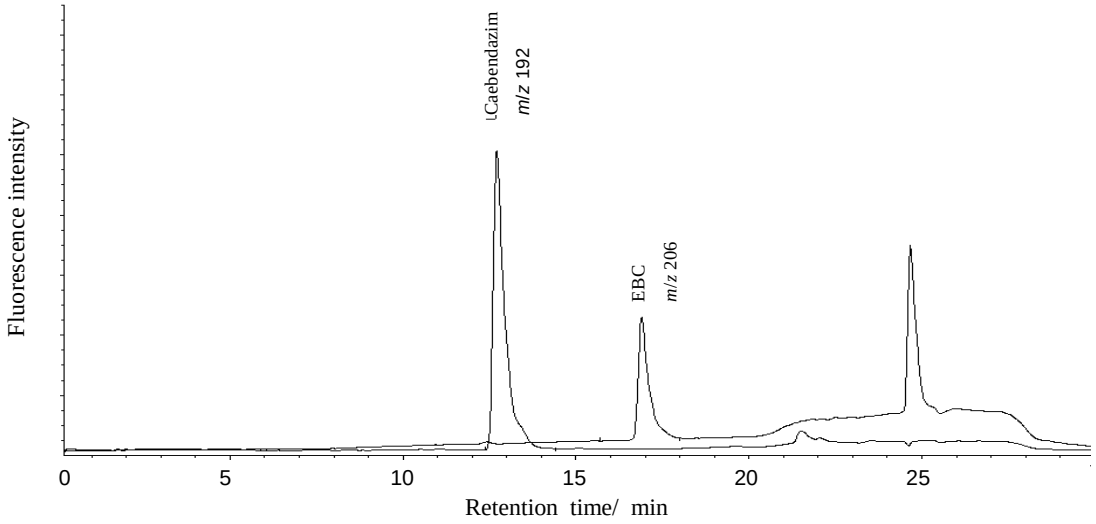
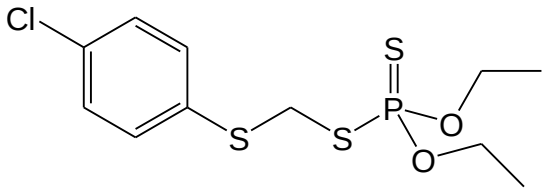


Figure 6.1.48-2. SIM chromatogram of carbendazim and EBC
(Ethyl-2-benzimidazole carbamate obtained by ring-closing reaction of thiophanate)

49 Carbophenothion



S-4-chlorophenylthiomethyl *O*,*O*-diethyl phosphorodithioate

$C_{11}H_{16}ClO_2PS_3$ MW: 342.87 CAS No.: 786-19-6

[Summary of carbophenothion]

Carbophenothion is an organic phosphorus insecticide, white to amber liquid. It is applied to aphids, red mites, scale insects in citrus.

Carbophenothion has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

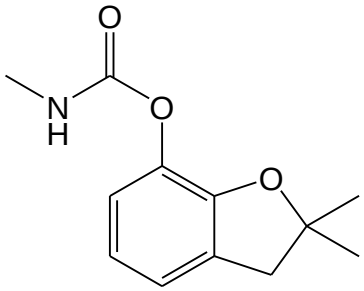
Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

50 Carbofuran
(including 3-OH carbofuran)
51 3-OH carbofuran

Carbofuran

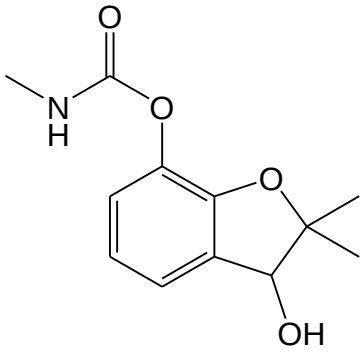


2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate

$C_{12}H_{15}NO_3$ MW: 221.3

CAS No.: 1563-66-2

3-OH carbofuran



2,3-dihydro-2,2-dimethyl-3,7-benzofurandiol-7-methyl carbamate

$C_{12}H_{15}NO_4$ MW: 237.25

CAS No.: 16655-82-6

[Summary of carbofuran]

Carbofuran is a carbamate insecticide with a wide insecticidal spectrum, being also used as a miticide. Carbofuran has not registered as an agricultural chemical in Japan.

3-OH carbofuran (3-hydroxy carbofuran) is a metabolite of carbosulfan (a carbofuran derivative, an *N*-methylcarbamate insecticide). This compound is partially as glycoside in plants, and therefore, can not be extracted sufficiently with an organic solvent such as acetone. Therefore, residue analysis methods for plants, etc. employ a heating-reflux extraction method under hydrochloric acid acidity.

Carbofuran and 3-OH carbofuran are listed separately in Analytical Standards of Feeds; however, defined as a total amount of both compound for the residue standard value based on the in Law Concerning Safety Assurance and Quality Improvement of Feeds.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(A total amount of carbofuran and 3-OH carbofuran (a metabolite of metabolite of carbofuran) converted to carbofuran)

Corn: 0.05 ppm, oat, milo and rye: 0.1 ppm, barley and wheat: 0.2 ppm, pasture grass: 13 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Analysis methods for carbofuran

1.1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)

Target Analytes: XMC, aldicarb (including aldicarb sulfoxide and aldicarb sulfone), isoprocarb, carbaryl, carbofuran, xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb (12 compounds)

Refer to Article 3, Section 3 in this chapter.

2. Analysis methods for 3-OH carbofuran

2.1. Liquid chromatography [Analytical Standards of Feeds Chapter 6, Section 1, Article 51.1]

A. Reagent preparation

1) 3-OH carbofuran standard solution[1]. Weigh accurately 20 mg of 3-OH carbofuran [$C_{12}H_{15}NO_4$], place in a 100 mL volumetric flask, add acetonitrile to dissolve, and further add the solvent up to the marked line to prepare the 3-OH carbofuran standard stock solution (1 mL of this solution contains an amount equivalent to 0.2 mg of 3-OH carbofuran).

At the time of use, dilute a definite quantity of the standard stock solution accurately with acetonitrile to prepare several 3-OH carbofuran standard solutions containing amounts equivalent to 0.025-2.5 μg/mL of 3-OH carbofuran.

2) Magnesium silicate[2]. Dry synthetic magnesium silicate (particle size: 149-250 μm (100-60 mesh)) at 130 °C for 16 hours.

3) Diatom earth. Wash diatom earth[*1] with warm water and methanol, and air-dry.

B. Quantification

Extraction. Weigh 5-10 g of analysis sample[3], place in a 500 mL recovery flask, add 100 mL of hydrochloric acid (1+29) solution, 3-4 pieces of boiling stone and approximately 1mL of silicon oil, connect to a reflux condenser, and heat for 1 hour to extract[4]. Place a 200 mL volumetric flask under a Büchner funnel[5], and filter the extract through with filter paper (No. 5B) mounted with 5 g of diatom earth [6] by suction. Wash the said recovery flask and the residue serially with 70 mL of hydrochloric acid (1+29) solution – acetone (5 : 2), and filter the washings by suction in a similar way. Further add hydrochloric acid (1+29) solution up to the marked line of the volumetric flask to prepare the sample solution subject to the column treatment I[7].

Column treatment I. Place 50 mL of the sample solution in a porous diatom earth column (for 50 mL retention), and allow to stand for 5 minutes. Place a 300 mL recovery flask under the column, add 200 mL of ethyl acetate to the column to elute 3-OH carbofuran, and condense the eluate to approximately 2 mL under reduced pressure in a water bath at 40 °C or lower[8], to prepare the sample solution subject to the column treatment II.

Column treatment II. Suspend 5 g of magnesium silicate in hexane, run down into a column tube (internal diameter: 15 mm), flow out until the liquid surface reaches at 30 mm from the top of the column packing material to prepare the column.

Place the sample solution in the column, wash the recovery flask having contained the sample solution 3 times with 5 mL each of hexane – acetone (9 : 1), add the washings serially to the column, flow out until the liquid surface reaches at 30 mm from the top of the column packing material, and further flow out in a similar way by adding 35 mL of the solvent[9]. Place a 200 mL recovery flask under the column, add 50 mL of hexane – acetone (4 : 1) to the column to elute 3-OH carbofuran, condense the eluate almost into dryness under reduced pressure in a water bath 40 °C or lower, further dry up by the flow of nitrogen gas.

Add accurately 1 mL of acetonitrile to dissolve the residue, and filter through membrane filter (pore size: 0.5 μm or less) to obtain the sample solution subject to liquid chromatography.

Liquid chromatography. Inject 20 μL each of the sample solution and respective 3-OH carbofuran standard solution into a liquid chromatograph to obtain the chromatogram.

Example of measurement conditions

Detector: Fluorescence Detector (Excited wave length: 340 nm, fluorescence wave length: 445 nm)

Column: Octadecylsilylated silica gel column (internal diameter: 3.9 mm, length 150 mm, particle size: 4 μm)[*2] [10]

Eluent: Water – methanol (9 : 1) → 25 min → (7 : 3) → 1 min → (5 : 95) (hold 14 min) → (9 : 1)[11]

Reaction liquid[*3]: Liquid I (alkaline solution): Dissolve 1 g of sodium hydrate in water to make 500 mL.

Liquid II (OPA reagent): dissolve 50 mg of *o*-phthalaldehyde with 5 mLof methanol, further add sodium borate solution (dissolve 19.1 g of sodium tetraborate+ hydrate in water to make 1 L) to make 500 mL, and mixed with 50 μL of 2-mercaptoethanol (freshly prepare at the time of use).

Flow rate: Eluent: 1.0 mL/min, Reaction liquid: 0.2 mL/min each

Temperature: Column oven: 45 °C, reaction tank: 90 °C

Calculation. Calculate the peak height or peak area from the obtained chromatogram[12] to prepare a calibration curve, calculate the amount of 3-OH carbofuran in the sample, and multiply 0.933 to convert to the amount of carbofuran.

* 1. Hyflo Supercel (Celite Corporation) or an equivalent.

2. Carbamate Analysis (Waters) or an equivalent.

3. Add the reaction liquid I to the eluent from the column to hydrolyze in the reaction coil in the reaction tank, cool the solution to room temperature, add the reaction liquid II to make fluorescing, and immediately send to the fluorescence detector. As for the reaction coil, use RXN 1000 Coil (internal diameter: 0.5 mm, length approximately 5 m, reaction volume: 1 mL, made of Teflon, Waters) or an equivalent.

«Summary of analysis methods»

This method is intended to determine the amount of 3-OH carbofuran in feeds by extracting with heat-refluxing under hydrochloric acidity, purifying with a porous diatom earth column and synthetic magnesium silicate column, and quantifying with a post-column fluorescence derivatization liquid chromatograph.

The flow sheet of analysis methods is shown in Figure 6.1.51-1.

Sample (10 g) (5 g for hay)
- Add 100 mL of hydrochloric acid (1 : 29).
- Add an antiform agent and boiling stones.
- Heat to reflux for 60 min.
- Filter by suction (No. 5B)
- Wash with 70 mL of hydrochloric acid (1 : 29)-acetone (5 : 2).
- Determine the volume to 200 mL with hydrochloric acid (1 : 29).

Chem Elut cartridge (for 50 mL retention)
- Load 50 mL of the sample solution, and allow to stand for 5 min.
- Elute with 200 mL of ethyl acetate.
- Condence under reduced pressure (to approx 2 mL).

Magnesium silicate column
- Pack 5 g of florisil with hexan.
- Load 50 mL of hexane-acetone (9 : 1), and wash.
- Elute with 50 mL of hexane-acetone (4 : 1).
- Condence under reduced pressure (to approx 1 mL), and evaporate into dryness with nitrogen gas.
- Add 1 mL of acetonitrile.

LC-post-column fluorescence derivatization-FL (Ex: 340 nm, Em: 445 nm)

Figure 6.1.51-1. Flow sheet of analysis method for 3-OH carbofuran

References: Kiyoshi Someya: Research Report of Animal Feed, 24, 62 (1999)

«Method validations»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Adult chicken formula feed	50-500	3	80.3-87.7	9.3
Finishing beef cattle formula feed	50-500	3	78.7-88.0	10.4
Sudan grass	50-500	3	71.3-84.7	8.2

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Finishing beef cattle formula feed	6	250	86.5	2.9	4.2	0.21

• Quantification lower limit: 25 μg/kg for formula feed, 50 μg/kg for hay

«Notes and precautions»

[1] The standard preparation is commercially available from HAYASHI PURE CHEMICAL.

[2] Freshly prepare and cool in a drying desiccator just before use.

[3] The appropriate amounts of sample to be collected are 10.0 g for formula feed, and 5.0 g for hay.

[4] It is no special problem that the sample is adsorbed to wall of the recovery flask and carbonized in a small amount. When the amount of carbonized sample is large, lower the heating temperature.

[5] Use a Kiriyama funnel with a caliber of approximately 9 cm.

[6] Rapidly filter the extract by suction just after extraction. Extracts at low temperature cause clogging, and require fair amount of time to filter.

[7] After determine the volume to 200 mL, stir the filtrate adequately.

[8] Excessive condensation generates precipitation, causing decreased recovery rates of 3-OH carbofuran. When precipitation is generated, dissolve the precipitation by adding a small amount of ethyl acetate.

[9] By adding the sample solution to the florisil column, precipitation may be generated; however, there is no special problem.

When clogging of a column due to precipitation causes extremely slow or impossible outflow, add pressure to flow out. Loading sodium sulfate (anhydrous) on florisil may causes decreased recovery rates; therefore, do not load sodium sulfate (anhydrous).

[10] Any column is applicable as long as its end-capped packing material meets the requirements. The column used at the time of development is the column for carbamate analysis (Waters).

[11] The equilibrating time for returning the eluting conditions after the end of the gradient analysis (water-methanol (5 : 95)) to those at the start of analysis (water-methanol (9 : 1)) is approximately 15 minutes.

[12] An example of chromatogram for 3-OH carbofuran is shown in Figure 6.1.51-2.

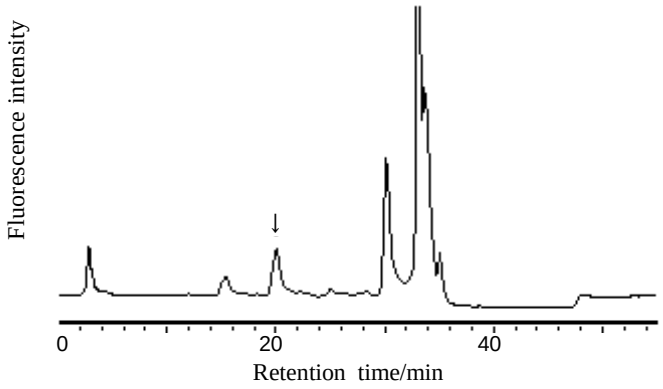


Figure 6.1.51-2. Chromatogram for 3-OH carbofuran added to a formula feed in amount equivalent to 250 µg/kg
(The arrow indicates the peak of 3-OH carbofuran derivative)

Measurement conditions

Detector: Fluorescence detector (exciting wave length: 340 nm, fluorescence wave length: 445 nm)

Column: Column for carbamate analysis (internal diameter: 3.9 mm, length: 150 mm, particle size: 4 µm)

Eluent:

Time (minutes)	0	25	26	40
Water	90	70	5	5
Methanol	10	30	95	95

Reaction liquid: Liquid I (for hydrolysis): Dissolve 1 g of sodium hydrate in water to make 500 mL.

Liquid II (for fluorescing): dissolve 50 mg of *o*-phthalaldehyde with 5 mL of methanol, further add sodium borate solution (dissolve 19.1 g of sodium tetraborate+ hydrate in water to make 1 L) to make 500 mL, further add 50 µL of

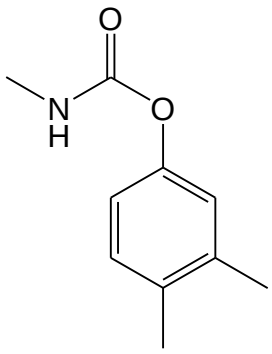
2-mercaptoethanol (Freshly prepare at the time of use) and mix.

Flow rate: Eluent: 1.0 mL/min, Reaction liquid: 0.2 mL/min each

Temperature: Column oven: 45 °C, reaction tank: 90 °C

Reaction coil: RXN 1000 Coil (internal diameter: 0.5 mm, length: approximately 5 m, reaction volume: 1 mL, made of teflon, Waters)

52 Xylylcarb (MPMC)



3,4-xylyl methylcarbamate

$C_{10}H_{13}NO_2$ MW: 179.22 CAS No.: 2425-10-7

[Summary of xylylcarb]

Xylylcarb is a carbamate insectcide, white crystal, developed by Sumitomo Chemical, and easily hydrolyzed at pH 12 or more. Xylylcarb has effects on plant hopper and leafhoppers injurious to rice plants. Xylylcarb was registered as an agricultural chemical in 1967, in Japan. Registered name had been MPMC. However, it was expired in 1994.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)

Target Analytes: XMC, aldicarb (including aldicarb sulfoxide and aldicarb sulfone), isoprocarb, carbaryl, carbofuran, xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb (12 compounds)

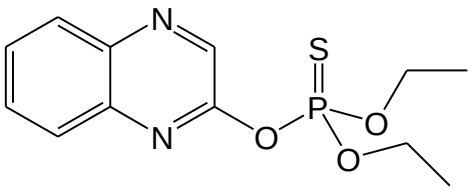
Refer to Article 3, Section 3 in this chapter.

2. Simultaneous analysis method of carbamate agricultural chemicals by gas chromatography

Target Analytes: XMC, isoprocarb, carbaryl, xylylcarb, fenobucarb, propoxur and metolcarb (7 compounds)

Refer to Article 5, Section 3 in this chapter.

53 Quinalphos



O,O-diethyl *O*-quinoxalin-2-yl phosphorothioate

$C_{12}H_{15}N_2O_3PS$ MW: 298.3 CAS No.: 13593-03-8

[Summary of Quinalphos]

Quinalphos is an organic phosphorus insectcide, white crystal, developed by Sandos (Switzerland).

Quinalphos specially has effects on arrowhead scale, ceroplates ceriferus fabricius injurious to citrus.

Quinalphos was registered as an agricultural chemical in 1987, in Japan. The trade name is “Ekalux”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn: 0.05 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

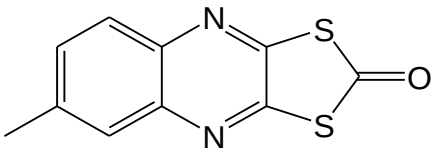
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

54 Chinomethionat



6-methyl-1,3-dithiolo[4,5-*b*]quinoxalin-2-one

$C_{10}H_6N_2OS_2$ MW: 234.3 CAS No.: 2439-01-2

[Summary of chinomethionat]

Chinomethionat is a quinoxaline miticide, bactericide developed by Bayer (Germany), which has also effects on *Erysiphe necator*.

Chinomethionat was registered as an agricultural chemical in 1964, in Japan. Registered name is quinoxaline. The trade name is “Molestan”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grain: 0.1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Gas chromatography [Analytical Standards of Feeds Chapter 6, Section 1, 54.1]

A. Reagent preparation

Chinomethionat standard solution. Weigh accurately 20 mg of chinomethionat [$C_{10}H_6N_2OS_2$][1], place in a 100 mL brown volmetric flask, add acetone to dissolve, and further add acetone up to marked line to prepare a chinomethionate standard solution (1 mL of this solution contains an amount equivalent to 0.2 mg of chinomethionat).

At the time of use, accurately dilute a quantity of the said standard stock solution with acetone to prepare several chinomethionat standard solutions containing amounts equivalent to 0.05-2.5 μg of chinomethionat in 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of the analysis sample, place in a stoppered 200 mL Erlenmeyer flask, add 2 mL of hydrochloric acid (1+4) solution[2] and 15 mL of water to moisten, allow to still standing for 30 minutes, further add 80 mL of acetone, and stir for 30 minutes to extract.

Place a 300 mL recovery flask under a Büchner funnel, filter the extract by suction with filter paper (No.5B) by suction, wash the Erlenmeyer flask and residue sequentially with 50 mL of acetone, and filter by suction in the similar way.

Condense the filtrate to 15 mL or less under reduced pressure in a water bath at 40 °C or lower, and add 5 g of sodium chloride to prepare the sample solution subject to column treatment.

Column treatment. Place the sample solution in a porous diatomite column (for 20 mL retention), and allow to still standing for 5 minutes. Place a 200 mL recovery flask under the column, wash the recovery

flask having contained the sample solution 3 times with 10 mL each of hexane, add the washing liquid to the column, and allow the liquid to flow out until the liquid level reaches the top of the column packing material to elute chinomethionat. Further add 70 mL of hexane to the column to elute in a similar way, condense the eluate almost into dryness in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add accurately 8 mL of cyclohexane-acetone (4 : 1) to dissolve the residue. Place this solution in a 10 mL centrifuge tube, centrifuge at 1,500×*g* for 5 minutes, and filter the supernatant through membrane filter (pore size not exceeding 0.5 μm) to prepare the sample solution subject to gel permeation chromatography.

Gel permeation chromatography. Inject 4.0 mL of the sample solution to the gel permeation chromatograph, take the eluted fraction of chinomethionat into a 50 mL recovery flask, condense into almost dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add accurately 2 mL of acetone to dissolve the residue to prepare the sample solution subject to the gas chromatography.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column[3] (internal diameter: 20mm, length 300 mm, particle size: 15 μm)

Guard column: Styrene-divinylbenzene copolymer column[3] (internal diameter: 20 mm, length: 100 mm, particle size: 15 μm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction: 115-145 mL

Gas chromatography. Inject 2 μL each of the sample solution and respective chinomethionat standard solutions into a gas chromatograph to obtain the chromatogram.

Example of measurement conditions

Detector: Flame thermionic detector

Column: Fused silica capillary column (5 % diphenyl - 95 % dimethylpolysiloxane-coating, 0.32 mm in internal diameter, 30 m in length, 0.25 μm in film thickness) [4]

Carrier gas: He (1.5 mL/min)

Make-up gas: He (30 mL/min)

Hydrogen: 3 mL/min

Dry air: 90 mL/min

Sample injection: Splitless (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial stage temperature 80 °C (hold 1 min) → ramp 20 °C/min → 280 °C (hold 10 min)

Detector temperature: 280 °C

Calculation. Calculate the peak height or peak area from the obtained chromatogram [5] to prepare a

calibration curve, and determine the amount of chinomethionat in the sample.

«Summary of analysis method»

This method is intended to determine the amount of chinomethionat, by extracting with hydrochloric acid acetone, purifying with a porous diatomite column and GPC, and quantifying with a gas chromatograph attached with a flame thermionic detector (or nitrogen-phosphorus detector).

The flow sheet of the analysis method is shown in Figure 6.1.54-1.

Sample (10 g)
- Add 2 mL of hidrochloric acid (1 : 4) and 15 mL of water, and allow to still standing for 30 min.
- Add 80 mL of acetone, and stir for 30 min.
- Filter under vacuum (No.5B).
- Wash with 50 mL of acetone.
- Condense under reduced pressure (40 °C or lower, to approx. 15 mL).
- Add 5 g of sodium chloride.

Chem Elut cartridge (for 20 mL retention)
- Load the sample solution and allow to still standing for 5 min.
- Add 100 mL of hexan.
- Condense under reduced pressure (40 °C or lower), and evaporate into dryness with nitrogen gas.
- Add 8 mL of cycliohexane-acetone (8 : 2).
- Filter through membrane filter (pore diameter: 5 µm or less).

GPC (inject 4 mL of the sample solution)
- Collect 115-145 mL of fraction.
- Condense under reduced pressure (40 °C or lower, to approx. 15 mL).
- Add 2 mL of acetone.

GC-FTD or GC-NPD

Figure 6.1.54-1 Flow sheet of analysis method of chinomethionat

References: Norio Aita: Research Report of Animal Feed, 26, 10 (2001)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (µg/kg)	Repeat	Spike recovery (%)	Repeatability RSD (% or less)
Adult chicken formula feed	50-250	3	91.1-98.5	1.0
Growing beef cattle formula fe	50-250	3	91.9-97.9	5.6
Oat hay	50-250	3	83.1-91.1	3.8
Alfalfa hay-cube	50-250	3	82.1-95.9	5.5

• Collaborative study

Sample type	No. of labs	Spike concentration (µg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Adult chicken formula feed	5	100	98.4	3.4	7.2	0.83

• Lower quantification limit: 10 µg/kg in sample

«Notes and precautions»

[1] The standard preparation was commercially available from GL Science, Kanto Chemical, Wako Pure Chemical Industries or others.

[2] Since chinomethionat is easily hydrolyzed under alkaline pH, add hydrochloric acid to prevent hydrolysis at the time of extraction.

[3] A column filled with styrene – divinyl benzene copolymer hard gel with the eluent.

[4] DB-5 (Agilent Technologies) and others were available.

[5] An example of the chromatogram is shown in Figure 6.1.54-2.

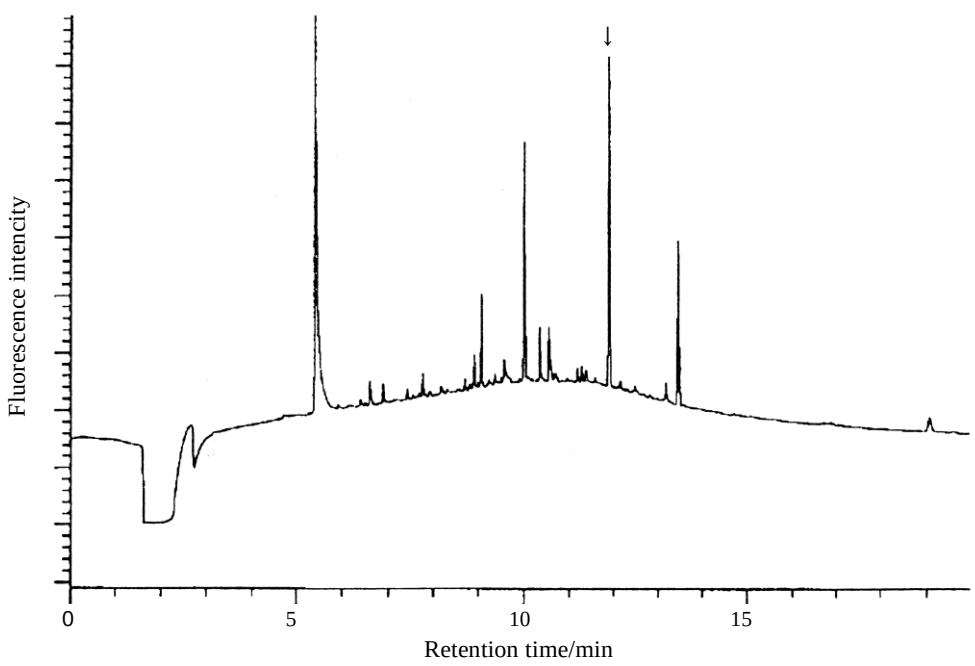


Figure 6.1.54-2 Chromatogram for chinomethionat added to a formula feed in amount equivalent to 0.5 mg/kg (The arrow indicates the peak of chinomethionat)

Measurement conditions

Detector: Alkali flame ionization detector

Column: J&W Scientific DB-5 (internal diameter: 0.32 mm, length: 30 m, film thickness: 0.25 mm)

Carrier gas: He (initial stage flow: 1.5 mL/min,)

Make up gas: He (30 mL/min)

Hydrogen: 3 mL/min

Dry air: 90 mL/min

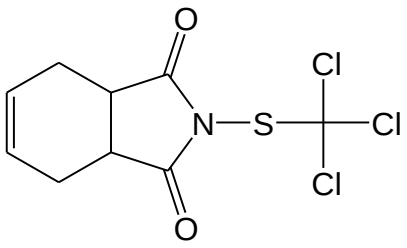
Sample injection: Splitless

Injection port temperature: 250 ℃

Column oven temperature: Initial temperature 80 °C (hold 1 min) → ramp 20 °C/min → 280 °C (hold 10 min)

Detector temperature: 280 ℃

55 Captan



N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide
$C_9H_8Cl_3NO_2S$ MW: 300.59 CAS No.: 133-06-2

[Summary of captan]

Captan is one of phthalimide insecticides containing sulfur and chlorine. This insecticide has less risk of chemical antagonism; therefore, widely used in dusting to vegetables, fruits and flowers. The residue is permitted up to 50 ppm as post-harvest pesticide for dried grapes in USA.

Captan is registered as an agricultural chemical in 1953, in Japan. The trade name is “Orthocide”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Corn：10 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Gas chromatography [Analytical Standards of Feeds Chapter 6, Section 1, Article 55.1]

A. Reagent preparation

1) Captan standard solution. Weigh accurately 20 mg of captan [$C_9H_8Cl_3NO_2S$][1], place in a 100 mL volmetric flask, add 20 mL of acetone to dissolve, further add 2,2,4-trimethylpentane up to the marked line to prepare the captan standard stock solution (1 mL of this solution contains 0.2 mg of captan).

At the time of use, accurately dilute a quantity of the standard stock solution with 2,2,4-trimethyl pentan – acetone (4 : 1) to prepare several captan standard solutions containing amounts equivalent to 0.02-0.4 µg of captan in 1 mL.

2) Magnesium silicate. Dry synthetic magnesium silicate (particle size: 149-250 µm (100-60 mesh)) at 130 °C for 16 hours.

3) Activated carbon mixture. Mix 10 g of activated carbon[*1] and 90 g of cellulose[*2].

B. Quantification

Extraction. Weigh 10.0-20.0 g of the analysis sample[2], place in a stoppered 200 mL Erlenmeyer flask, add 30 mL of phosphoric acid (1+9) solution to moisture[3], allow to still standing for 30 minutes, further add 70 mL of acetone, and shake for 30 minutes to extract.

Place a 500 mL recovery flask under a Büchner funnel, filter the extract through filter paper (No.5B) by suction, wash the Erlenmeyer flask and residue serially with 50 mL of acetone – water (7 : 3), and filter by suction in a similar way. Condense the filtrate to approximately 60 mL under reduced pressure

in a water bath at 50 °C or lower to prepare the sample solution subject to purification.

Purification. Add the sample solution to the 500 mL separating funnel A containing 200 mL of sodium chloride solution (5 w/v%) and 100 mL of hexane. Wash the recovery flask having contained the sample solution twice with 10 mL each of acetone – water (7 : 3), and add the washing liquid to the separating funnel A. Shake the separating funnel A for 5 minutes, allow to still standing, transfer the water layer (upper layer) to the 500 mL separating funnel B, and the hexane layer (upper layer) to an Erlenmeyer flask. Add 50 mL of hexane to the separating funnel B, stir for 5 minutes, allow to still standing, and transfer the hexane layer to the Erlenmeyer flask. Dehydrate the hexane layer with an appropriate amount of sodium sulfate (anhydrous), filter through filter paper (No.2S) into a 300 mL recovery flask, wash the said Erlenmeyer flask and filter serially with a small amount of hexane, and add the washing liquid to the filtrate through the filter paper. Condense the filtrate almost into dryness under reduced pressure in a water bath at 50 °C or lower, further dry up by the flow of nitrogen gas.

Transfer the residue to a 100 mL separating funnel with 30 mL of hexane, further add 30 mL of acetonitrile to the separating funnel. Shake the separating funnel for 5 minutes, allow to still standing, and transfer the acetonitrile layer (lower layer) to a 200 mL recovery flask. Add 30 mL of acetonitrile to the separating funnel, shake for 5 minutes, allow to still standing, and transfer the acetonitrile layer to the recovery flask. Condense the acetonitrile layer almost into dryness under reduced pressure in a water bath at 50 °C or lower, further dry up by the flow of nitrogen gas. Add 5 mL of hexane to dissolve the residue to prepare the sample solution subject to the column treatment I.

Column treatment I. Suspend 5 g of sodium sulfate (anhydrous), 10 g of magnesium silicate and 5 g of sodium sulfate (anhydrous) in hexane, serially flow into a column tube (internal diameter: 15 mm), and allow to flow out until the liquid surface reaches at 3 mm from the top of the column packing material to prepare the column.

Place the sample solution in the column, wash the recovery flask having contained the sample solution 5 times with 5 mL each of hexane, add the washing liquid serially to the column, and flow out until the liquid surface reaches at 3 mm from the top of the column packing material. Add 150 mL of hexane – diethylether (17 : 3) to the column, and flow out in a similar way. Place a 300 mL recovery flask under the column, add 150 mL of hexane – ethyl acetate (1 : 1) to the column to elute captan, condense the eluate under reduced pressure almost into dryness in a water bath at 50 °C or lower, further dry up by the flow of nitrogen gas.

Add 5 mL of hexane to dissolve the residue to prepare the sample solution subject to the column treatment II.

Column treatment II. Suspend 5 g of sodium sulfate (anhydrous), 5 g of activated carbon mixture and 5 g of sodium sulfate (anhydrous) in hexane, respectively, flow out serially into the column tube (internal diameter: 15 mm), and allow to flow out until the liquid surface reaches at 3 mm from the top of the column packing material to prepare the column.

Place a 500 mL recovery flask under the column, place the sample solution into the column, wash the recovery flask having contained the sample solution 5 times with 5 mL each of hexane – diethyl ether (1 : 1), and serially add the washings to the column. Flow out captan until the liquid surface reaches at 3

mm from the top of the column packing material. Further add 200 mL of the solvent to the column, and flow out in a similar way. Condense the effluent under reduced pressure into almost dryness in a water bath at 50 °C or lower, further dry up by the flow of nitrogen gas.

Add accurately 5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) to dissolve the residue to prepare the sample solution subject to gas chromatography.

Gas chromatography. Inject 1 µL each of the sample solutions and respective captan standard solutions in a gas chromatograph to obtain the chromatogram.

Operating conditions (example)

Detector: Electron capture detector

Column: Fused silica capillary column (50 % diphenyl/ 50 % dimethyl-polysiloxane coating, internal diameter: 0.32 mm, length: 30 m, film thickness: 0.25 µm)[4]

Carrier gas: He (3.5 mL/min)

Make up gas: N_2 (75 mL/min)

Sample injection: Splitless (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 60 °C (hold 0.5 min) → ramp 20 °C/min → 220 °C (hold 10 min) → ramp 10 °C/min → 250 °C (hold 30 min)

Detector temperature: 280 °C

Calculation. Calculate the peak height or peak area from the obtained chromatogram[5] to prepare a calibration curve, and obtain the amount of captan in the sample.

* 1. Activated carbon for column chromatogram (Wako Pure Chemical Industries) or an equivalent.
2. Cellulose for microcrystal column chromatogram (Merck) or an equivalent.

«Summary of analysis method»

This method is intended to determine the amount of residual captan in feed by extracting with acidic acetone, purifying with liquid-liquid distribution, magnesium silicate column and activated carbon column, and quantifying using a gas chromatograph attached with electron capture detector. The flow sheet of the analysis method is shown in Figure 6.1.55-1.

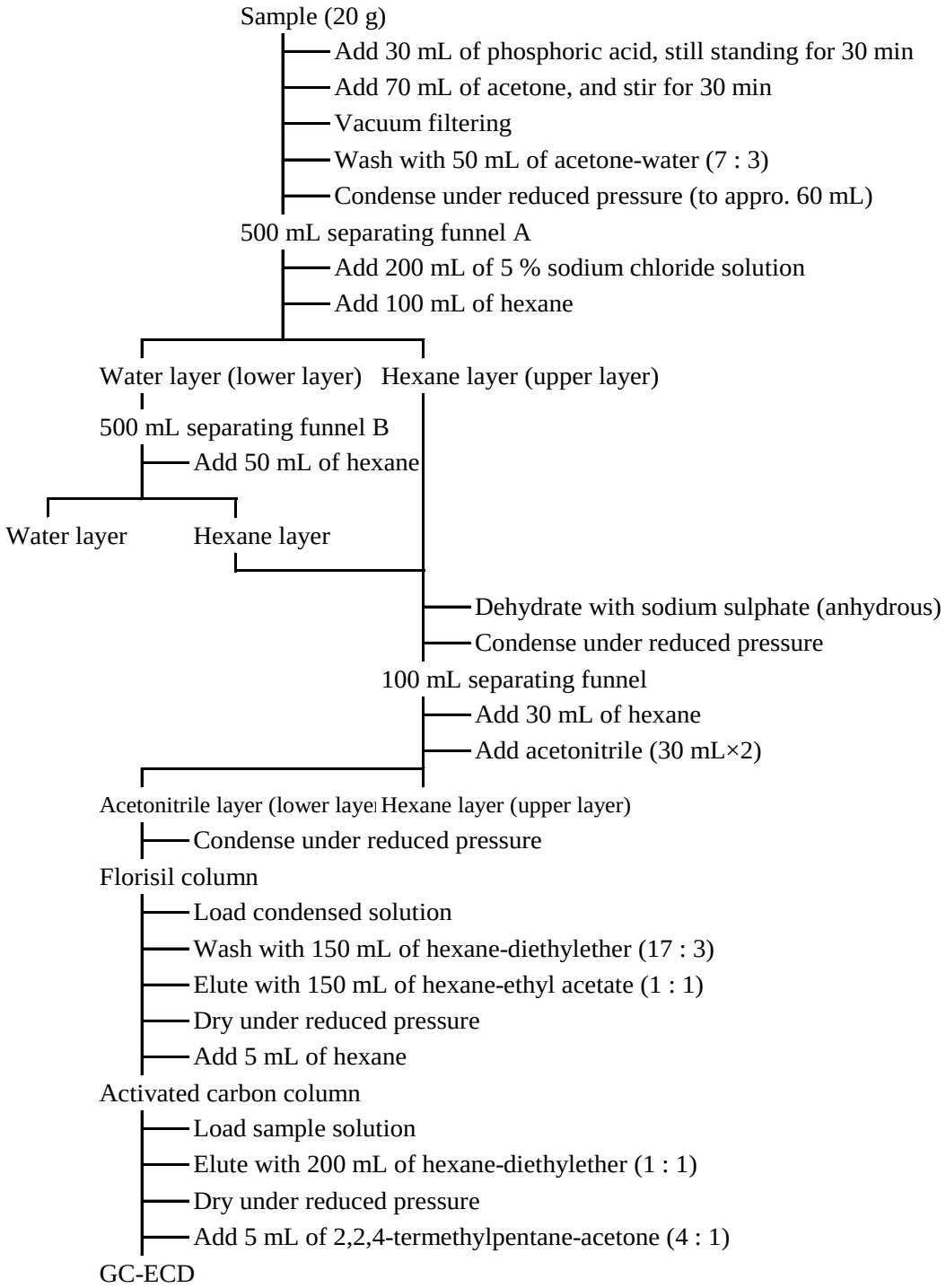


Figure 6.1.55-1 Flow sheet of analysis method of captan

References: Manabu Matsuzaki: Research Report of Animal Feed, 21, 1 (1996)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Adult chicken formula feed	20-80	3	87.7-92.3	17.9
Dairy cattle formula feed	20-80	3	91.3-99.3	16.4
Alfalfa	20-80	3	91.0-94.0	11.7

• Collaborative study

Sample type	No. of Labs	Spike concentration (μg/kg)	Spike recovery (%)	Repeatability RSD_r (%)	Inter-lab reproducebility RSD_R (%)	HorRat
Finishing Broiler formula feed	6	80	92.0	8.0	20.5	0.93

«Notes and precautions»

[1] The standard preparation is commercially available from Wako Pure Chemical Industries, Kanto Chemical, HAYASHI PURE CHEMICAL, GL Sciences or others.

[2] Sampling amount is 10 g for deeply pigmented samples such as hay.

[3] Caution is demanded for samples containing mineral because they generate hydrogen.

[4] DB-17 (Agilent Technologies) and others are available.

[5] An example of the chromatogram is shown in Figure 6.1.55-2.

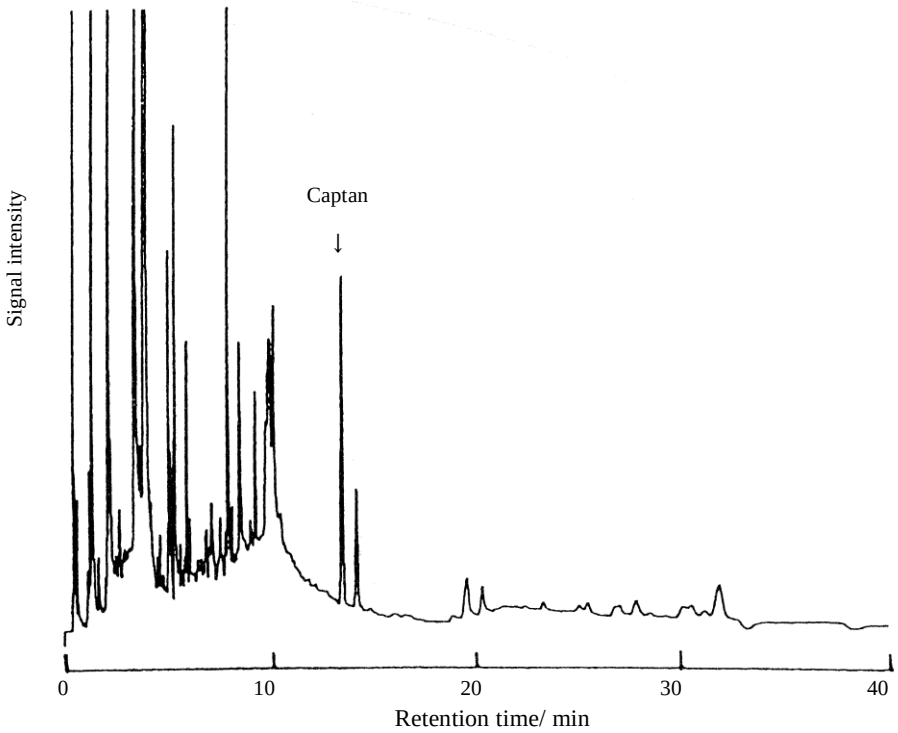


Figure 6.1.55-2 Chromatogram of a sample of formula feed added with captan in an amount equivalent to 40 μg/kg

Measurement conditions

Detector: Electron capture detector (ECD)

Column: J&W scientific DB-17 (internal diameter: 0.53 mm, length: 30 m, film thickness: 1.0 μm)

Carrier gas: He (initial stage flow: 5.0 mL/min)

Make up gas: N_2 (75 mL/min)

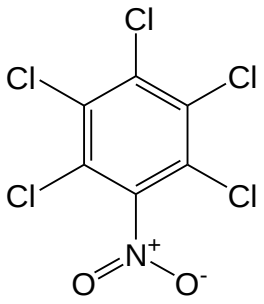
Sample injection: Splitless

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 60 °C (hold 0.5 min) → ramp 20 °C/min → 220 °C (hold 10 min) → ramp 10 °C/min → 250 °C (hold 30 min)

Detector temperature: 280 °C

56 Quintozene



Pentachloronitrobenzene

$C_6Cl_5NO_2$ MW: 295.3 CAS No.: 82-68-8

[Summary of quintozene]

Quintozene is an organic chlorine bactericide developed by Bayer (Germany). Quintozene was applied to clubroot, dieback, etc. of vegetables.

Quintozene had been registered as an agricultural chemical in 1956, in Japan. However, it was expired in 2000.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley and corn: 0.01 ppm, rye: 0.02 ppm

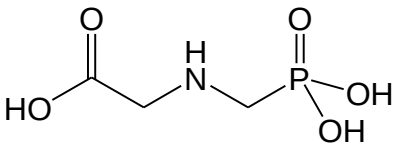
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

57 Glyphosate



N-(phosphonomethyl)glycine

$C_3H_8NO_5P$ MW: 169.073 CAS No.: 1071-83-6

[Summary of glyphosate]

Glyphosate is a nonselective foliage treatment type phosphorus-containing amino acid herbicide developed by Monsanto (USA).

Glyphosate was registered as an agricultural chemical in 1980, in Japan (as isopropylamine salt). The registered designation is glyphosate. The trade name is "Roundup". Later, glyphosate was also registered as ammonium salt, trimesium salt, potassium salt and sodium salt.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

(glyphosate, glyphosate ammonium salt, glyphosate isopropylamine salt, glyphosate trimesium salt and glyphosate sodium salt are included)

Rye: 0.2 ppm, corn: 1 ppm, wheat: 5 ppm, oat, barley and milo: 20 ppm, pasture grass: 120 ppm, rice straw and rice plant silage: 0.2 ppm.

[Methods listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for glyphosate, glufosinate and related compounds by gas chromatography

Target Analytes:

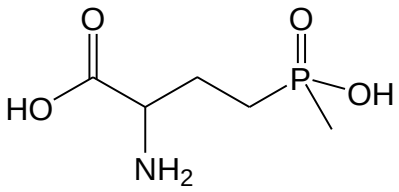
Group A: Glyphosate and 3-(methylphosphinico)propionic acid (2 compounds)

Group B: Glufosinate (1 compound)

Refer to Article 6, Section 2 in this chapter.

58 Glufosinate (including 3-(methylphosphinico)propionic acid)

Glufosinate

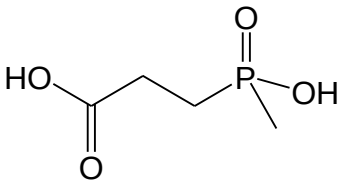


(2*RS*)-2-amino-4-[hydroxy(methyl)phosphinoyl] butyric acid

$C_5H_{12}NO_4P$ MW: 181.1

CAS No.: 51276-47-2

3-(Methylphosphinico) propionic acid

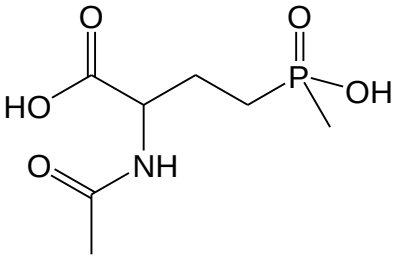


3-(methylphosphinico)propionic acid

$C_4H_9O_4P$ MW: 152.1

CAS No.: 15090-23-0

(Reference)
N-acetylglufosinate



(2*RS*)-2-acetamido-4-[hydroxy(methyl)phosphinoyl] butyric acid

$C_7H_{14}NO_5P$ MW: 223.2

CAS No.: 73634-73-8

[Summary of glufosinate]

Glufosinate is a nonselective foliage treatment type phosphorus-containing amino acid herbicide developed by Hoechst (Germany).

Glufosinate was registered as an agricultural chemical in 1984, in Japan (as ammonium salt). The registered designation is glufosinate. The trade name is "Basta".

Both 3-(methylphosphinico)propionic acid and *N*-acetylglufosinate are metabolites of glufosinate, and the latter is a metabolite generated in glufosinate-resistant recombinant plants.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

(As for grain, the value is defined as the sum of the contents of glufosinate, *N*-acetylglufosinate equivalent to glufosinate and 3-(methylphosphinico)propionic acid equivalent to glufosinate. For pasture grass, the value is defined as the sum of the contents of glufosinate and 3-(methylphosphinico)propionic acid equivalent to glufosinate. Further, glufosinate includes glufosinate ammonium salt)

Corn : 0.1 ppm, wheat: 0.2 ppm, barley: 5 ppm, pasture grass: 15 ppm, and rice straw: 0.5 ppm.

[Methods listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for glyphosate, glufosinate and related compounds by gas chromatography

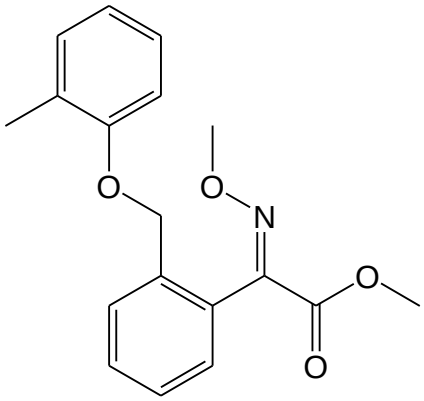
Target Analytes:

Group A: Glyphosate and 3-(methylphosphinico)propionic acid (2 compounds)

Group B: Glufosinate (1 compound)

Refer to Article 6, Section 2 in this chapter.

59 Kresoxim-methyl



Methyl (*E*)-methoxyimino[*α*-(*o*-tolyloxy)-*o*-tolyl]acetate

$C_{18}H_{19}NO_4$ MW: 313.4 CAS No.: 143390-89-0

[Summary of kresoxim-methyl]

Kresoxim-methyl is a strobilurin bactericide.

Kresoxim-methyl was registered as an agricultural chemical in 1997, in Japan, targeting *mugi*, vegetables, etc. The trade name is “Stroby”.

«Standard values to grain defined by Food Sanitation Law»

Wheat: 0.1 ppm, barley, rye, corn and other grain: 5 ppm

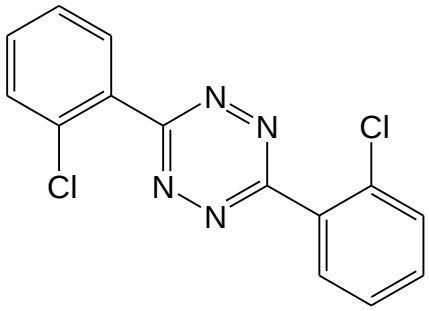
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

60 Clofentezine



3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine

$C_{14}H_8Cl_2N_4$ MW: 303.15 CAS No.: 74115-24-5

[Summary of clofentezine]

Clofentezine is a tetrazine miticide developed by Fisons (UK), being considered to act to mites with inhibiting cuticle formation during the embryo-period, inhibiting the development of mites for a long time. Clofentezine is reddish violet crystal, soluble in organic solvent, and stable to light and heat.

Clofentezine was registered as an agricultural chemical in 1989, in Japan. The trade names are “Karla” or “Apollo”.

«Standard values to grain defined by Food Sanitation Law»

Wheat, barley and rye: 0.02 ppm, corn: 0.05 ppm, and other grain: 0.02 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Liquid chromatography [Analytical Standards of Feeds Chapter 6, Section 1, Article 60.1]

A. Reagent preparation

Clofentezine standard solution. Weigh accurately 20 mg of clofentezine [$C_{14}H_8Cl_2N_4$][1], place in a 100 mL volmetric flask, add acetonitrile to dissolve, further add the solvent to the marked line to prepare the clofentezine standard stock solution (1 mL of this solution contains an amount equivalent to 0.2 mg of clofentezine).

At the time of use, dilute a quantity of the standard stock solution accurately with acetonitrile to prepare several clofentezine standard solutions containing amounts equivalent to 0.02-1 μg of clofentezine in 1 mL.

B. Quantification

Extraction. Weigh accurately 5 g of the analysis sample, place in a stoppered 200 mL Erlenmeyer flask, add 15 mL of water to moisten, allow to still standing for 30 min, further add 50 mL of acetone, and shakr for 30 minutes to extract.

Place a 200 mL beaker under a Büchner funnel, filter the extract through filter paper (No.5B) by suction, wash serially the Erlenmeyer flask and residue with 50 mL of acetone, and filter by suction in a similar way, to prepare the filtrate as the sample solution for purification.

Purification. Add the sample solution to the 500 mL separating funnel A having received 150 mL of sodium chloride solution (5 w/v%) and 50 mL of hexane, shake vigorously for 5 minutes, allow to still standing, and transfer the water layer (lower layer) and hexane layer (upper layer) to 500 mL separating funnel B and Erlenmeyer flask, respectively. Add 50 mL of hexane to separating funnel B, shake gently, allow to still standing, and transfer the hexane layer to the Erlenmeyer flask. Dehydrate the hexane layer with an appropriate quantity of sodium sulfate (anhydrous), filter through filter paper (No.2S) into a 300 mL recovery flask, wash the Erlenmeyer flask and filter paper serially with a small amount of hexane, and add the washings to the filtrate through the filter paper. Condense the filtrate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add accurately 5 mL of cyclohexane – acetone (7 : 3) to dissolve the residue, and filter with membrane filter (pore size: 0.5 µm or less) to prepare the sample solution subject to gel permeation chromatography.

Gel permeation chromatography. Inject 2.0 mL of the sample solution into a gel permeation chromatograph, take the fraction containing the clofentezine eluate into a 100 mL recovery flask, condense almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add accurately a 5 mL of hexane to dissolve the residue to prepare the sample solution subject to column treatment.

Example of operating conditions

Column: Styrene – divinylbenzene copolymer column[2] (internal diameter: 20 mm, length: 300 mm, particle size 15 µm)

Guard column: Styrene – divinylbenzene copolymer column[2] (internal diameter: 20 mm, length: 100 mm, particle size: 15 µm)

Eluent: Cyclohexane – acetone (7 : 3)

Flow rate: 5 mL/min

Fraction collected: 80-110 mL[3]

Column treatment. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane.

Place the sample solution in the minicolumn, flow out until the remaining amount in the reserver of the minicolumn reaches 0.5 mL, wash the recovery flask having containing the sample solution with 5 mL of hexane, add the washings to the minicolumn, and flow out in a similar way. Place a 50 mL recovery flask under the minicolumn, add 15 mL of hexane-ethyl acetate (19 : 1) to the minicolumn to elute clofentezine. Condense the eluate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add 2 mL of acetonitrile accurately to dissolve the residue, filter through membrane filter (pore size: 0.5 µm or less) to prepare the sample solution subject to liquid chromatography.

Liquid chromatography. Inject 20 µL each of the sample solution and respective clofentezine standard solutions into liquid chromatograph to obtain the chromatogram.

Example of measurement conditions

Detector: Ultraviolet spectrophotometer (measured wavelength: 267 nm)

Column: Octadecylsilylated silica gel column[4] (internal diameter: 4 mm, length: 250 mm, particle size: 5 μm)*1

Eluent: Acetonitrile – water (3 : 1)

Flow rate: 1.0 mL/min

Column oven temperature: 40 °C

Calculation. Calculate the peak height or peak area to prepare a calibration curve from the obtained chromatogram[5], and determine the amount of clofentezine in the sample.

* 1. Wakosil II5C18 HG (Wako Pure Chemical Industries) or an equivalent.

«Summary of analysis method»

This method is intended to determine the amount of clofentezine in feed by extracting by hydrous acetone, changing solvent with hexane, purifying with gel permeation chromatography and the Florisil minicolumn, and quantifying using a liquid chromatograph equipped with an ultraviolet spectrophotometer.

The flow sheet of the analysis method is shown in Figure 6.1.60-1.

Sample (5 g)
- Add 15 mL of water, and allow to still standing for 30 min.
- Add 50 mL of acetone, and shake for 30 min.
- Filter under vacuum.
- Wash with 50 mL of acetone.

Liquid-liquid extraction
- Add 150 mL of 5 w/v% sodium chloride solution.
- Add 50 mL of hexar
- Shake for 5 min.

] Repeat twice, and add the hexane layer.

- Dehydrate the hexane layer with sodium sulfate (anhydrous)
- Condense under reduced pressure (approx. 1 mL), and evaporate into dryness with nitrogen gas.
- Add 5 mL of Cyclohexane-acetone (7 : 3)

GPC (Inject 2 mL of the sample)
- Collect 80-110 mL of fraction.
- Condense under reduced pressure (approx. 1 mL), and evaporate into dryness with nitrogen gas.

Sep-Pak Plus Florisil cartridge

(Preliminarily wash with 5 mL of hexane)
- Load with 5 mL of hexane (twice).
- Elute with 15 mL of hexane-ethyl acetate (19 : 1).
- Condense under reduced pressure (approx. 1 mL), and evaporate into dryness with nitrogen gas.
- Add 2 mL of acetonitrile.

LC-UV (267 nm)

Figure 6.1.60-1. Flow sheet of analysis method of clofentezine

References: Sayaka Hashimoto, Hiroshi Hibino: Research Report of Animal Feed, 23, 24 (1998)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Adult chicken formula feed	100-1,000	3	87.7-91.7	7.7
Finishing beef cattle formula feed	100-1,000	3	91.3-96.0	4.2
Alfalfa hay	100-1,000	3	94.0-95.3	7.7

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Adult chicken formula feed	6	500	90.9	3.8	5.7	0.32

• Lower quantification limit: 25 μg/kg sample for formula feeds, 50 μg/kg sample for hay

«Notes and precautions»

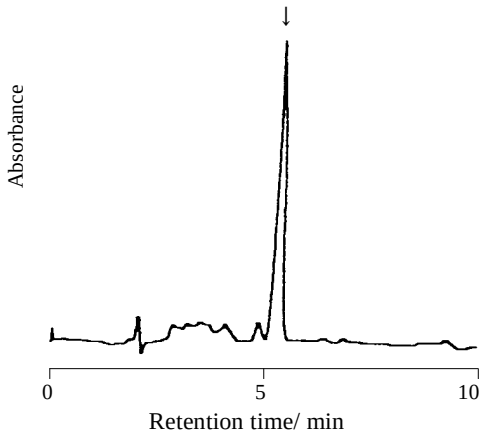
[1] The standard preparation is commercially available from Wako Pure Chemical Industries, Kanto Chemical or others.

[2] A column filled with styrene – divinylbenzene copolymer hard gel by eluent

[3] Confirm the eluting fraction before the time of use, because it may be changed by using conditions or using frequency of the column.

[4] Any column is applicable as long as its end-capped packing material meets the requirements.

[5] An example of chromatogram is shown in Figure 6.1.60-2.



Measurement conditions

Detector: Ultraviolet spectrophotometer (measured wavelength: 267 nm)

Column: Wakosil II 5C18 HG

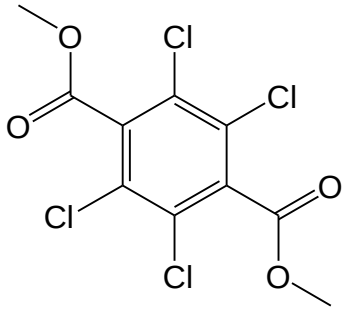
Eluent: Acetonitrile-water (3 : 1)

Flow rate: 1.0 mL/min

Column oven temperature: 40 °C

Figure 6.1.60-2. Chromatogram for clofentezine added to formula feed in an amount equivalent to 0.5 mg/kg (The arrow indicates the peak of clofentezine)

61　Chlorthal-dimethyl (TCTP)



Dimethyl tetrachloroterephthalate

$C_{10}H_6Cl_4O_4$　MW: 332.0　CAS No.: 1861-32-1

[Summary of chlorthal-dimethyl]

Chlorthal-dimethyl is a non-hormone type phthalic acid herbicide.

Chlorthal-dimethyl had been registered as an agricultural chemical in 1971, in Japan. Registered name had been TCTP. However, it was expired in 2005.

«Standard value in grain defined by Food Sanitation Law»

Corn: 3 ppm

[Methods listed in the Analytical Standards of Feeds]

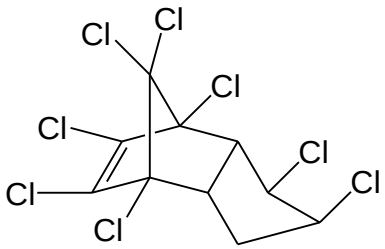
1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

62 Chlordane (*cis*-chlordane and *trans*-chlordane)

cis-chlordane

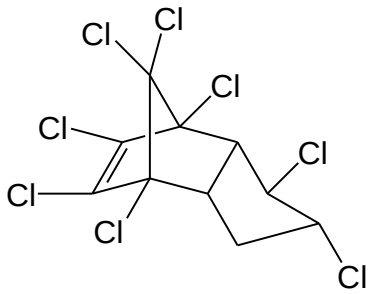


(1*α*,2*α*,3a*α*,4*β*,7*β*,7a*α*)-1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*H*-indene

$C_{10}H_6Cl_8$ MW: 409.8

CAS No.: 5103-71-9

trans-chlordane



(1*α*,2*β*,3a*α*,4*β*,7*β*,7a*α*)-1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*H*-indene

$C_{10}H_6Cl_8$ MW: 409.8

CAS No.: 5103-74-2

[Summary of chlordane]

Chlordane is an organic chlorine insecticide developed by Velsicol (USA) similar to dieldrin, and had been used as a mixed formulation containing heptachlor. Chlordane had been used in Japan from 1950; however, fell into abeyance in 1969, and, after then, generally used as a termiticide. Because of extremely high persistence, it designated to a class 1 specific chemical substance in 1986, and banned to manufacture, import or use.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(The amount is designated as the sum of *cis*-chlordane and *trans*-chlordane for agricultural products, and sum of *cis*-chlordane, *trans*-chlordane and its metabolite, oxychlordane in animal products and aquatic products)

Wheat, barley, rye, corn and other grain: 0.02 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

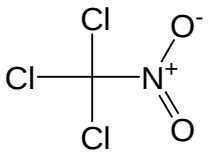
Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor

(24 compounds)

Group B: Alachlor, β-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

63 Chloropicrin



Trichloronitromethane

CCl_3NO_2 MW: 164.375 CAS No.: 76-06-2

[Summary of chloropicrin]

Chloropicrin is soil treatment type insecticide, bactericide and herbicide synthesized in UK in 1948, which is oily colorless liquid. Chloropicrin is a deleterious substance.

Chloropicrin was registered as an agricultural chemical in 1948, in Japan. The trade name is "Chlopic".

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Gas chromatography [Analytical Standards of Feeds Chapter 6, Section 1, Article 63.1]

A. Reagent preparation

Chloropicrin standard solution. Use chloropicrin [CCl_3NO_2][1] standard solution[*1] as the standard stock solution (1 mL of this solution contains 1 mg of chloropicrin.).

At the time of use, dilute a quantity of the standard stock solution accurately with hexane to prepare several chloropicrin standard solutions containing amounts equivalent to 0.025-1 μg of chloropicrin in 1 mL.

B. Quantification

Extraction. Weigh 10.0-20.0 g of the analysis sample[2], place in a 1 L flask for Dean Stark distillation apparatus[3]. To this flask, add 500 mL of water[*2], 10 mL of hydrochloric acid, a few drops of silicon oil and 20 mL of hexane, connect this flask to the distillation apparatus, distill for 1 hour, take chloropicrin with hexane, and allow being cool.

Remove the water in a trap of the distillation apparatus, wash the inside of the cooling tube with approximately 10 mL of water[*2], and filter the hexane layer through filter paper (No. 2S) into a 20 mL test tube to prepare the sample solution subject to gas chromatography.

Gas chromatography. Inject 1 μL each of the sample solution and respective chloropicrin standard solutions into a gas chromatograph to obtain the chromatogram.

Example of measurement conditions

Detector: Electron capture detector

Column: Fused silica capillary column (coated with polyethyleneglycol (molecular weight: 20,000), internal diameter: 0.32 mm, length: 30 m, film thickness: 0.5 μm)[4]

Carrier gas: He (2.0 mL/min)
Makeup gas: N_2 (50 mL/min)
Sample injection: Splitless (60 s)
Injection port temperature: 200 °C
Column even temperature: Initial temperature: 50 °C (hold 5 min) → ramp 10 °C/min → 200 °C (hold 10 min)
Detector temperature: 280 °C

Calculation. Calculate the peak area from the obtained chromatogram[5] to prepare a calibration curve to determine the amount of chloropicrin in the sample.

* 1. Reagent for testing water quality (hexane solution) (Wako Pure Chemical Industries) or an equivalent.
2. One liter of distilled water washed by shaking with 200 mL of hexane.

«Summary of analysis method»

This method is intended to determine the amount of chloropicrin by extracting the sample solution with distillation, and quantifying with a gas chromatograph attached with an electron capture detector.

References: Sayaka Hashimoto, Soichiro Matsumura, Yuji Fukumoto, Atsushi Kito: Research Report of Animal Feed, 21, 13 (1996)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Adult chicken formula feed	125-500	3	94.3-100.3	8.0
Finishing pig formula feed	125-500	3	100.0-102.7	10.1
Finishing beef cattle formula feed	125-500	3	96.7-102.3	7.6
Alfalfa	125-500	3	98.0-106.0	13.3

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Adult chicken formula feed	7	250	96.1	2.9	5.6	0.28

• Lower quantification limit: Formula feed: 25 μg/kg sample, hay: 50 μg/kg sample

«Notes and precautions»

[1] The standard preparation is commercially available from Kanto Chemical or HAYASHI PURE CHEMICAL.

[2] Sampling amount of hay is 10 g.

[3] Dean-Stark distillation apparatus is as shown in Figure 6.1.63-1.

Add the sample, water, hexane and a drop of silicon oil as antifoam to 1 L flask, attach a mantle heater at the site of flask, and distill with heating. Hexane is pooled in the part of the condenser with scale marks, where steam is cooled while the solution passes through continuously, and thus

chloropicrin is trapped by hexane.

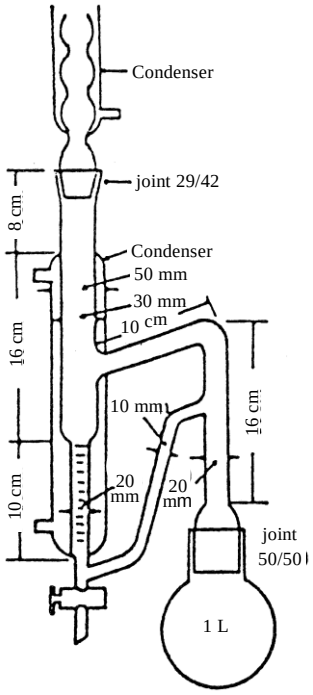


Figure 6.1.63-1. Dean-Stark distillation apparatus

[4] DB-WAX (Agilent Technologies) and others were available.

[5] An example of chromatogram is shown in Figure 6.1.63-2.

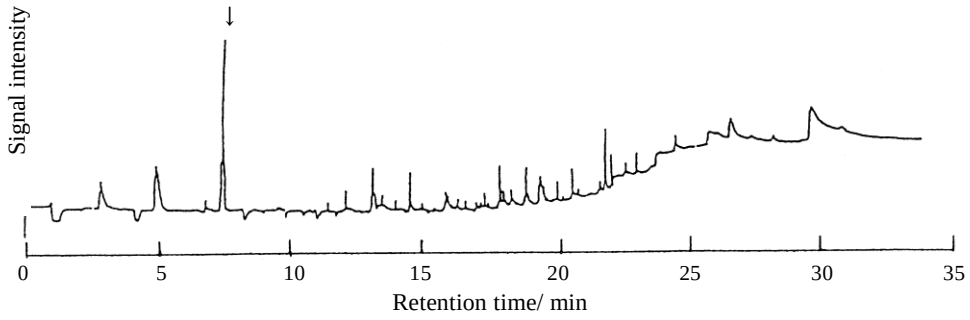


Figure 6.1.63-2 Chromatogram for chloropicrin added to formula feed in an amount equivalent to 0.25 mg/kg (The arrow indicates the peak of chloropicrin)

Measurement conditions

Detector: Electron capture detector (ECD)

Column: J&W Scientific DB-WAX (internal diameter: 0.32 mm, length: 30 m, film thickness: 0.5 μm)

Carrier gas: He (initial flow rate: 2.0 mL/min)

Makeup gas: N_2 (50 mL/min)

Sample injection: Splitless

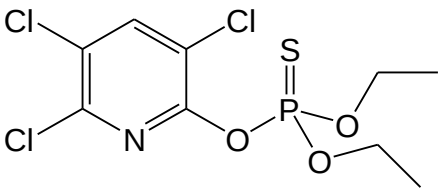
Injection port temperature: 200 °C

Column oven temperature: Initial temperature 35 °C (hold 5 min) → ramp 10 °C/min → 240 °C

(hold 10 min)

Detector temperature: 280 ℃

64 Chlorpyrifos



O,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate

$C_9H_{11}Cl_3NO_3PS$ MW: 350.6 CAS No.: 2921-88-2

[Summary of chlorpyrifos]

Chlorpyrifos is an organic phosphorus insecticide developed by Dow Chemical (USA), colorless crystal generally used for fruit pest control, having effect on larvas of Bell moth, with immediate and residual efficacies. Chlorpyrifos is used as a post-harvest pesticide in USA.

Chlorpyrifos was registered as an agricultural chemical in 1971, in Japan. The trade name is "Dazban".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Rye: 0.01 ppm, corn: 0.1 ppm, barley: 0.2 ppm, wheat: 0.5 ppm, oat and milo: 0.75 ppm, and pasture grass: 13 ppm.

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

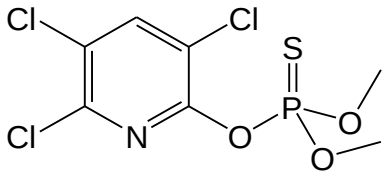
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

65 Chlorpyrifos-methyl



O,*O*-dimethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate
$C_7H_7Cl_3NO_3PS$ MW: 322.5 CAS No.: 5598-13-0

[Summary of chlorpyrifos-methyl]

Chlorpyrifos-methyl is a low-toxic organic phosphorus insecticide developed by Dow Chemical (USA), colorless crystal, readily soluble in organic solvent and stable in normal conditions. Chlorpyrifos-methyl has contact toxicity and dietary toxicity, and is an insecticide having immediate effects on many kinds of pests. Chlorpyrifos-methyl is used as a post-harvest pesticide in USA.

Chlorpyrifos-methyl was registered as an agricultural chemical in 1975, in Japan. The trade name is "Reldan".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Barley: 6 ppm, corn and milo: 7 ppm, oat and wheat: 10 ppm.

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

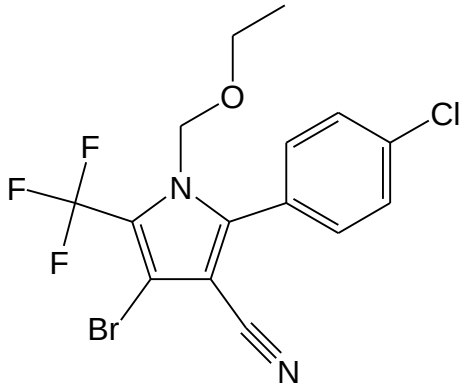
Refer to Article 2, Section 2 in this chapter.

3. Simultaneous analysis method for chlorpyrifos-methyl and pirimiphos-methyl by gas chromatography

Target Analytes: Chlorpyrifos-methyl and pirimiphos-methyl (2 compounds)

Refer to Article 12, Section 3 in this chapter.

66 Chlorfenapyr



4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-5-trifluoromethyl-1*H*-pyrrole-3-carbonitrile

$C_{15}H_{11}BrClF_3N_2O$ MW: 407.6 CAS No.: 122453-73-0

[Summary of chlorfenapyr]

Chlorfenapyr is a pyrrol miticide developed by American Cyanamid (USA, current BASF).

Chlorfenapyr was registered as an agricultural chemical for vegetables, fruits, red beans in 1996, in Japan. The trade name is "Kotetsu".

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grain: 0.05 ppm

[Methods listed in the Analytical Standards of Feeds]

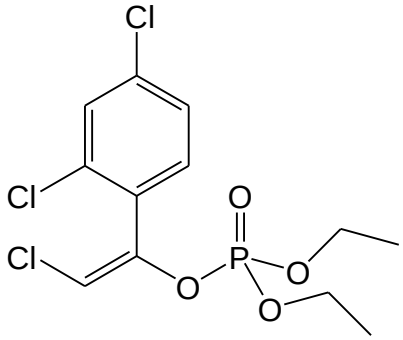
1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

67　Chlorfenvinphos (CVP)
(chlorfenvinphos (*E*-isomer) and chlorfenvinphos (*Z*-isomer))

Chlorfenvinphos (*E*-isomer）

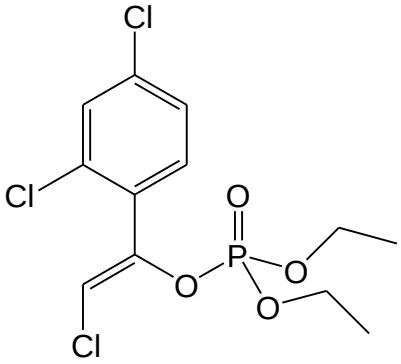


(*E*)-2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate

$C_{12}H_{14}Cl_3O_4P$　MW: 359.6

CAS No.: 18708-86-6

Chlorfenvinphos (*Z*-isomer)



(*Z*)-2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate

$C_{12}H_{14}Cl_3O_4P$　MW: 359.6

CAS No.: 18708-87-7

[Summary of chlorfenvinphos]

Chlorfenvinphos is an organic phosphorus insecticide developed by Shell (USA), which is amber liquid. The isomers of chlorfenvinphos include *cis* (*Z*) isomer and *trans* (*E*) isomer.

Chlorfenvinphos is relatively low toxic, and generally effective to many kind of pests. It has effects on soil pests by powdering to soil, as well as stem and leaf pests.

Chlorfenvinphos had been registered as an agricultural chemical in 1966, in Japan. Registered name had been CVP. However, it was expired in 2004.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(Sum of chlorfenvinphos (*E*-isomer) and chlorfenvinphos (*Z*-isomer))

Wheat and corn: 0.05 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion,

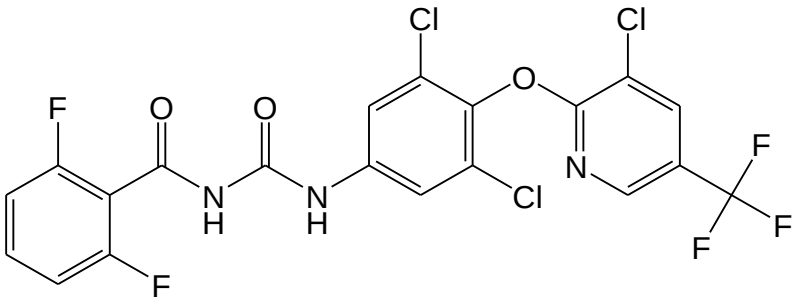
fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

68 Chlorfluazuron



1-[3,5-dichloro-4-(3-chloro-5-trifluoromethyl-2-pyridyloxy)phenyl]-3-(2,6-difluorobenzoyl)urea

$C_{20}H_9Cl_3F_5N_3O_3$ MW: 540.65 CAS No.: 71422-67-8

[Summary of chlorfluazuron]

Chlorfluazuron is a benzoylphenyl urea insect growth inhibitor developed by ISHIHARA SANGYO KAISYA LTD., inhibits chitin synthesis, and is colorless crystal, being used for soybean and sugar beets.

Chlorfluazuron was registered as an agricultural chemical in 1989, in Japan. The trade name is "Atabron".

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grain: 0.05 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Liquid chromatography [Analytical Standards of Feeds Chapter 6, Section 1, Article 68.1]

A. Reagent preparation

1) Chlorfluazuron standard solution. Weigh accurately 20 mg of chlorfluazuron [$C_{20}H_9Cl_3F_5N_3O_3$] [1], place in a 100 mL brown volmetric flask, add acetonitrile to dissolve, further add the solvent up to the marked line to prepare the chlorfluazuron standard stock solution (1 mL of this solution contains an amount equivalent to 0.2 mg of chlorfluazuron).

At the time of use, dilute a quantity of chlorfluazuron standard stock solution accurately with acetonitrile to prepare several chlorfluazuron standard solutions containing amounts equivalent to 0.1-2 μg of chlorfluazuron in 1 mL.

2) Magnesium silicate. Dry synthetic magnesium silicate (particle size: 149-250 μm (100-60 mesh)) at 130 °C for 16 hours.

B. Quantification

Extraction. Weigh accurately 5 g of the analysis sample, place in a stoppered 200 mL Erlenmeyer flask, add 0.1 g of dibutylhydroxytoluene [2] and 5 mL of water, allow to still standing for 30 minutes, further add 100 mL of acetonitrile, and extract by shaking for 30 minutes. Place a 200 mL volmetric flask under a Büchner funnel, filter the extract through filter paper (No.5B) by suction, wash the Erlenmeyer flask and residue serially with 50 mL of acetonitrile, and filter by suction in a similar way. Further, add acetonitrile up to the marked line of the volmetric flask, place 100 mL of this solution in a 300 mL

recovery flask, condense almost into dryness under reduced pressure in a water bath at 40 °C or lower. Add 20 mL of sodium chloride saturated solution to the residue to prepare the sample solution subject to column treatment I.

Column treatment I. Place the sample solution in a porous diatomite column[3] (for 20 mL retention), allow to still standing for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask having contained the sample solution 4 times with 25 mL each of cyclohexane, add the washings serially to the column, flow out until the liquid surface reaches the top of the column packing material to elute chlorfluazuron. Further, add 50 mL of cyclohexane to the column to elute in a similar way. Condense the eluate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add 5 mL of hexane – acetone (9 : 1) to dissolve the residue to prepare the sample solution subject to column treatment II.

Column treatment II. Suspend 5 g each of sodium sulfate (anhydrous), magnesium silicate and sodium sulfate (anhydrous) in hexane – acetone (9 : 1), serially run down into a column tube (internal diameter: 15 mm), flow out until the liquid surface reaches at 3 mm from the top of the column packing material to prepare the column.

Place the sample solution in the column, wash the recovery flask having contained the sample solution 2 times with 5 mL each of hexane – acetone (9 : 1), serially add the washings to the column, and flow out until the liquid surface reaches at 3 mm from the top of the column packing material. Further add 35 mL of hexane – acetone (9 : 1) to the column, and wash the column. Then, place a 200 mL recovery flask under the column, and add 100 mL of hexane – acetone (7 : 3) to elute chlorfluazuron. Condense the eluate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add 3 mL of hexane – diethylether (17 : 3) to dissolve the residue to prepare the sample solution subject to column treatment III.

Column treatment III. Wash a silica gel minicolumn (690 mg) with 10 mL of hexane – diethyl ether (17 : 3).

Place the sample solution in the minicolumn, wash the recovery flask having contained the sample solution twice with 3 mL each of hexane – diethyl ether (17 : 3), serially add the washings to the minicolumn, and flow out. Place a 50 mL recovery flask under the minicolumn, add 10 mL of hexane – diethyl ether (7 : 3) to the minicolumn to elute chlorfluazuron, condense the eluate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add 2.5 mL of acetonitrile accurately to dissolve the residue, and filter with membrane filter (pore size: 0.5 μm or less) to prepare the sample solution subject to liquid chromatograpy.

Liquid chromatography. Inject 20 μL each of the sample solution and respective chlorfluazuron standard solutions into a liquid chromatograph to obtain the chromatogram.

Example of measurement conditions

Detector: Ultraviolet spectrophotometer (wave length : 260 nm)

Column: Octadecylsilylated silicagel column (internal diameter: 4.6 mm, length: 250 mm, particle

size: 5 μm)*1 [4]

Eluent: Acetonitrile – water (4 : 1)

Flow rate: 1 mL/min

Calculation. Calculate the peak height and peak area from the obtained chromatogram[5] to prepare a calibration curve to determine the amount of chlorfluazuron in the sample.

* 1. Mightysil RP18 (Kanto Chemical) or an equivalent.

«Summary of analysis method»

This method is intended to determine the amount of chlorfluazuron in the sample by extracting with hydrous acetonitrile, purifying with a porous diatomite column, synthetic magnesium silicate column and silica gel minicolumn, and measuring with a liquid chromatograph attached with an ultraviolet spectrophotometer.

The flow sheet of analysis method is shown in Figure 6.1.68-1.

Sample (5 g)
- Add 5 mL of water and 0.1 g of HT 0.1 g, and allow to still standing for 30 min.
- Add 100 mL of scetonitrile and stir for 30 min.
- Filter under vacuum (No. 5B) .
- Wash with 50 mL of acetonitrile.
- Add acetonitrile up to a total volume of 200 mL.

Extract (100 mL)
- Condense under vacuum and dry with nitrogen gas..
- Add 20 mL of saturated sodium chloride solution.

Chem Elut cartridge (for 20 mL retention)
- Allow to still standing for 5 min.
- Wash with 150 mL of cyclohexane to elute.
- Condense under reduced pressure, and evaporate into dryness with nitrogen gas.
- Add 5 mL of hexane-acetone (9 : 1).

Synthetic magnesium silicate column (wet-filling with hexane-acetone (9 : 1))
- Wash with 5 mL of hexane-acetone (9 : 1).
- Elute with 3 mL of hexane-acetone (7 : 3).
- Condense under reduced pressure, and evaporate into dryness with nitrogen gas.
- Add 3 mL of hexane-diethylether (7 : 3).

Sep-Pak Plus Silica cartridge (previously wash with hexane-diethylether (7 : 3))
- Wash with 6 mL of hexane-diethylether (17 : 3).
- Elute with 10 mL of hexane-diethylether (17 : 3).
- Condense under reduced pressure, and evaporate into dryness with nitrogen gas.
- Add 2.5 mL of acetonitrile

LC-UV (260 nm)

Figure 6.1.68-1 Flow sheet of analysis method of chlorfluazuron

References: Shigetaka Suzuki, Wakana Sekiguchi, Yuji Shirai: Research Report of Animal Feed, 21, 23 (1996)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Pig formula feed	200-2,000	3	88.3-95.7	18.8
Alfalfa	200-2,000	3	78.7-86.0	11.3
Cotton seed	200-2000	3	82.7-91.3	10.0

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Pig formula feed	7	1,000	87.7	2.3	4.8	0.28

«Notes and precautions»

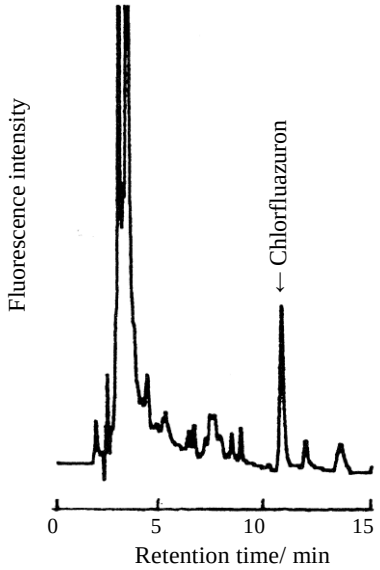
[1] The standard preparation is commercially available from Wako Pure Chemical Industries, Kanto Chemical and HAYASHI PURE CHEMICAL.

[2] This is BHT used as a food additive. Add BHF for quantification of chlorfluazuron in cotton seeds, if not, chlorfluazuron possibly can not be recovered.

[3] This is a method to rapidly perform liquid-liquid distribution. The sample solution (aqueous solution) injected in the cartridge adsorbs in thin membrane to the surface of porous diatom earth. By adding organic solvent nonmiscible to water, the target substance is extracted from the water layer by flowing solvent. This method offers an advantage of high extraction efficiency without trouble by emulsion.

[4] Any column is applicable as long as its endocapped packing material meets the requirements. Additionally, the column used in the validation of this analysis method was Mightysil RP18.

[5] An example of chromatogram is shown in Figure6.1.68-2.



Measurement conditions:

Detector: Ultraviolet absorptiometer (measured wavelength: 260 nm)

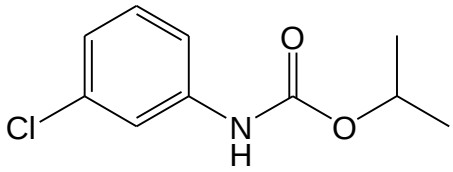
Column: Kanto Chemical Mightysil RP18 (internal diameter: 4.6 mm, length: 250 mm, particle size: 5 μm)

Eluent: Acetonitril – water (4 : 1)

Flow rate: 1 mL/min

Figure 6.1.68-2 Chromatogram for chlorfluazuron added to formula feed in an amount equivalent to 0.2 mg/kg

69 Chlorpropham (IPC)



Isopropyl 3-chlorocarbanilate

$C_{10}H_{12}ClNO_2$ MW: 213.7 CAS No.: 101-21-3

[Summary of chlorpropham]

Chlorpropham is a carbamate herbicide, non-transitional hormone type soil treatment agent. It more strongly act to bent grass as compared to broad leafweed. Chlorpropham is absorbed from radicles of plants just after budding and cause abnormal cell divisions, respiration disorder, etc.

The crystal is solid, poorly soluble in water, readily soluble in general organic solvent, and degraded in acidic and alkaline conditions.

Chlorpropham was registered as an agricultural chemical in 1954, in Japan. Registered name is IPC. The trade names are “Chloro IPC”, or others.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Barley, wheat, corn and rye: 0.05 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for alachlor, allethrin, chlorpropham, dichloran and methoxychlor by gas chromatography

Target Analytes:

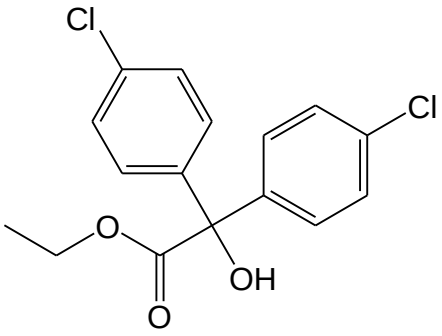
Group A: Alachlor, allethrin and dichloran (3 compounds)

Group B: Methoxychlor (1 compound)

Group C: Chlorpropham (1 compound)

Refer to Article 5, Section 2 in this chapter.

70 Chlorobenzilate



Ethyl 4,4'-dichlorobenzilate

$C_{16}H_{14}Cl_2O_3$ MW: 325.2 CAS No.: 510-15-6

[Summary of chlorobenzilate]

Chlorobenzilate is an organic chlorine miticide, DDT related chemical. Chlorobenzilate has effects on eggs, larvae and imagoes of a wide variety of red mite, especially on citrus rust mites.

Chlorobenzilate is light yellow solid of which solubility to water is 10 ppm, and readily soluble in organic solvents.

Chlorobenzilate was registered as an agricultural chemical in 1955, in Japan. The trade name is "Akar".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Corn: 0.02 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

3. Gas chromatography [Analytical Standards of Feeds Chapter 6, Section 1, Article 70.3]

A. Reagent preparation

1) Chlorobenzilate standard solution. Weigh accurately 10 mg of chlorobenzilate [$C_{16}H_{14}Cl_{12}O_3$][1], place in a 100 mL brown volmetric flask, add 2,2,4-trimethylpentane – acetone (4 : 1) to dissolve, further add the solvent up to the marked line to prepare the chlorobenzilate standard stock solution (1 mL of this solution contains an amount equivalent to 0.1 mg of chlorobenzilate).

At the time of use, dilute accurately a quantity of the standard stock solution with 2,2,4-trimethylpentane – acetone (4 : 1) to prepare several chlorobenzilate standard solutions containing amounts equivalent to 0.1-2 μg of chlorobenzilate in 1 mL.

2) Magnesium silicate. Dry synthetic magnesium silicate (particle size: 149-250 μm (100-60 mesh)) at 130 °C for 5 hours, allow to be cool, add water equivalent to 5 v/w% while mixing, and allow to still standing overnight (prepare at the time of use).

B. Quantification

Extraction. Weigh 10.0 g of the analysis sample, place in a stoppered 200 mL Erlenmeyer flask, add 30 mL of water to moisten, allow to still standing for 30 minutes, further add 70 mL of acetonitrile, and stir for 30 minutes to extract. Place a 200 mL volmetric flask under a Büchner funnel, filter through filter paper (No.5B) by suction, wash the Erlenmeyer flask and residue serially with 50 mL of acetonitrile – water (7 : 3), and filter by suction in a similar way. Further add acetonitrile – water (7 : 3) up to the marked line of the volmetric flask to prepare the sample solution subject to purification.

Purification. Add 100 mL of the sample solution to the 500 mL separating funnel A having received 250 mL of sodium chloride solution (5 w/v%) and 50 mL of hexane. Stir the separating funnel A vigorously for 5 minutes, allow to still standing, transfer the water layer (lower layer) to the 500 mL separating funnel B, and the hexane layer (upper layer) in a 200 mL Erlenmeyer flask. Add 50 mL of hexane to the separating funnel B, stir gently, allow to still standing, and transfer the hexane layer to the Erlenmeyer flask. Dehydrate the hexane layer with appropriate quantity of sodium sulfate (anhydrous), filter through separating filter into a 300 mL recovery flask, serially wash the Erlenmeyer flask and the filter paper with a small amount of hexane, and add the washing liquid to the filtrate through the filter paper. Condense the filtrate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Transfer the residue to the 100 mL separating funnel C with 30 mL of hexane, and further add 30 mL of acetonitrile – water (100 : 1). Stir the separating funnel C vigorously for 5 minutes, allow to still standing, and transfer the acetonitrile layer (lower layer) to the 200 mL separating funnel D. Add 30 mL of acetonitrile – water (100 : 1) to the separating funnel C, process in a similar way, and add the acetonitrile layer to the separating funnel D. Add 30 mL of hexane to the separating funnel D, stir vigorously for 5 minutes, allow to still standing, and transfer the acetonitrile layer to a 300 mL recoverty flask. Condense the acetonitrile layer almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add 5 mL of hexane to dissolve the residue to prepare the sample solution subject to column treatment.

Column treatment. Suspend 10 g of magnesium silicate and 2 g of sodium sulfate (anhydrous) in hexane,

respectively, serially run down into a column tube (internal diameter: 15 mm), and flow out until the liquid surface reaches at 3 mm from the top of the column packing material to prepare the column.

Place the sample solution in the column, wash the recovery flask having contained the sample solution 5 times with 5 mL each of hexane, serially add the washing liquid to the column, and flow out until the liquid surface reach at 3 mm from the top of the column packing material. Add 100 mL of hexane – diethyl ether (19 : 1) to the column, and flow out in a similar way. Place a 300 mL recovery flask under the column, and add 100 mL of hexane – diethyl ether (4 : 1) to the column to elute chlorobenzilate. Condense the eluate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add 5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately to dissolve the residue to prepare the sample solution subject to gas chromatography.

Gas chromatography. Inject 1 µL each of the sample solution and respective chlorobenzilate standard solutions into a gas chromatograph to obtain the chromatogram.

Example of measurement conditions

Detector: Electron capture detector

Column: Fused silica capillary column (14 % cyanopropylmethyl-86 % dimethylpolysiloxane coating, internal diameter: 0.53 mm, length: 30 m, film thickness: 0.50 µm)[2]

Carrier gas: He (5 mL/min)

Makeup gas: N_2 (60 mL/min)

Sample injection: Splitless (60 s)

Injection port temperature: 260 °C

Column oven temperature: Initial temperature 60 °C (hold 1 min) → ramp 20 °C/min → 200 °C → ramp 2 °C/min → 260 °C (hold 2 min)

Detector temperature: 300 °C

Calculation. Calculate the peak height or peak area from the obtained chromatogram[3] to prepare a calibration curve, and determine the amount of chlorobenzilate in the sample.

«Summary of analysis method»

This method is intended to determine the amount of chlorobenzilate in feed by extracting with hydrous acetonitrile, purifying with changing solvent with hexane, liquid-liquid distribution and a synthetic magnesium silicate column, and by quantifying with a gas chromatograph attached with an electron capture detector.

References: Yutaka Kunugi, Yukie Ishida: Research Report of Animal Feed, 21, 33 (1996)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Adult chicken formula feed	200-2,000	3	84.7-102.3	6.1
Growing pig formula feed	200-2,000	3	92.7-100.0	10.8
Timothy	200-2,000	3	92.0-101.3	13.2

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD^r (%)	Inter-lab reproducibility RSD^R (%)	HorRat
Adult chicken formula feed	7	1,000	95.0	2.1	5.8	0.36

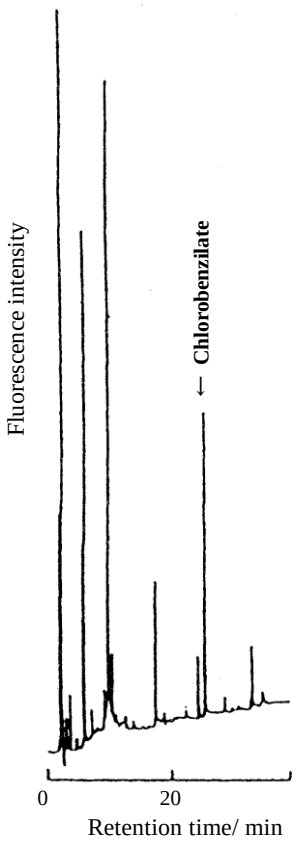
«Notes and precautions»

[1] The standard preparation is commercially available from Wako Pure Chemical Industries, Kanto Chemical, HAYASHI PURE CHEMICAL, GL Science or others.

[2] DB-1701 (Agilent Technologies) is available.

Since chlorobenzilate has hydroxyl in its structure, the peak shows tailing in regular type columns; therefore, a wide bore type column is preferable.

[3] An example of chromatogram is shown in Figure 6.1.70-1.



Measurement conditions:

Detector: Electron capture detector (ECD)

Column: Supelco SPB-1701 (internal diameter: 0.53 mm, length: 30 m, film thickness: 0.50 μm)

Carrier gas: He (initial flow rate: 5 mL/min)

Makeup gas: N_2 (60 mL/min)

Sample injection: Splitless

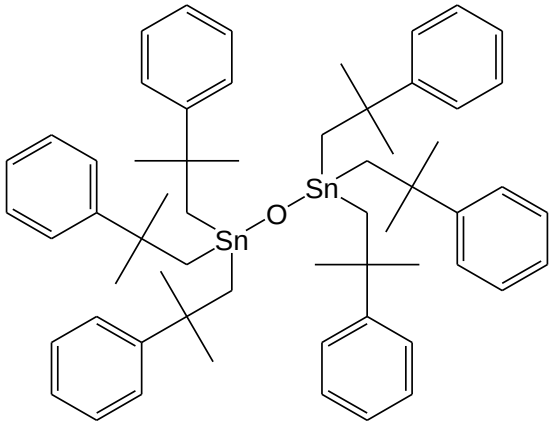
Injection port temperature: 260 °C

Column oven temperature: initial temperature 60 °C (hold 1 min) → ramp 20 °C/min → 200 °C → ramp 2°C/min → 260 °C (hold 2 min)

Detector temperature: 300 °C

Figure 6.1.70-1 Chromatogram for chlorobenzilate added to formula feed in an amount equivalent to 1 mg/kg

71　Fenbutatin oxide



Bis[tris(2-methyl-2-phenylpropyl)tin] oxide

$C_{60}H_{78}OSn_2$　MW: 1052.68　CAS No.: 13356-08-6

[Summary of fenbutatin oxide]

Fenbutatin oxide is an organic tin miticide developed by Shell (USA), said to block the reaction of energy generation system. Especially, it has effects on larvae and imagoes just after molting, said to be rather slow-acting, but have high residual efficacy.

Fenbutatin oxide was registered as an agricultural chemical in 1986, in Japan. The trade name is "Osadan".

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grain: 0.05 ppm

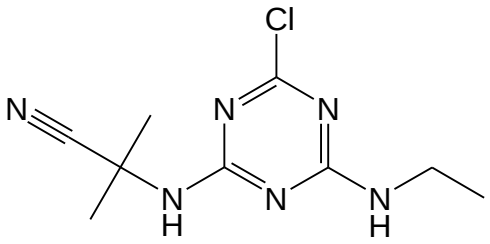
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for fenbutatin oxide and cyhexatin by gas chromatography

Target Analytes: Fenbutatin oxide and cyhexatin (2 compounds)

Refer to Article 13, Section 3 in this chapter.

72 Cyanazine



2-(4-chloro-6-ethylamino-1,3,5-triazin-2-ylamino)-2-methylpropiononitrile

$C_9H_{13}ClN_6$ MW: 240.7 CAS No.: 21725-46-2

[Summary of cyanazine]

Cyanazine is a triazine herbicide developed by Shell (USA), and used in dry field.

Cyanazine was registered as an agricultural chemical in 1983, in Japan.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Oat, milo, rye and pasture grass: 0.01 ppm, barley: 0.05 ppm, wheat and corn: 0.1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for ametryn, cyanazine and prometryn by liquid chromatograph-mass spectrometer

Target Analytes: Ametryn, cyanazine and prometryn (3 compounds)

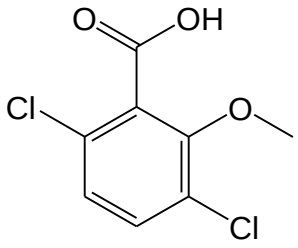
Refer to Article 11, Section 3 in this chapter.

2. Simultaneous analysis method for cyanazine and myclobutanil by gas chromatograph-mass spectrometer

Target Analytes: Cyanazine and myclobutanil (2 compounds)

Refer to Article 14, Section 3 in this chapter.

73 Dicamba (MDBA)



3,6-dichloro-*o*-anisic acid

$C_8H_6Cl_2O_3$ MW: 221.0 CAS No.: 1918-00-9

Dicamba-isopropylammonium CAS No.: 55871-02-8

Dicamba-dimethylammonium CAS No.: 2300-66-5

Dicamba-potassium CAS No.: 10007-85-9

Dicamba-sodiium CAS No.: 1982-69-0

[Summary of dicamba]

Dicamba is a hormone type selective aromatic carboxylic acid herbicide developed by Velsicol (USA). Dicamba has effects on broad leaf plants without regard to annual or perennial, although has no effects on gramineous plant.

Dicamba was registered as an agricultural chemical in 1965 (dimethylamine salt), 1981 (dicamba), 1993 (sodium salt) and 1998 (isopropylamine salt), in Japan. The registered name is MDBA (as dicamba and dimethylamine salt), MDBA sodium salt and MDBA isopropylamine salt. After then, MDBA sodium salt and MDBA isopropylamine salt fell into abeyance in 2005. The trade names are "MDBA" or others.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(Dicamba, dicamba isopropylamine salt, dicamba dimethylamine salt, dicamba potassium salt and dicamba sodium salt are included)

Rye: 0.1 ppm, barley, wheat and corn: 0.5 ppm, oat and milo: 3 ppm, and pasture grass: 200 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Analysis method for dicamba by gas chromatograph-mass spectrometer [Analytical Standards of Feeds Chapter 6, Section 1, Article 73.1]

A. Reagent preparation

Dicamba standard solution. Weigh accurately 25 mg of dicamba [$C_8H_6Cl_2O_3$], place in a 50 mL brown volmetric flask, add acetone to dissolve, further add the solvent up to the marked line (1 mL of this solution contains an amount equivalent to 0.5 mg of dicamba).

At the time of use, dilute a quantity of this solution with acetone to prepare the dicamba standard solution containing an amount equivalent to 5.0 μg/mL of dicamba.

B. Quantification

Extraction. Weigh 20.0 g of analysis sample (10.0 g for hay), place in a stoppered 200 mL brown Erlenmeyer flask, add 30 mL of water and 1 mL of hydrochloric acid (6 mol/L) to moisten, allow to still standing for 30 minutes, further add 70 mL of acetone, and shake for 30 minutes to extract[*1]. Place a 200 mL volmetric flask under a Büchner funnel, filter the extract through filter paper (No. 5B) by suction, serially wash the Erlenmeyer flask and residue with 50 mL of acetone, and filter by suction in a similar way. Further add acetone up to the marked line[1] to prepare the sample solution subject to hydrolysis.

Hydrolysis. Place accurately 20 mL of the sample solution (when the sample is hay, the amount is 40 mL) in a 100 mL recovery flask, and condense to approximately 5 mL under reduced pressure in a water bath at 40 °C or lower. Add 5 mL of sodium hydrate solution (1 mol/L) to the condensed solution, and allow standing at room temperature for 30 minutes, while gently stirring in intervals to prepare the sample solution subject to column treatment I.

Column treatment I. Add 2 mL of hydrochloric acid (6 mol/L) to the sample solution, confirm that pH of the solution is 1.0 or lower with a pH test paper[2], place this solution in a porous diatomite column (for 20 mL retention), and allow to still standing for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask containing the sample solution 3 times with 10 mL each of ethyl acetate, add the washings serially to the column and naturally flow down until the liquid surface reaches the top of the column packing material to elute dicamba. Further add 70 mL of ethyl acetate to the column and elute in a similar way. Condense the eluate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas[3].

Transfer the residue to a 10 mL volmetric flask with 2 mL of cyclohexane – ethyl acetate (4 : 1), wash the recovery flask having contained the residue 3 times with 2 mL each of cyclohexane – ethyl acetate (4 : 1), and add the washings to the volmetric flask. Further add cyclohexane – ethyl acetate (4 : 1) up to the marked line of the volmetric flask, and filter with membrane filter (pore size: 0.5 μm or less) to prepare the sample solution subject to gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into a gel permeation chromatograph, take the fraction containing dicamba into a 100 mL pear-shaped flask, condense to approximately 4 mL under reduced pressure in a water bath at 40 °C or lower, and transfer in a 20 mL test tube. Wash the pear-shaped flask 3 times with 2 mL each of acetone, and add the washings to the test tube. Dry up this solution by the flow of nitrogen gas[4] in a water bath at 50 °C or lower to obtain the residue subject to trifluoroethylesterification.

Example of operating conditions

Column: Styrene – divinylbenzene copolymer column[5] (internal diameter: 20 mm, length: 300 mm, particle size: 15 μm)

Guard column: Styrene – divinylbenzene copolymer column[5] (internal diameter: 20 mm, length: 100 mm, particle size: 15 μm)

Eluent: Cyclohexane – ethyl acetate (4 : 1)

Flow rate: 5 mL/min

Sampling fraction: 75-125 mL[6]

Trifluoroethylesterification. Add 1 mL of 2,2,2-trifluoroethanol and 0.2 mL of sulfuric acid to the residue, air-tightly stopper, and allow to still standing in a water bath[7] at 90 °C for 30 minutes while shaking at intervals to obtain the sample solution subject to changing solvents.

Simultaneously, place accurately 1 mL of dicamba standard solution in another 20 mL test tube, dry by introducing nitrogen gas, and perform trifluoroethylesterification in a similar way to derivatize dicamba to trifluoroethylesterified substance to obtain the standard solution subject to changing solvents.

Changing solvents. Add 10 mL of sodium chloride solution (5 w/v%) and 5 mL of hexane to the sample solution, shake for 5 minutes, allow to still standing, and take the hexane layer (upper layer) with a Pasteur pipette into a 50 mL Erlenmeyer flask. Further add 5 mL of hexane to the test tube and process in a similar way. Add the hexane layer to the Erlenmeyer flask to obtain the sample solution subject to column treatment II.

Simultaneously, treat the standard solution (derivatized) in a way similar to sample solution, and filter the hexane layer into 50 mL pear-shaped flask with a funnel having stuffed with absorbent cotton and holding 5 g of sodium sulfate (anhydrous). Wash the tube having contained the standard solution (derivatized) twice with 2 mL each of hexane, add the washings to the filtrate through the said funnel, further wash the said sodium sulfate twice with 2 mL each of hexane, and add the washings to the filtrate. Condense the filtrate to approximately 1 mL under reduced pressure in a water bath at 40 °C or lower, transfer the residue to a 5 mL volmetric flask, wash the pear-shaped flask having contained the residue 3 times with 1 mL each of hexane, and add the washings to the volmetric flask. Further add hexane up to marked line of the volmetric flask to prepare the derivatized standard stock solution containing an amount equivalent to 1.0 µg of dicamba in 1 mL. Dilute this derivatized standard stock solution accurately with hexane to prepare several derivatized standard solutions containing amounts equivalent to 0.01-0.5 µg of dicamba in 1 mL.

Column treatment II. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane. Filter[9] the sample solution through the funnel of minicolumn having stuffed with absorbent cotton and containing 5 g of sodium sulfate (anhydrous)[8]. Wash the Erlenmeyer flask having contained the sample solution twice with 2 mL each of hexane, add the washings to the minicolumn through the funnel, further wash the sodium sulfate twice with 2 mL each of hexane, add the washings to the minicolumn, naturally flow out until the liquid surface reaches the top of the column packing material, and take off the funnel. Place a 50 mL pear-shaped flask under the minicolumn, add 10 mL of hexane – diethyl ether (24 : 1) to the minicolumn, and naturally flow down to elute the trifluoroethylesterificated substance of dicamba. Condense the eluate to approximately 1 mL under reduced pressure[10] in a water bath at 40 °C or lower, place in a 5 mL volmetric flask, wash the pear-shaped flask having contained the eluate 3 times with 1 mL each of hexane, and add the washings to the said volmetric flask. Further add hexane up to the marked line of the volmetric flask to prepare the sample solution subject to measurement by gas chromatograph-mass spectrometer.

Measurement by gas chromatograph-mass spectrometer. Inject 2 µL each of the sample solution and respective derivatized standard solutions into a gas chromatograph mass spectrometer to obtain the selected ion monitoring chromatogram.

Example of measurement conditions

Column: Fused silica capillarycolumn (5 % diphenyl/ 95 % dimethyl-polysiloxane coating, internal diameter: 0.25 mm, length: 30 m, film thickness: 0.25 μm)[11]

Carrier gas: He (1.0 mL/min)

Sample injection: Splitless (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 2 min) → ramp 5 °C/min → 180 °C → ramp 15 °C/min → 280 °C (hold 5 min)

Detector: Quadrupole mass spectrometer*2

Iinterface： 280 °C

Ion source temperature: 200 °C

Ionizing voltage: 70 eV

Ionizing method: Electron bombardment ionization (EI) method

Monitor ion: *m*/*z* 302

Calculation. Calculate the peak area from the obtained selected ion monitoring chromatogram[12] to prepare a calibration curve, and determine the amount of dicamba in the sample.

* 1. When the amount of dicamba in the sample is large, perform the subsequent operations after diluting the extract with acetone.

2. An example of conditions by GCMS-QP2010 (Shimazu Corporation)

«Summary of analysis method»

This method is intended to determine the amount of dicamba and its salts in feed by extracting with acidic acetone, hydrolyzing dicamba salts, purifying with a porous diatomite column and GPC, esterifying, further purifying with a Florisil minicolumn, and quantifying with a gas chromatograph mass spectrometer.

The flow sheet of the analysis method is shown in Figure 6.1.73-1.

20 g of sample (10 g for hay)
- Add 30 mL of water and 1 mL of hydrochloric acid (6 mol/L), and allow to still standing for 30 min.
- Add 70 mL of acetone, and shake for 30 min.
- Filter under vacuum, wash with 50 mL of acetone, and fix the volume with acetone at 200 mL.

Hydrolysis (20 mL of sample solution (40 mL for hay))
- Condense under reduced pressure (to 5 mL).
- Add 5 mL of sodium hydrate solution (1 mol/L), and allow to still staning for 30 min.
- Add 2 mL of hydrochloric acid (6 mol/L).

Chem Elut cartridge (for 20 mL retention)
- Load sample solution, and allow to still standing for 5 min.
- Elute with 100 mL of ethyl acetate.
- Condense under reduced pressure, and evaporate into dryness with nitrogen gas.
- Fix the volume with cyclohexane-ethyl acetate (4 : 1) at 10 mL.
- Filter with membrane filter.

GPC (inject 5 mL of sample solution)
- Collect 75-125 mL of fraction.
- Condense under reduced pressure (to 4 mL), and transfer to a 20 mL test tube.
- Evaporate into dryness with nitrogen gas (in water bath at 50 °C).

Esterification
- Add 1 mL of 2,2,2-trifluoroethanol and 0.2 mL of sulfuric acid.
- Reacting at 90 °C for 30 min.
- Add 10 mL of sodium chloride (5 w/v%)and 5 mL of hexane.
- Shake for 5 min, and dehydrate the hexane layer (upper layer) with sodium sulfate (anhydrous).

Sep-pak Plus Florisil cartridge (readily washed with 5 mL of hexane)
- Load with sample solution.
- Elute with 10 mL of hexane-diethylether (24:1).
- Condense under vacuum (to 1 mL), and fix the volume at 5 mL with hexane.

GC-MS

Figure 6.1.73-1 Flow sheet of analysis method for dicamba by gas chromatograph-mass spectrometer

References: Mitsunori Yakata, Takako Nagahara: Research Report of Animal Feed, 31, 65 (2006)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Chicken formula feed	50-500	3	88.8-89.3	10.6
Cattle formula feed	50-500	3	98.5-100.2	12.5
Timothy	50-500	3	85.7-101.3	9.0
Rice straw	50-500	3	92.3-104.3	8.6
Fescue	200 mg/kg	3	87.8	3.5
Rye grass	200 mg/kg	3	82.8	4.1

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Fescue	6	200 mg/kg	84.6	7.5	13.6	1.85
Corn	6	500 μg/kg	88.7	4.2	8.5	0.47

• Lower quantification limit: 10 μg/kg (spike recovery and standard deviation)

• Lower detection limit: 3 μg/kg (spike recovery and standard deviation)

«Notes and precautions»

[1] When allow the extract to still standing for long time, dicamba possibly precipitate together with lipid in the extract and adhere to the bottom of the volmetric flask as dried solid. After fixing the volume in the volmetric flask, rapidly take the sample solution subject to hydrolysis.

[2] Since pH in almost all feeds becomes 1.0 or lower by adding 2 mL of hydrochloric acid (6 mol/L), pH is confirmed simply with pH test papers.

[3] Although drying to solid with nitrogen gas may be impossible due to a lot of remaining lipid in the residue of hydrochloric acid acidified extract after condensing under reduced pressure, there is no matters so far as organic solvents is removed.

[4] It can afford to blow nitrogen gas somewhat strongly within the extent not scatter the sample solution in the test tube.

[5] A column filled with styrene divinylbenzene copolymer hard gel by using the eluent. The column and guard column prescribed in Analytical Standards of Feeds Appendix-2 are SHOWA DENKO Shodex CLNpak EV-2000 AC and Shodex CLNpak EV-G AC, respectively.

[6] Eluted fraction may vary by lots, frequencies of use, etc.; therefore, prior confirmation in each laboratory is necessary.

[7] It can afford to derivatize by using a thermostat, etc. so far as the temperature can be maintained; however, pay adequate attention to keep airtight state in the test tube.

[8] It can afford to make an adjustment to amount of sodium sulfate (anhydrous) by condition of the hexane layer collected with a Pasteur pipette.

[9] Place the funnel on the reserver connected to the minicolumn so that the sample solution directly run into the reserver.

[10] Do not evaporate dicamba trifluoroethylester-derivatized substances into dryness, because they are largely lost by drying. Further, quickly perform the process after derivatization, because trifluoroethylester-derivatized substances are lost by still standing in open air for a long time.

[11] The capillary column used in validation of this method was Agilent Technologies HP-5MS.

[12] An example of selected ion monitoring (SIM) chromatogram is shown in Figure 6.1.73-2.

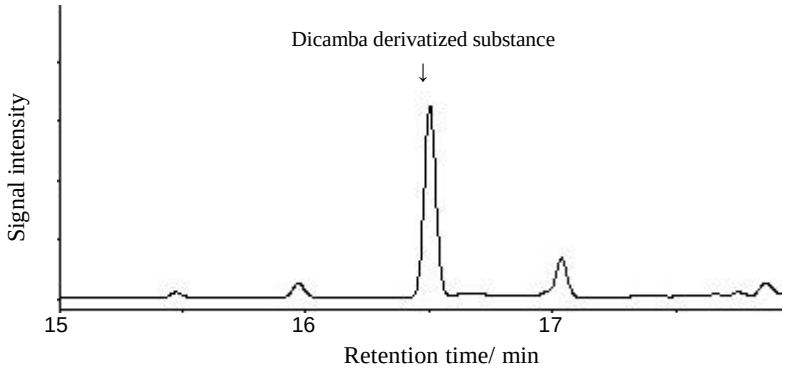
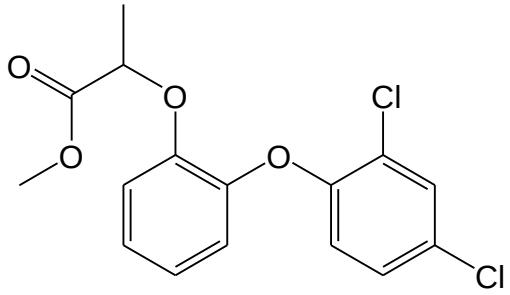


Figure 6.1.73-2　SIM chromatogram of dicamba
added to starting chick formula feed in an amount equivalent to 500 μg/kg

74 Diclofop-methyl



Methyl (*RS*)-2-[4-(2,4-dichlorophenoxy)phenoxy]propionate

$C_{16}H_{14}Cl_2O_4$ MW: 341.2 CAS No.: 51338-27-3

[Summary of diclofop-methyl]

Diclofop-methyl is an allyloxyphenoxypropionic acid herbicide.

Diclofop-methyl is not registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grain: 0.1 ppm

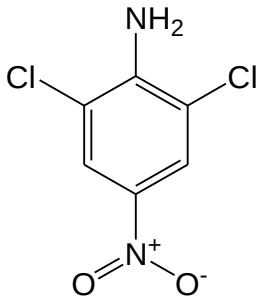
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

75 Dichloran (CAN)



2,6-dichloro-4-nitroaniline

$C_6H_4Cl_2N_2O_2$ MW: 207.0 CAS No.: 99-30-9

[Summary of dichloran]

Dichloran had been used as an intermediate of synthetic dye, and its effect as a insecticide was recognized in 1957. Dichloran is an aromatic bactericide having selective effects, preventively as well as therapeutically, on crown rot in vegetables including lettuce, strongly inhibiting growth of fungal filaments. Dichloran is one of post-harvest pesticides in USA.

Dichloran is yellow crystal, insoluble in water, and readily soluble in polar organic solvents.

Dichloran had been registered as an agricultural chemical in 1964, in Japan. Registered name had been CAN. However, it was expired in 1994.

«Standard value defined by Food Sanitation Law»

0.01 ppm (uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

3. Systematic analysis methods for alachlor, allethrin, chlorpropham, dichloran and methoxychlor by gas chromatography

Target Analytes:

Group A: Alachlor, allethrin and dichloran (3 compounds)

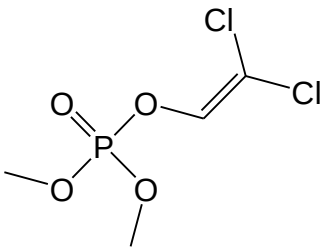
Group B: Methoxychlor (1 compound)

Group C: Chlorpropham (1 compound)

Refer to Article 5, Section 2 in this chapter.

76 Dichlorvos (Dichlorvos (DDVP) and Naled (BRP))

Dichlorvos

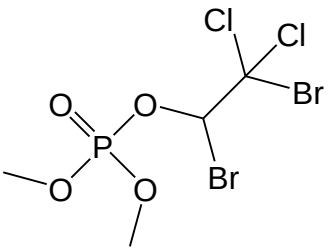


2,2-dichlorovinyl dimethyl phosphate

$C_4H_7Cl_2O_4P$ MW: 221.0

CAS No.: 62-73-7

Naled



(*RS*)-1,2-dibromo-2,2-dichloroethyl dimethyl phosphate

$C_4H_7Br_2Cl_2O_4P$ MW: 380.8

CAS No.: 300-76-5

[Summary of dichlorvos and naled]

Dichlorvos is an organic phosphorus insecticide with short-lasting residual efficacy, which is used for preventing pests until just before harvesting.

Naled is an organic phosphorus insecticide used for preventing pests. The activity is due to dichlorvos generated from naled by leaving of bromine atom.

Dichlorvos was registered as an agricultural chemical in 1957, in Japan. Registered name is DDVP. Naled was registered as an agricultural chemical in 1961, in Japan. Registered name is BRP.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(Sum of the amount of dichlorvos and amount of naled converted to that of dichlorvos)

Oat, barley, wheat, corn, milo and rye: 0.2 ppm, and pasture grass: 10 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Analysis method for dichlorvos and naled by gas chromatograph-mass spectrometer [Analytical Standards of Feeds Chapter 6, Section 1, Article 76.1][*1, 2]

A. Reagent preparation

1) Dichlorvos standard solution. Weigh accurately 25 mg of dichlorvos [$C_4H_7Cl_2O_4P$][1], place in a 50 mL brown volmetric flask, add acetone to dissolve, further add acetone up to the marked line to prepare the dichlorvos standard stock solution (1 mL of this solution contains an amount equivalent to 0.5 mg of dichlorvos).

At the time of use, dilute a quantity of standard stock solution accurately with acetone, to prepare several dichlorvos standard solutions containing amounts equivalent to 0.01-2 μg of dichlorvos in 1 mL.

2) Phosphoric acid buffer solution. Add a solution of 17.9 g disodium hydrogenphosphate 12-water/ 500 mL water to 230 mL of solution of 7.8 g sodium dihydrogenphosphate dehydrate/500 mL water, and adjust the pH at 7.2 with 2 mol/L sodium hydrate solution.

3) Cysteine solution[*3]. Dissolve 4 g of L-cysteine hydrochloride salt monohydrate in 50 mL of water, and adjust the pH at 7.0 with 2 mol/L sodium hydrate solution.

B. Quantification

Extraction. Weigh 10.0 g of the analysis sample, place in a stoppered 200 mL brown Erlenmeyer flask, add 15 mL of 1 mol/L hydrochloric acid, and allow to still standing for 15 minutes. Add 50 mL of acetone (150 mL for the sample of hay), and shake for 30 minutes to extract. Place a 100 mL brown volmetric flask (200 mL brown volmetric flask for the sample of hay) under a Büchner funnel, filter the extract through filter paper (No.5B) by suction, wash the Erlenmeyer flask and residue serially with 30 mL of acetone, and filter by suction in a similar way. Further add acetone up to the marked line of the brown volmetric flask. Place 20 mL of extract (4 mL for the sample of hay) accurately in a 100 mL recovery flask, and condense to 2 mL or less under reduced pressure in a water bath at 40 °C or lower to prepare the sample solution subject to column treatment I.

Column treatment I. Place the sample solution in a porous diatomite column (for 20 mL retention), wash the recovery flask having contained the sample solution with 5 mL of water, add the washing to the column, and allow to still standing for 10 minutes. Place a 200 mL recovery flask under the column, wash the recovery flask 3 times with 10 mL each of hexane, serially add the washings to the column, flow down until the liquid surface reaches the top of the column packing material to elute dichlorvos and naled. Add 50 mL of hexane to the column, elute in a similar way, and condense the eluate to 40 mL or less under reduced pressure in a water bath at 40 °C or lower to prepare the sample solution subject to the transformation to dichlorvos.

Transformation to dichlorvos. Place the sample solution in the 200 mL separating funnel A, wash the recovery flask having contained the sample solution with 30 mL of phosphoric acid buffer solution, and add the washings to the separating funnel A. Add 4 mL of cysteine solution and 5 g of sodium chloride to the separating funnel A, and shake for 5 minutes to transform naled to dichlorvos. Place the water layer (lower layer) in the 200 mL separating funnel B, and the hexane layer in a 200 mL Erlenmeyer flask. Add 40 mL of hexane to the separating funnel B, shake for 5 minutes, allow to still standing, and discard the water layer, while add the hexane layer to the Erlenmeyer flask. Add an appropriate amount of sodium sulfate (anhydrous) to the Erlenmeyer flask to dehydrate the hexane layer, and filter through filter paper (No.5B) into a 200 mL recovery flask. Wash the Erlenmeyer flask with a small amount of hexane, and add the washings to the filtrate through the filter paper. Add 0.5 mL of acetone – diethylene glycol (49 : 1), condense to 1 mL or less under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas[*4]. Add 5 mL of hexane – diethyl ether (17 : 3) to prepare the sample solution subject to column treatment II.

Column treatment II. Wash a silica gel minicolumn (690 mg)[4] with 5 mL of hexane – diethyl ether (17 : 3). Place the sample solution in a column, and flow out until the liquid surface reaches the top of the column packing material[*5]. Wash the recovery flask having contained the sample solution 3 times with 5 mL each of hexane – diethyl ether (17 : 3), add the washings serially to the column, and flow out in a similar way. Place a 50 mL recovery flask under the column, and add 20 mL of hexane – acetone (19 : 1) to the column to elute dichlorvos.

Add 0.5 mL of acetone – diethylene glycol (49 : 1) to the eluate, condense to 1 mL or less under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas[*4].

Add accurately 2 mL of acetone to dissolve the residue to prepare the sample solution subject to measurement by gas chromatograph-mass spectrometer.

Measurement by gas chromatograph-mass spectrometer. Inject 1 µL each of the sample solution and respective dichlorvos standard solutions into a gas chromatograph-mass spectrometer to obtain a selected ion monitoring chromatogram.

Example of measurement conditions

Column: Fused silica capillary column (5 % phenyl/ 95 % methyl polysil phenylene siloxane chemical binding type, internal diameter: 0.25 mm, length: 30 m, film thickness: 0.25 µm)[*6] [2]

Carrier gas: He (initial stage flow rate: 1.0 mL/min)

Sample injection: Splitless (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature: 60 °C (hold 1 min) → ramp 15 °C/min → 280 °C (hold 5 min)

Interface temperature: 280 °C

Detector: Quadrupole mass spectrometer[*7]

Ion source temperature: 230 °C

Ionization method: Electron ionization (EI) method

Ionization voltage: 70 eV

Monitor ion: Quantification ion: *m*/*z* 185, confirmation ion: *m*/*z* 109

Calculation. Calculate the peak area or peak height from the obtained selected ion monitoring chromatogram[3] to prepare a calibration curve, and determine the amount of dichlorvos (including the values converted naled to dichlorvos).

* 1. In this method, naled is converted to dichlorvos, and the amount is determined as a total amount of dichlorvos in the sample and that converted from naled.

2. Process under protection from light.

3. Prepare at the time of use, because the solution can not be stored.

4. Evaporate into dryness by introducing nitrogen gas gently, because dichlorvos is easily volatiled.

5. Set the flow rate at 2-3 mL/min. Use a vacuum manifold if necessary.

6. Thermo TR-5MS (the retention time of dichlorvos under the operating conditions is approximately 7 minutes) or an equivalent.

7. Example for GCMS-QP2010 (Shimadzu corporation)

«Summary of analysis method»

This method is intended to determine the amount of dichlorvos and naled in feed by extracting with acidic acetone, treating with a porous diatomite column, converting naled to dichlorvos, purifying with a silica gel minicolumn, and quantifying with a gas chromatograph-mass spectrometer.

The flow sheet of analysis method is shown in Figure 6.1.76-1.

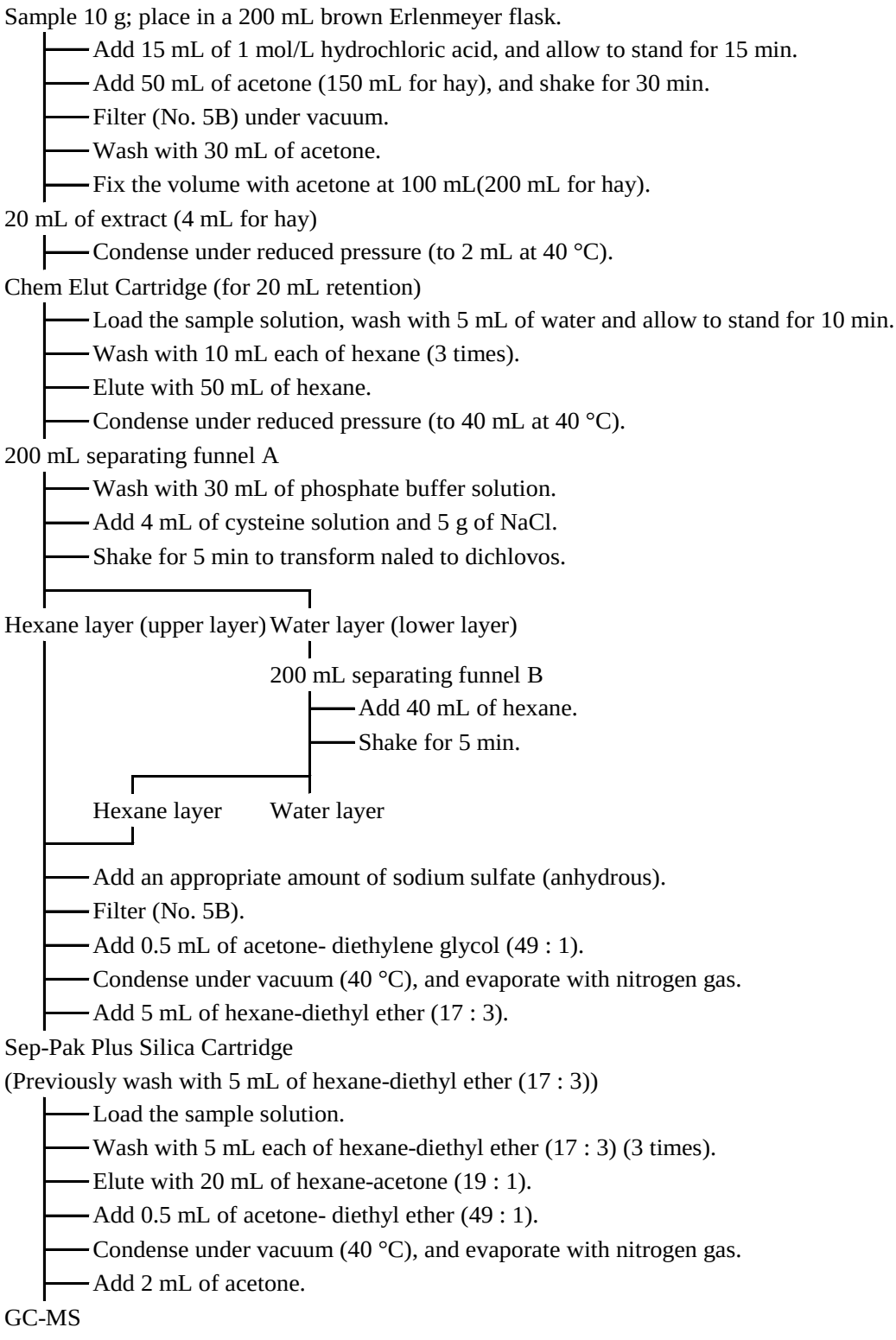


Figure 6.1.76-1. Flow sheet of analysis method for dichlorvos and naled by gas chromatograph mass spectrometer

References: Yasutoshi Sugimoto, Toshiharu Yagi, Takahisa Kato, Jun Ito, Yukiko Mitsui, Sae Shirai: Research Report of Animal Feed, 33, 39 (2008)

«Method validation»

• Spike recovery and repeatability

Spiked component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Dichlorvos	Finishing beef cattle formula feed	40-200	3	94.1-101.7	16
	Adult chicken formula feed	40-200	3	85.4-99.1	11
	Corn	40-200	3	93.4-96.7	17
	Bermuda hay	1,000-10,000	3	73.2-84.1	2.3
Naled	Finishing beef cattle formula feed	40-200	3	86.7-93.2	13
	Adult chicken formula feed	40-200	3	73.5-83.6	12
	Corn	40-200	3	75.7-87.9	14
	Bermuda hay	1,000-10,000	3	76.3-77.2	6.7

• Collaborative study

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Naled	Corn	9	200	92.7	4.3	12	0.54
	Alfalfa	9	10,000	83.1	4.1	12	0.96

• Lower quantification limit: 20 μg/kg (spike recovery and relative standard deviation)

• Lower detection limit: 7 μg/kg (*SN* ratio)

«Notes and precautions»

[1] The standard preparation is commercially available from Kanto Chemical or others.

[2] General 5% phenyl-95% dimethylpolysiloxane chemical binding type capillary column (Agilent Technologies DB-5MS, Restek Rtx-5ms, etc.) also can be used.

[3] An example of selected ion monitoring (SIM) chromatogram is shown in Figure 6.1.76-2.

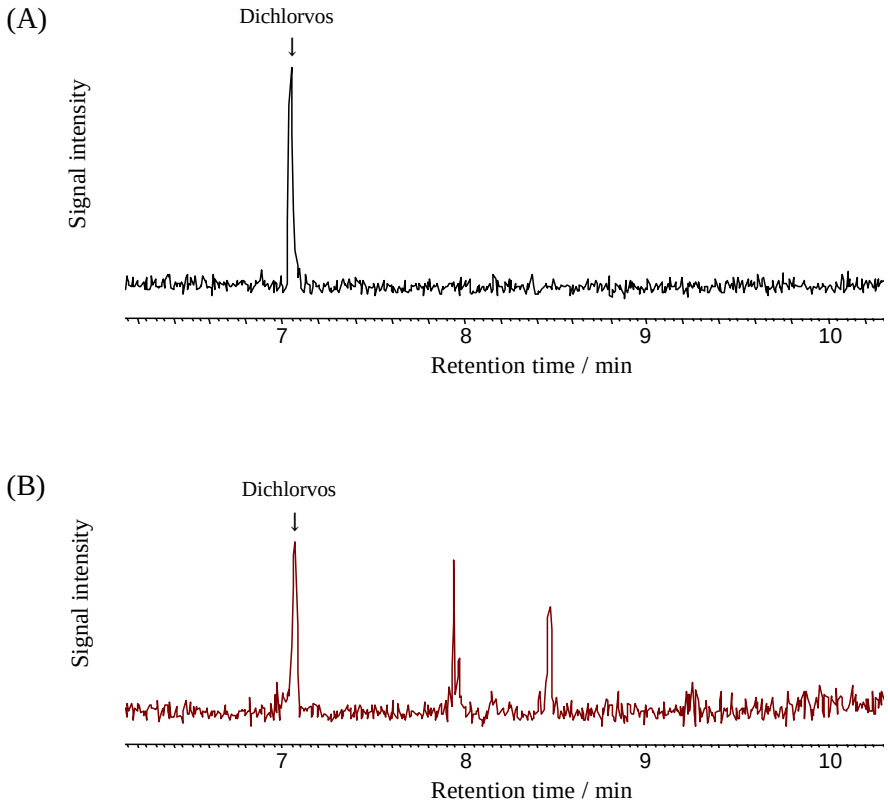


Figure 6.1.76-2. SIM chromatogram of the standard solution and sample solution
(A) Dichlorvos standard solution (100 ng/mL)
(B) Sample solution of an adult chicken formula feed (spiked at 200 µg/kg equivalent)

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

3. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (2)

Target Analytes:

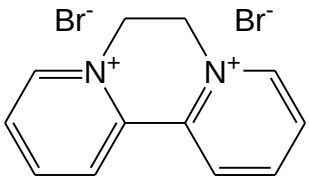
Group A: EPN, diazinon, parathion, fenitrothion, fenthion, phenthoate, phosalone and malathion (8

compounds).

Group B: Dichlorvos (1 compound)

Refer to Article 3, Section 2 in this chapter.

77 Diquat (Diquat dibromide)



9,10-dihydro-8a,10a-diazoniaphenanthrene dibromide

Diquat	$C_{12}H_{12}N_2$	MW: 184.24	CAS No.: 2764-72-9
Diquat dibromide	$C_{12}H_{12}Br_2N_2$	MW: 344.05	CAS No.: 85-00-7

[Summary of diquat]

Diquat is a non-hormone type, non-selective quaternary ammonium salt herbicide developed by ICI (UK). Diquat shows high grass-cide intensity via foliage treatment, and is used for weeding underbrush in fruit gardens, weeding before seeding crops, weeding in non-agricultural land, etc. Diquat is used in Japan, by itself or as a mixed formulation with paraquat at a low concentration.

Diquat was registered (as a dibromide) as an agricultural chemical in 1963, in Japan. The trade name is "Regrocks".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Guideline for rice straw and rice plant silage]

Rye: 0.03 ppm, corn: 0.05 ppm, oat, wheat and milo: 2 ppm, barley: 5 ppm, pasture grass: 100 ppm, and rice straw: 0.05 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Liquid chromatography [Analytical Standards of Feeds Chapter 6, Section 1, Article 77.1]

A. Reagent preparation

1) Diquat standard stock solution. Weigh accurately 20 mg of diquat [$C_{12}H_{12}N_2Br_2$][1], place in a 100 mL brown volmetric flask, add hydrochloric acid (0.01 mol/L) to dissolve, further add the solvent up to the marked line to prepare the diquat standard stock solution (1 mL of this solution contains an amount equivalent to 0.2 mg of diquat).

2) Cation-exchange resin (Na^+ type). Weigh 100 g of strongly acidic cation-exchange resin[*1], place in a 500 mL Erlenmeyer flask, add 300 mL of water, stir, and remove the supernatant. Repeat this process until pH of the supernatant becomes 6.8-7.2, add 300 mL of water, and allow to still standing overnight. Then, add 200 mL of sodium hydrate solution (2 mol/L) to this resin, stir, and remove the supernatant. Repeat this process until pH of the supernatant becomes 12 or more, add 200 mL of sodium hydrate solution (2 mol/L), and allow to still standing overnight. Then, add 300 mL of water to this resin, stir, and remove the supernatant. Repeat this process until pH of the supernatant becomes 6.8-7.2, add 300 mL of water and store in water.

B. Quantification

Extraction. Weigh 10.0 g of analysis sample, place in a 500 mL recovery flask, add 90 mL of sulfuric acid (1+2) solution, 3 to 4 particles of boiling stone and 2 to 3 drops of silicon oil, connect with a reflux cooler, and heat mildly for 5 hours to extract[2].

Place a 500 mL beaker under a Büchner funnel, filter the extract through glass filter*2 by suction, wash the recovery flask and residue serially with 50 mL of water, and filter by suction in a similar way. Further add water to this filtrate to make approximately 200 mL, and adjust the pH to 8.9-9.1 with sodium hydrate solution (12 mol/L) [3]. Place a 500 mL Erlenmeyer flask under a Büchner funnel, filter the filtrate through glass fiber filter*2 by suction, wash the beaker and filter serially with a small amount of water, and filter by suction in a similar way to obtain filtrate as the sample solution subject to column treatment.

Column treatment. Add cation-exchange resin (Na^+ type) to column tube[4] (internal diameter: 15 mm), run down to 6 cm of height, add 20 mL of water, and flow out until the liquid surface reaches at 3 mm from the top of the column packing material to prepare the column.

Place the sample solution in the column, wash the Erlenmeyer flask having contained the sample solution with a small amount of water, add the washings to the column, and flow out until the liquid surface reaches at 3 mm from the top of the column packing material*3. Add 100 mL of water to the column, and flow out in a similar way to wash the column. Then, serially add 50 mL of hydrochloric acid (2 mol/L), 100 mL of water, 50 mL of ammonium chloride solution (5 w/v%) and 100 mL of water in a similar way to wash the column[5].

Place a 100 mL volmetric flask under the column, add 50 mL of ammonium chloride solution (5 mol/L) to the column to elute diquat*3. Further add ammonium chloride solution (5 mol/L) up to the marked line of the volmetric flask to prepare the sample solution subject to fluorescence derivatization.

Fluorescence derivatization[6]. Place accurately 5 mL of the sample solution in a 100 mL separating funnel, add 25 mL of sodium hydrate solution (12 mol/L) and 1 mL of potassium ferricyanide solution (1 w/v%), and mix while mildly stirring. Add 20 mL of chloroform to the said separating funnel, shake vigorously for 5 minutes, allow to still standing, and transfer the chloroform layer (lower layer) to an Erlenmeyer flask. Add 20 mL of chloroform to the residual solution, process in a similar way, and add the chloroform layer to the said Erlenmeyer flask. Dehydrate the chloroform layer with an appropriate quantity of sodium sulfate (anhydrous), filter through filter paper (No.5A) into a 100 mL recovery flask, wash the Erlenmeyer flask and filter paper serially with a small amount of chloroform, and add to filtrate through the filter paper. Condense the filtrate almost into dryness under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add accurately 2 mL of water-acetonitrile (9 : 1) to dissolve the residue, and filter through membrane filter (pore size: 0.5 μm or less) to prepare the sample solution subject to liquid chromatography.

Simultaneously, place accurately 1 mL of diquat standard stock solution and 5 mL of ammonium chloride solution (5 mol/L) in a 100 mL separating funnel to derivatize under the conditions same to the sample solution.

Add accurately 2 mL of water-acetonitrile (9 : 1) to dissolve the residue, filter through membrane filter

(pore size: 0.5 μm or less), further dilute accurately with the solvent to prepare several standard solutions containing amounts equivalent to 0.01-0.5 μg of diquat in 1 mL.

Liquid chromatography. Inject 20 μL each of the sample solution and respective standard solutions into a liquid chromatograph to obtain the chromatogram.

Example of measurement conditions

Detector: Fluorescent Detector (excitation wave length: 368 nm, emission wave length: 430 nm)

Column: Octadecylsilylated silica gel column (internal diameter: 4.6 mm, length: 250 mm, particle size: 5 μm)*4 [7]

Eluent: Water-acetonitrile (9 : 1)

Flow rate: 1.0 mL/min

Column oven temperature: 40 °C

Calculation. Calculate the peak area from the obtained chromatogram[8] to prepare a calibration curve, and determine the amount of diquat in the sample.

* 1. AG 50W-X8 H^+ type (particle size: 200-100 mesh) (Bio-Rad Laboratories) or an equivalent.

2. GF-A (Whatman) or an equivalent.

3. The flow rate at the time of washing is 10 mL/min, and the flow rate at the time of eluting is 10 mL/h.

4. Symmetry C_{18} (Waters) or an equivalent.

«Summary of analysis method»

This method is intended to determine the amount of diquat in the sample by extracting by boiling under the acidic condition with sulfuric acid, purifying with a cation exchange column, fluorescence derivatizing, and quantifying with a liquid chromatograph.

The flow sheet of analysis method is shown in Figure 6.1.77-1.

Sample (10 g)
- Add 90 mL of sulfuric acid (1:2), boiling stone and silicon oil.
- Connect with a reflux cooling tube, and heat with a mantle heater for 5 hr.
- Filter under vacuum with glass fiber filter.
- Wash the container and residue with 50 mL of water, and add the washings to the filtrate up to a total amount of approx. 200 mL.
- Adjust the pH of the filtrate to 9 with sodium hydrate solution (10 mol/L).
- Filter with glass fiber filter.

Cation exchange resin column
- Load all amount of sample, and flow out.
- Wash with 100 mL of water.
- Wash with 50 mL of hydrochloric acid (1 : 5), 100 mL of water and 50 mL of ammonoium chloride solution (5 w/v%) and 50 mL of water.
- Add 50 mL of ammonium chrolide (27 w/v%), elute into a 50 mL volumetric flask.
- Fix the volume with ammonium chloride solution (27 w/v%) at 50 mL.

Fluorescence derivatization (100 mL separating funnel)
- Add 5 mL of sample solution (accurately).
- Add 25 mL of sodium hydrate solution (10 mol/L).
- Add sodium ferricyanide solution (1 w/v%), and mildly stir.
- Extract twice with 20 mL each of chloroform.
- Shake for 5 min.
- Add the chlorofolm layer, and filter under vacuum.
- Condense under reduced pressure, and evaporate into dryness.
- Add 2 mL of water-acetinitrile (9 : 1).

LC-FL (Ex: 368 nm, Em: 430 nm)

Figure 6.1.77-1. Flow sheet of analysis method of diquat

References: Manabu Matsuzaki, Yukinobu Nakamura, Yukie Ishida, Tomoharu Nozaki, Ikumi Kobayashi, Toshiaki Hayakawa: Research Report of Animal Feed, 26, 20 (2001)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Adult chicken formula feed	50-200	3	76.4-98.4	13.9
Dairy cattle formula feed	50-200	3	79.8-104.3	9.9
Sudan grass	50-200	3	72.7-91.6	10.8

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Adult chicken formula feed	7	100	86.8	3.8	9.3	0.42

• Lower quantity limit: Formula feed: 30 μg/kg sample, hay: 50 μg/kg sample

«Notes and precautions»

[1] The standard preparation is commercially available from Wako Pure Chemical Industries or others.

[2] Heat to release small bubbles. Overheating may causes sudden boiling. Further, at the time of heat and reflux in the extraction process, volatile organic substances may adhere to the cooling apparatus, possibly adsorbing diquat. Therefore, wash the cooling apparatus with sulfuric acid (1 : 1) before use.

[3] The amount of sodium hydrate solution needed for neutralization is approximately 120 mL. Since heat is emitted by neutralization, neutralization should be performed under cooling.

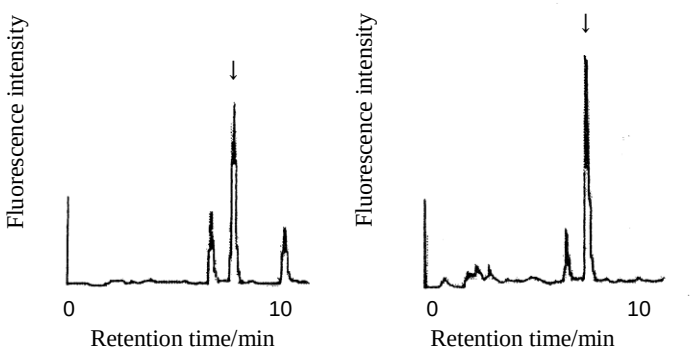
[4] Use one with a fluid reserver.

[5] Since the drip rate is changed by the loading amount, adjust the drip rate to be steady as much as possible. Further, it is noteworthy that when the drip rate is too slow at the time of loading the sample solution, crystalline substances may be precipitated, possibly making impossible to drip.

[6] Rapidly perform the derivatizing process.

[7] Any column is applicable as long as its end-capped packing material meets the requirements.

[8] An example of chromatogram is shown in Figure 6.1.77-2.



Measurement conditions

Detector:
excitation wave length: 368 nm, emission wave length: 430 nm

Column: Symmetry C18 (internal diameter: 4.6 mm, length: 250 mm, particle size: 5 μm)

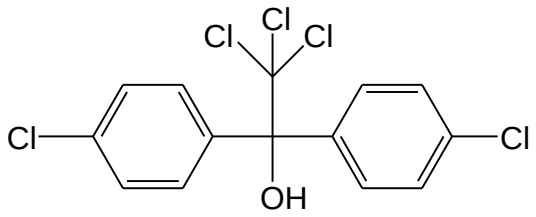
Eluent: Water – acetonitrile (9 : 1)

Flow rate: 1.0 mL/min

Column oven temperature: 40 °C

Figure 6.1.77-2 Chromatogram for diquat added to formula feed and Sudan grass in an amount equivalent to 0.1 mg/kg
(Arrows indicate the peaks of fluorescent derivative of diquat.)

78 Dicofol (Kelthane)



2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol

$C_{14}H_9Cl_5O$ MW: 370.5 CAS No.: 115-32-2

[Summary of dicofol]

Dicofol is an organic chlorine insecticide developed by Rohm and Haas (USA), and has effect on many kinds of red mites.

Dicofol had been registered as an agricultural chemical in 1956, in Japan. Registered name had been kelthane. However, it was expired in 2004.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley and rye: 0.02 ppm, corn: 3 ppm, and other grain: 0.02 ppm

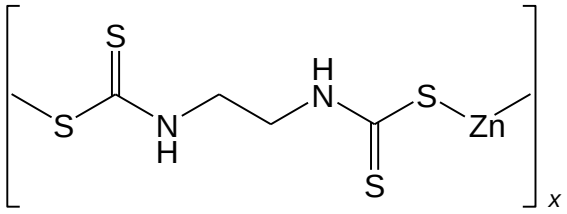
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for dicofol and trifluralin by gas chromatography

Target Analytes: Dicofol and trifluralin (2 compounds)

Refer to Article 15, Section 3 in this chapter.

79 Zineb



Zinc ethylenebis(dithiocarbamate) (polymeric)

$C_4H_6N_2S_4Zn$ MW: 275.74 CAS No.: 12122-67-7

[Summary of zineb]

Zineb is a widely used ethylenebisdithiocarbamate bactericide. Zineb is one of agricultural chemical s being used in a most large amount. Zineb has bactericidal effects on melanose in fruit trees including apples and pears and on downy mildew in vegetables, as well as acaricidal effects.

Zineb is light yellow powder soluble in water at 10 mg/L, insoluble in general organic solvents, unstable to light, heat, humidity and alkali.

Zineb was registered as an agricultural chemical in 1952, in Japan. The trade name is “Daisen”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(as an amount of dithiocarbamate (the sum of respective contents of zineb, ziram, thiram, nickel bis(dithiocarbamate), ferbam, propineb, polycarbamate, mancozeb, maneb and methiram equivalent to carbon disulfide)

Corn: 0.1 ppm, wheat, barley and rye: 1 ppm, and other grain: 0.1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Analysis method[*1] for zineb and mancozeb by liquid chromatography [Analytical Standards of Feeds Chapter 6, Section 1, Article 79.1]

Target analytes: Zineb and mancozeb (2 compounds)

Scope of application: Formula feed

A. Reagent preparation

1) Cysteine-EDTA solution. Add 800 mL of water to 50 g of L-cysteine hydrochloride salt monohydrate and 50 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate, further add 70 mL of sodium hydrate solution (12 mol/L) to dissolve, and adjust the pH to 9.6 with sodium hydrate solution (12 mol/L).

2) Zineb standard solution. Weigh accurately 20 mg of zineb $[(C_4H_6ZnN_2S_4)_n]$[1], place in a 100 mL brown volmetric flask, add cysteine-EDTA solution to dissolve, and further add the solvent up to the

marked line to prepare the standard solution (1 mL of this solution contains an amount equivalent to 0.2 mg of zineb) (prepare at the time of use[2]).

3) Mancozeb standard solution. Weigh accurately 20 mg of mancozeb [$(C_4H_6MnN_2S_4)_x(Zn)_y$], place in a 100 mL brown volmetric flask, add cysteine-EDTA solution to dissolve, further add the solvent up to the marked line to prepare the standard solution (1 mL of this solution contains an amount equivalent to 0.2 mg of mancozeb) (prepare at the time of use).

4) Tetrabutyl ammonium hydrogen sulfate solution. Dissolve 14 g of tetrabutyl ammonium hydrogen sulphate in water to make 100 mL.

5) Methyl iodide reagent. Mix 750 mL of chloroform, 250 mL of hexane and 3 mL of methyl iodide.

B. Quantification [3]

Extraction. Weigh 10.0 g of the analysis sample, place in a 300 mL separating funnel, add 80 mL of cysteine-EDTA solution and 40 mL of dichloromethane, and shake for 30 minutes to extract. Place the extract in a 350 mL centrifuge tube, centrifuge at 1,500×*g* for 5 minutes, and transfer accurately 40 mL of the water layer (upper layer) to a 100 mL beaker to prepare the sample solution subject to methylation.

Methylation. Add 5 mL of tetrabutyl ammonium hydrogen sulphate solution to the sample solution, adjust the pH to 7.5-7.8 with hydrochloric acid (2 mol/L), and place in a 100 mL separating funnel. Wash the beaker having contained the sample solution with water, and add the washings to the separating funnel. Add 20 mL of the methyl iodide reagent to this separating funnel, shake vigorously for 3 minutes, methylate zineb and mancozeb to generate ethylene bismethyl dithiocarbamate, allow to still standing, and transfer the lower layer (the part separated as gel) to a 100 mL centrifuge tube. Add 20 mL of the methyl iodide reagent to the said separating funnel, shake gently, allow to still standing, and add the lower layer to the centrifuge tube. Further process similarly, and centrifuge the lower layer at 1,500×*g* for 5 minutes. Remove the water layer (upper layer), dehydrate the lower layer with an appropriate quantity of sodium sulfate (anhydrous), and filter immediately through glass fiber filter[*2] into a 200 mL recovery flask. Serially wash the said centrifuge tube and glass fiber filter several times with chloroform, and add the washings to the filtrate through the said glass fiber filter. Add 0.1 g of L-cysteine hydrochloride salt monohydrate to the filtrate, condense to approximately 1 mL under reduced pressure in a water bath at 40 °C or lower, further dry up by the flow of nitrogen gas.

Add accurately 5 mL of acetonitrile to dissolve the residue to prepare the sample solution subject to column treatment.

Simultaneously, place accurately 1 mL of the zineb standard solution or mancozeb standard solution in a 100 mL beaker, add 40 mL of cysteine-EDTA solution, and process in a similar way to the sample solution. Add accurately 20 mL of acetonitrile to dissolve the residue, and filter through membrane filter[4] (pore size: 0.5 µm or less). Dilute a quantity of filtrate accurately with acetonitrile to prepare several standard solutions (methylated) containing amounts equivalent to 0.1-2 µg/mL of zineb or mancozeb subject to liquid chromatography.

Column treatment. Wash a neutral alumina minicolumn (1,710 mg) with 10 mL of acetonitrile.

Place a 50 mL recovery flask under the minicolumn, place accurately 3 mL of the sample solution in

the minicolumn, flow out ethylene bismethyl dithiocarbamate, further add 20 mL of acetonitrile to the column, and flow out in a similar way. Add 0.1 g of L-cysteine hydrochloride salt monohydrate to the effluent, condense almost into dryness under reduced pressure in a water bath at 40 °C or less, further dry up by the flow of nitrogen gas.

Add accurately 3 mL of acetonitrile to dissolve the residue, and filter through membrane filter (pore size: 0.5 µm or less) to prepare the sample solution subject to liquid chromatography.

Liquid chromatography. Inject 20 µL each of the sample solution and respective mixed standard solutions (methylated) into a liquid chromatograph to obtain the chromatogram.

Example of measurement conditions

Detector: Ultraviolet spectrophotometer (measured wavelength: 272 nm)[5]

Column: Octadecylsilylated silica gel column (internal diameter: 4 mm, length: 250 mm, particle size: 5 µm)*3 [6]

Eluent: Water-acetonitrile (3 : 2)

Flow rate: 1.0 mL/min

Calculation. Calculate the peak height or peak area from the obtained chromatogram[7] to prepare a calibration curve, and determine the amount of zineb or mancozeb in the sample.

* 1. In this method, the amounts of zineb and mancozeb in the sample are converted to ethylene bismethyl dithiocarbamate, and totally quantificated as the contents of zineb or mancozeb.

Further, in this method, when maneb [$(C_4H_6MnN_2S_4)_n$] is contained in the sample, maneb may be converted to ethylene bismethyl dithiocarbamate, and the amount of maneb in the sample is possibly included in the amount of zineb or mancozeb.

2. GA-100 (ADVANTEC) or an equivalent.

3. Wakosil-II 5C18 HG (Wako Pure Chemical Industries) or an equivalent.

«Summary of analysis method»

This method is intended to determine the amount of zineb and mancozeb in the sample by converting to water-soluble ethylenebisdithiocarbamate sodium salt with EDTA-NaOH mixture through extraction, adding an ion-pair reagent (tetrabutylammonium hydrogen sulfate), changing solvents to chloroform-hexane mixture containing methyl iodide, generating ethylene bismethyl dithiocarbamate, purifying with a neutral alumina minicolumn, and quantifying with a liquid chromatograph.

References: Yutaka Kunugi: Research Report of Animal Feed, 19, 56 (1994)

«Method validation»

• Spike recovery and repeatability

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Zineb	Chicken formula feed	100-2,000	3	78.6-94.0	5.8
	Cattle formula feed	100-2,000	3	75.9-87.2	6.8
	Corn	100-2,000	3	81.4-87.6	6.2
Manzeb	Chicken formula feed	100-2,000	3	81.5-84.2	8.7
	Cattle formula feed	100-2,000	3	77.5-96.1	9.6
	Corn	100-2,000	3	81.2-96.5	10.7

• Collaborative study

Component	Sample	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Zineb	Breeding swine formula feed	6	1,000	82.6	7.1	13.1	0.80
Mancozeb	Breeding swine formula feed	6	1,000	81.7	7.1	13.2	0.80

• Lower quantification limit: 50 μg/kg sample

«Notes and precautions»

[1] The standard preparation is commercially available from Wako Pure Chemical Industries, Kanto Chemical, HAYASHI PURE CHEMICAL, GL Science, or others.

[2] Sodium salt of ethylene bis dithiocarbamate in the cysteine-EDTA mixture is degraded with time; therefore, the standard solution should be prepared at the time of use.

[3] Zineb is degraded with time in each stage of quantification; therefore, the process from extraction to measurement is to be performed within a day.

[4] Use a non-aqueous filter (polytetrafluoro-ethylene (PTFE)) or a filter with dual purpose for aqueous and non-aqueous (fluorine polymer (PVDF)).

[5] An absorbance curve of ethylene bismethyl dithiocarbamate is shown in Figure 6.1.79-1.

[6] Any column is applicable as long as its end-capped packing material meets the requirements.

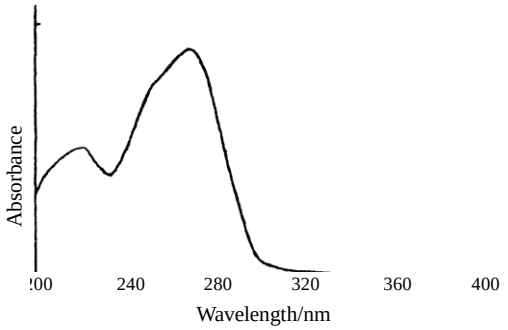
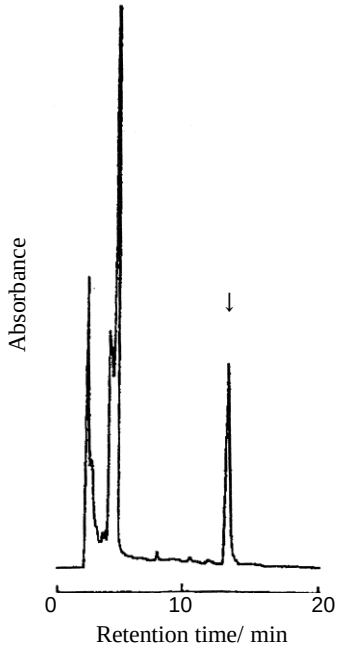


Figure 6.1.79-1. Absorption curve of ethylene bismethyl dithiocarbomate

[7] An example of chromatogram is shown in Figure 6.1.79-2.



Measurement conditions:

Detector: Ultraviolet spectrophotometer
(measured wavelength: 272 nm)

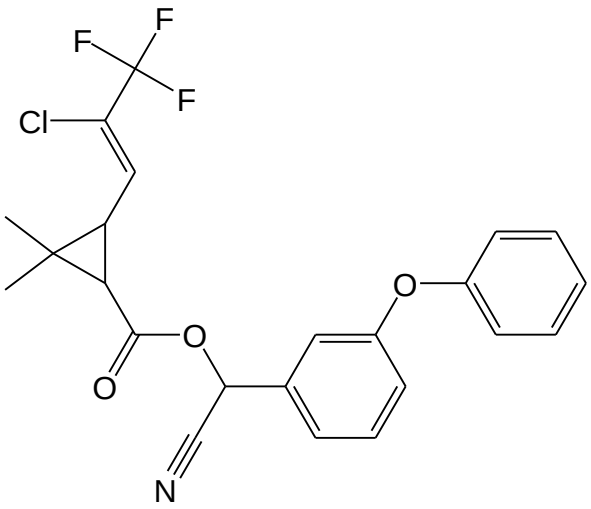
Column: Wakosil-II 5C18HG

Eluent: Water – acetonitrile (3 : 2)

Flow rate: 1 mL/min

Figure 6.1.79-2. A chromatogram for zineb added to chicken formura feed in an amount equivalent to 0.5 mg/kg
(the arrow indicates the peak of ethylene bismethyl dithiocarbamate)

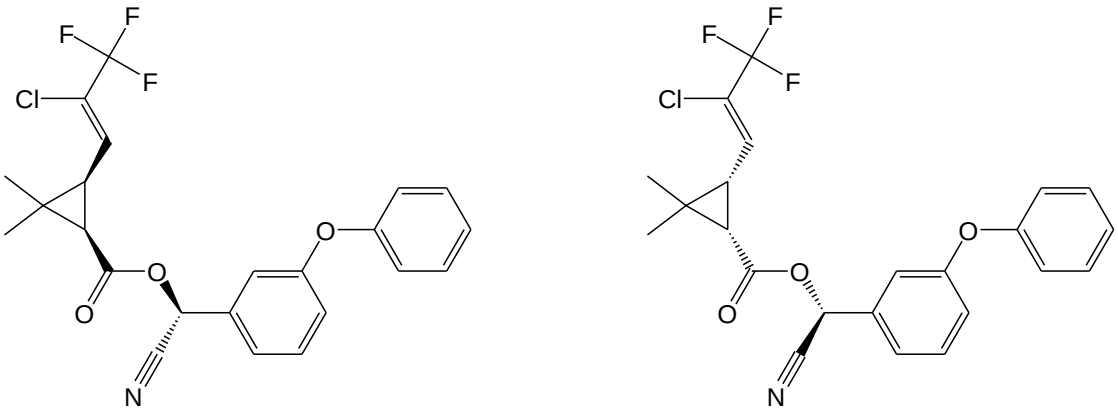
80 Cyhalothrin



(*RS*)-*α*-cyano-3-phenoxybenzyl (1*RS*)-*cis*-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate

$C_{23}H_{19}ClF_3NO_3$ MW: 449.9 CAS No.: 68085-85-8

(Reference) *λ*-cyhalothrin (CAS No.: 91465-08-6)



[Summary of cyhalothrin]

Cyhalothrin is a synthetic pyrethroid insecticide developed by ICI (UK), and used for fruit trees, vegetables and teas.

Cyhalothrin was registered as an agricultural chemical in 1988, in Japan. The trade name is “Saiharon”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(Cyhalothrin includes *λ*-cyhalothrin)

Rye: 0.02 ppm, corn: 0.04 ppm, wheat: 0.05 ppm, oat, barley and milo: 0.2 ppm, and pasture grass: 0.6 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

   Target analytes: agricultural chemicals (138 compounds)

   Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

   Target Analytes:

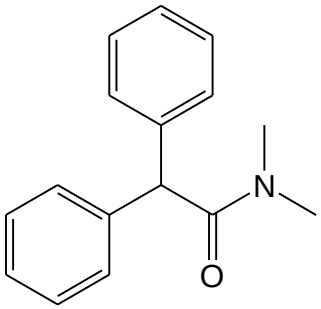
   Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

   Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

   Group C: Allethrin and tetramethrin (2 compounds)

   Refer to Article 4, Section 2 in this chapter.

81 Diphenamid



N,*N*-dimethyldiphenylacetamide

$C_{16}H_{17}NO$ MW: 239.3 CAS No.: 957-51-7

[Summary of diphenamid]

Diphenamid is an acid amide herbicide, a non-hormone type translocating field soil treatment agent.

Diphenamid had been registered as an agricultural chemical in 1965, in Japan. However, it was expired in 1995.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (uniform limit)

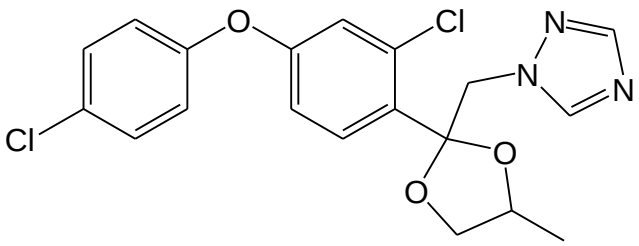
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

82 Difenoconazole



3-chloro-4-[(2*RS*,4*RS*;2*RS*,4*SR*)-4-methyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether

$C_{19}H_{17}Cl_2N_3O_3$ MW: 406.3 CAS No.: 119446-68-3

[Summary of difenoconazole]

Difenoconazole is a permeable triazole bactericide developed by Ciba-Geigy (Switzerland).

Difenoconazole was registered as an agricultural chemical targeting apples, pears, etc. in 1993, in Japan. The trade name is “Score”.

«Standard value to grain defined by Food Sanitation Law»

Wheat, barley, rye and corn: 0.1 ppm

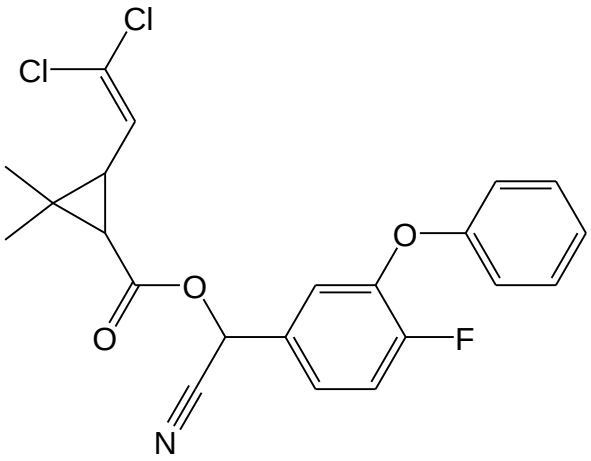
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

83 Cyfluthrin



(*RS*)-*α*-cyano-4-fluoro-3-phenoxybenzyl (1*RS*)-*cis*-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

$C_{22}H_{18}Cl_2FNO_3$ MW: 434.3 CAS No.: 68359-37-5

[Summary of cyfluthrin]

Cyfluthrin is a synthetic pyrethroid insecticide developed by Bayer (Germany), and used for fruit trees, vegetables, etc.

Cyfluthrin was registered as an agricultural chemical in 1989, in Japan. The trade name is "Baythroid".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(Sum of respective isomers)

Oat, barley, wheat, corn, milo and rye: 2 ppm, and pasture grass: 3 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

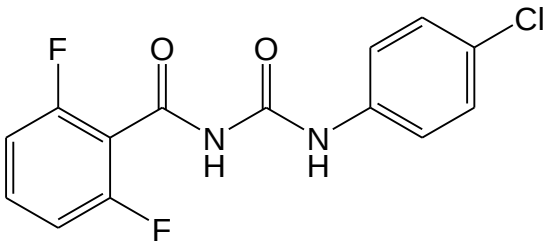
Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

Refer to Article 4, Section 2 in this chapter.

84 Diflubenzuron



1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea

$C_{14}H_9ClF_2N_2O_2$ MW: 310.68 CAS No.: 35367-38-5

[Summary of diflubenzuron]

Diflubenzuron is a benzoyl phenyl urea insencticide developed by Philips-Duphar GmbH (Netherlands), and is called Insect Growth Regulator (IGR) because of its chitin synthesis inhibitor activity against insects.

In Japan, diflubenzuron is mainly used as an insecticide for citrus and tea trees. While its toxicity in man, animal or fish is mild, diflubenzuron is highly toxic to silkworm. Diflubenzuron was registrered as an agricultural chemical in 1981, in Japan. The trade names are “Demi Lynn” or “Retardane”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat and barley: 0.1 ppm / Rye, corn and other grains: 2 ppm

[Method listed in the Analytical Standards of Feeds]

1. Liquid chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 84.1]

A. Reagent preparation

1) Diflubenzuron standard solution. Weigh accurately 20 mg of diflubenzuron [$C_{14}H_9ClF_2N_2O_2$][1], transfer to a 100 mL volmetric flask and dissolve by adding acetonitrile. Further, add the same solvent up to the graduation line of the flask to prepare the diflubenzuron standard stock solution (1 mL of this solution contains 0.2 mg as diflubenzuron).

Before use, dilute accurately a certain amount of the standard stock solution with water - acetonitrile (1 : 1) to prepare several diflubenzuron standard solutions that contain 0.02 – 2 μg of diflubenzuron in 1 mL.

2) Magnesium silicate[2]. Dry synthetic magnesium silicate (particle size 149-250 μm (100-60 mesh)) at 130 °C for 16 hours.

B. Quantification

Extraction. Weigh 5 – 10 g of an analysis sample, transfer it to a stopperd 200 mL Erlenmeyer flask, add 15 mL of water to moisten and leave to stand for 30 minutes. Further, add it 80 mL of acetonitrile and extract by shaking for 30 minutes. Place a 500 mL recovery flask under a Büchner funnel and filter the extract by suction through a filter paper (No. 5B). Then, wash the Erlenmeyer flask and the residue with 50 mL of acetonitrile sequentially, and filter by suction in the similar way. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or below to ca. 15 mL under vacuum, add 5 g of sodium

chloride, and use this solution as a sample solution for column treatment I.

Column treatment I. Place the sample solution on the porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that has contained the sample solution three times with 10 mL each of hexane – ethyl acetate (1 : 1), add the washings to the column in order of precedence and elute diflubenzuron by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 90 mL of hexane – ethyl acetate (1 : 1) to the column to elute in the similar way and concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness, further dry up by the flow of nitrogen gas.

Add accueately 10 mL of cyclohexane – acetone (7: 3) to dissolve the residue, filter the solution through a membrane filter (pore size: 0.5 µm or less), and use the filtrate as a sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph. Dispense each elution fraction into a 100 mL recovery flask, concentrate under reduced pressure in a water bath at 40 °C or lower to almost dryness, further dry up by the flow of nitrogen gas.

Add 5 mL of hexane – acetone (9 : 1) to dissolve the residue and use this solution as a sample solution for column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column[3] (20 mm in inner diameter, 300 mm in length, particle size 15 µm)

Guard column: Styrene-divinylbenzene copolymer column[3] (20 mm in inner diameter, 100 mm in length, particle size 15 µm)

Eluent: Cyclohexane – acetone (7 : 3)

Flow rate: 5 mL/min

Fraction collection: 65-80 mL

Column treatment II. Suspend 10 g of magnesium silicate and 2 g of sodium sulfate (anhydrous) respectively in hexane, pour into a column (15 mm inner diameter) sequentially and let them flow out so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Place the sample solution on the column. Wash the recovery flask that has contained the sample solution three times with 5 mL each of hexane – acetone (9 : 1), add the washings to the column in order of precedence and let them flow out by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Further, add 30 mL of hexane – acetone (9: 1) to the column and flow out in the similar way. Place a 200 mL recovery flask under the column and add 80 mL of hexane – acetone (4 : 1) to the column to elute diflubenzuron. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness, further dry up by the flow of nitrogen gas.

Add accurately 2.5 mL of water – acetonitrile (1 : 1) to dissoleve the residue, filter with a membrane filter (pore size: 0.5 µm or less), and use the filtrate as a sample solution for liquid chromatography.

Liquid chromatography. Inject 20 µL each of the sample solution and respective diflubenzuron standard solutions into a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Ultraviolet spectrophotometer (Measurement wavelength: 254 nm)

Column: Octadecylsilylated silica gel column (4.6 mm in inner diameter, 150 mm in length, particle size 3 μm)*1[4]

Eluent: Water – acetonitrile (11 : 9)

Flow rate: 1.0 mL/min

Column oven temperature: 40 °C

Calculation. Obtain the peak height or peak area from the resulting chromatograms[5] to prepare a calibration curve and subsequently calculate the amount of diflubenzuron in the sample.

* 1. Capcell pak C18 UG120 S-3 (Shiseido) or equivalents.

«Summary of analysis method»

In this method, diflubenzuron in feeds is extracted with acetonitrile/water, purified with a porous diatomite column, a GPC and a magnesium silicate column and quantified by a liquid chromatograph with an ultraviolet spectrophotometer.

The flow sheet of the analysis method is shown in Figure 6.1.84-1.

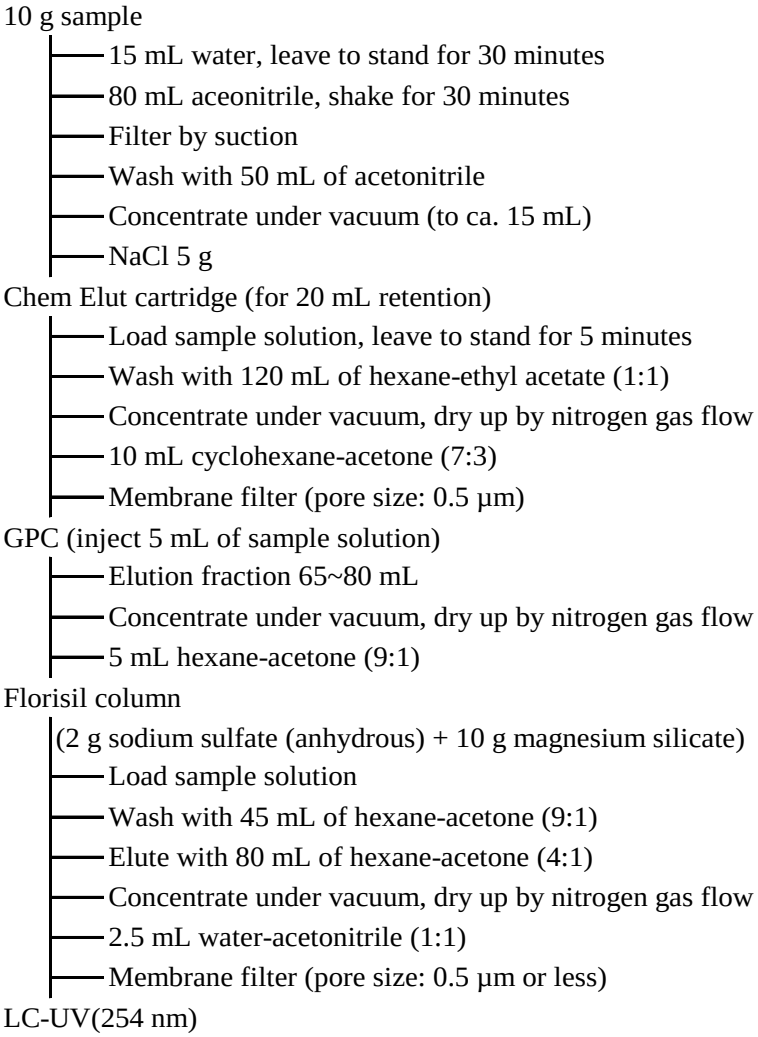


Figure 6.1.84-1. Flow sheet of the analysis method for diflubenzuron

Reference: Masato Shibata, Research Report of Animal Feed, 24, 49(1999).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
adult hen formula feed	50~500	3	90.7~95.7	7.5
finishing beef cattle formula feed	50~500	3	87.7~93.0	8.8
alfalfa hay	50~500	3	92.7~102.0	9.0

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
growing pig formula feed	7	500	91.6	2.4	7.7	0.43

• Lower limit of quantification: 20 μg/kg in samples

«Notes and precautions»

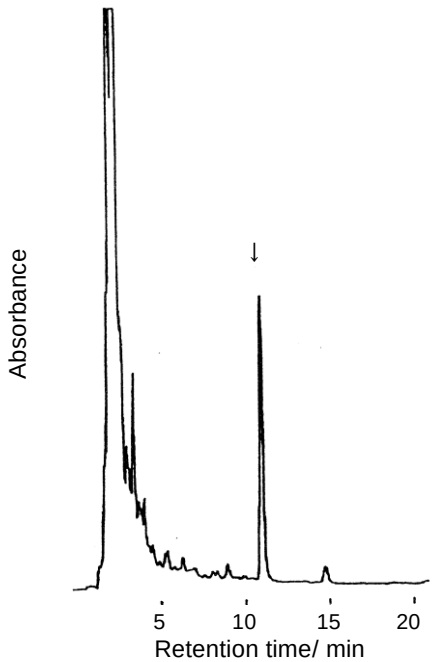
[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical and other manufacturers.

[2] Florisil PR (Floridin) immediately after opening usually contains ca. 1 % moisture, so it can be used directly.

[3] A column packed with styrene-divinylbenzene copolymer hard gel with the use of eluent.

[4] End-capped column packing materials should be used. When the octadecylsilylated silica gel column is used, components show unique elution trends different from those observed using the common ODS columns, because its packing material is octadecyl group bonded type porous spherical silica gel coated with a thin layer of silicone polymer. If some other ODS column is to be used, separation of target compound should be confirmed in advance.

[5] An example of chromatogram is shown in Figure 6.1.84-2.



Measurement conditions

Detector: Ultraviolet spectrophotometer (Measurement wavelength: 254 nm)

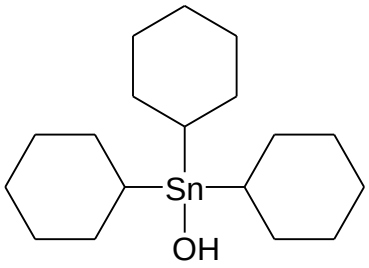
Column: Shiseido Capcell pak C18 UG120 S-3 (4.6 mm in inner diameter, 150 mm in length, particle size 3 μm)

Eluent: Water – acetonitrile (11 : 9)

Flow rate: 1.0 mL/min

Figure 6.1.84-2. Chromatogram of a formula feed spiked with an amount equivalent to 0.5 mg/kg diflubenzuron (The arrow indicates the peak of diflubenzuron.)

85 Cyhexatin



tricyclohexyltin hydroxide

$C_{18}H_{34}OSn$ MW: 385.17 CAS No.: 13121-70-5

[Summary of cyhexatin]

Cyhexatin is an organotin acaricide developed by the Dow Chemical Company (USA) and is effective against a wide range of phytophagous mites on fruit trees and vegetables.

Cyhexatin was registeted as an agricultural chemical in 1972, in Japan. However, it was expired in 1987.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(Azocyclotin and cyhexatin)

Not detected (Limit of detection: 0.02 ppm)

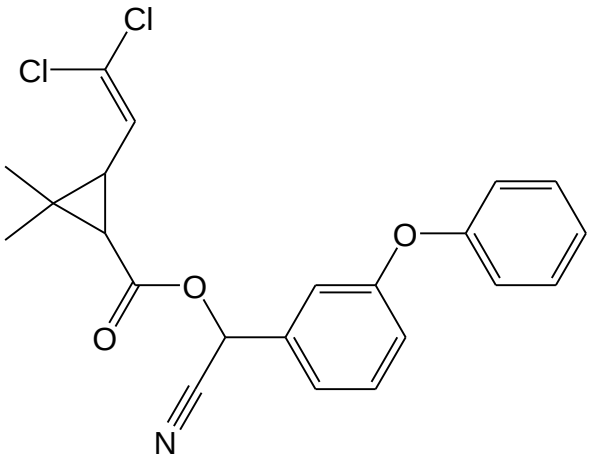
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for fenbutatin oxide and cyhexatin by gas chromatography

Target Analytes: Fenbutatin oxide and cyhexatin (2 compounds)

Refer to Article 13, Section 3 in this chapter.

86 Cypermethrin



(*RS*)-*α*-cyano-3-phenoxybenzyl (1*RS*)-*cis*-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

$C_{22}H_{19}Cl_2NO_3$ MW: 416.3 CAS No.: 52315-07-8

[Summary of cypermethrin]

Cypermethrin is a synthetic pyrethroid insecticide discovered by Elliott et al. and developed by Sumitomo Chemical Co., Ltd., which theoretically consists of a mixture of eight different isomers. It is a yellowish brown viscous liquid, and is practically insoluble in water, readily soluble in organic solvents. It shows extremely high insecticidal effect primarily against major insect pests in fruit trees, vegetables, tuber crops, pulse, tee trees and the like.

Cypermethrin was registered as an agricultural chemical in 1986, in Japan. The trade name is “Agrothrin”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(The sum of each isomer. Cypermethrin includes ζ-cypermethrin.)

Wheat and corn: 0.2 ppm / Barley: 0.5 ppm / Rye and other grains: 1.0 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

Refer to Article 4, Section 2 in this chapter.

2. Gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 1 86.2]

A. Reagent Preparation

1) Cypermethrin Standard Solution. Weigh accurately 25 mg of cypermethrin, transfer to a 50 mL volmetric flask and dissolve by adding 10 mL of acetone. Further, add 2,2,4-trimethylpentane to the graduation line of the flask to prepare the cypermethrin standard stock solution (1 mL of this solution contains 0.5 mg as cypermethrin).

Before use, dilute accurately a certain amount of the standard stock solution with 2,2,4-trimethylpentane – acetone (4 : 1) to prepare several cypermethrin standard solutions that contain 0.02 – 1 μg of cypermethrin in 1 mL.

2) Magnesium silicate[1]. Dry synthetic magnesium silicate (particle size: 149-250 μm (100-60 mesh)) at 130 °C for 16 hours.

B. Quantification

Extraction Weigh 10.0 – 20.0 g of an analysis sample[2], transfer it to a stoppered 200 mL Erlenmeyer flask, add 30 mL of water to moisten and leave to stand for 30 minutes. Further, add it 70 mL of acetonitrile and extract by shaking for 30 minutes. Place a 200 mL volmetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetonitrile – water (7 : 3) sequentially, and filter the wash by suction in the similar way. Further, add it acetonitrile – water (7 : 3) to the graduation line of the flask to prepare a sample solution for purification.

Purification. Transfer 100 mL of sample solution to a 500 mL separating funnel A already containing 250 mL of sodium chloride solution (5 w/v%) and 50 mL of hexane, shake vigorously for 5 minutes and leave to stand. Transfer the water layer (lower layer) to a 500 mL separating funnel B, and the hexane layer (upper layer) to a 200 mL Erlenmeyer flask, respectively. Add 50 mL of hexane to the separating funnel B, shake gently and leave to stand. Combine the hexane layer with the content of the Erlenmeyer flask above. Dehydrate the hexane layer with a suitable amount of sodium sulfate (anhydrous) and filter through a filter paper (No. 2S) into a 300 mL recovery flask. Wash the Erlenmeyer flask above and the filter paper with a small amout of hexane sequentially, filter the washes through this filter paper and combine the filtrates. Concentrate the filtrate under vacuum in a water bath at 40 °C or lower to almost dryness and dry up by nitrogen gas flow.

Dissolve the residue by adding 30 mL of hexane and transfer it to a 200 mL separating funnel. Further, add 30 mL of acetonitrile – water (100 : 1), shake the separating funnel for 5 minutes vigorously and leave to stand. Transfer the acetonitrile layer (lower layer) to a 200 mL recovery flask. Add 30 mL of acetonitrile – water (100 : 1) to the separating funnel above, shake gently and leave to stand. Combine the acetonitrile layer to the content of the recovery flask above. Concentrate the acetonitrile layer under vacuum in a water bath at 40 °C or below to almost dryness, further dry up by the flow of nitrogen gas.

Dissolve the residue with 5 mL of hexane and use this solution as a sample solution for Column treatment.

Column treatment. Suspend 10 g of magnesium silicate and 2 g sodium sulfate (anhydrous) in hexane respectively, pour the suspensions into a column (15 mm inner diameter) sequentially and let them flow

out so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Transfer the sample solution to the column. Wash the recovery flask that has contained the sample solution with 5 mL of hexane three times, add the washes to the column in order of precedence and let them flow out by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Further, add 100 mL of hexane – diethyl ether (19 : 1) to the column to obtain an outflow in the similer way. Place a 300 mL recovery flask under the column and add 150 mL of hexane – diethyl ether (4 : 1) to the column to elute cypermethrin[3]. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and dry up by nitrogen gas flow.

Dissolve the residue by adding 5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution for gas chromatography.

Gas chromatography. Inject 1 μL each of the sample solution and respective cypermethrin standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Electron capture detector

Column: Fused silica capillary column (50 % trifluoropropyl methyl/50 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)[4]

Carrier gas: He (1.5 mL/min)

Make up gas: N_2 (60 mL/min)

Sample injection : Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 2 min) → ramp 20 °C/min → 250 °C (hold 18 min)

Detector temperature: 300 °C

Calculation. Obtain the sum of the three peak heights or the three peak areas from the resulting chromatograms[5] to prepare a calibration curve and subsequently calculate the amount of cypermethrin in the sample.

«Summary of analysis method»

In this method, cypermethrin in samples is extracted with acetonitrile/water, purified with a magnesium silicate column and quantified by a capillary column chromatograph with an electron capture detector by summing up the three peaks obtained.

Reference: Yukie Ishida, Yutaka Kunugi, Sayaka Hashimoto: Research Report of Animal Feed, 22, 26 (1997).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
adult hen formula feed	100~1,000	3	83.3~91.7	11.6
dairy cattle formula feed	100~1,000	3	84.0~96.3	9.2
Alfalfa	100~1,000	3	90.0~93.0	8.0

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
dairy cattle formula feed	7	500	96.8	7.4	7.9	0.44

• Lower limit of quantification: 50 μg/kg in samples

«Notes and precautions»

[1] Prepare just before use and leave to cool in a dry desiccator. If the lot is changed to a new one, it is advisable to perform a fraction analysis for confirmation with the use of the standard solution.

[2] As for pigment-rich samples such as grass hay, the amount of sample shall be 10.0 g.

[3] The flow rate and the elution rate shall be ca. 1~2 mL/min.

[4] For example, Rtx-200 (Restek).

[5] An example of chromatogram is shown in Figure 6.1.86-1.

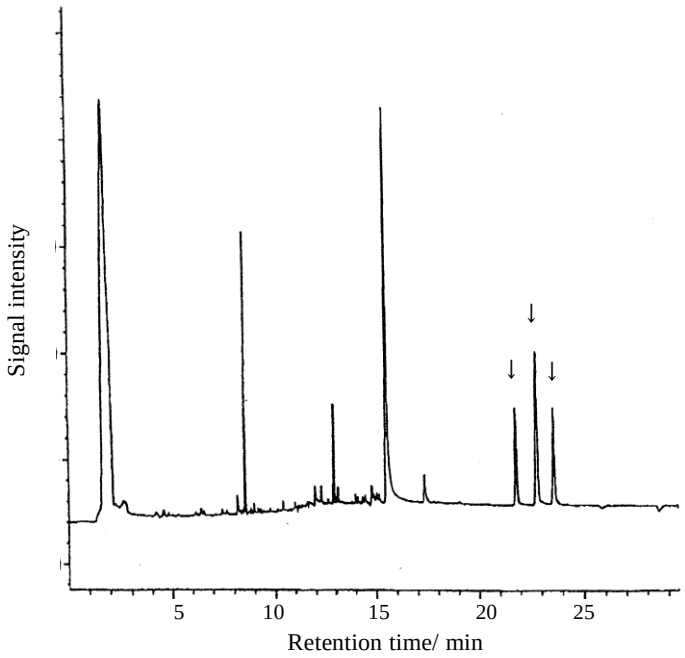


Figure 6.1.86-1. Chromatogram of a cypermethrin standard (1 ng)
(The arrows indicate the peaks of cypermethrin isomers.)

Measurement conditions

Detector: Electron capture detector

Column: Restek Rtx-200 (0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)

Carrier gas: He (1.5 mL/min, initial flow rate)

Make up gas: N_2 (60 mL/min)

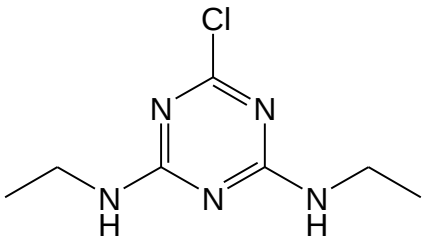
Sample injection: Splitless mode

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (2 min) → ramp 20 °C/min → 250 °C (18 min)

Detector temperature: 300 °C

87 Simazine (CAT)



6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine

$C_7H_{12}ClN_5$ MW: 201.66 CAS No.: 122-34-9

[Summary of simazine]

Simazine, a triazine herbicide developed by Ciba-Geigy (Switzerland), acts by inhibiting photosynthesis and is widely used to control weeds in cropland, lawns and the like. Although it is almost ineffective when applied to foliage, it is said to exhibit herbicidal activity against nearly all postemergent annual weeds by soil treatment. The registration standards of the Ministry of Environment state that simazine should not be detected in rice, wheat and barley, miscellaneous grain crops, fruits, vegetables, tubers, pulses and tea.

Simazine was registered as an agricultural chemical in 1958, in Japan. Registered name is CAT. The trade names are “Simazine” and “Primatol-S”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Corn: 0.3 ppm/pasture grass: 9 ppm

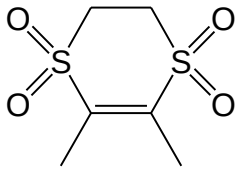
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for atrazine and simazine by gas chromatography

Target Analytes: Atrazine and simazine (2 compounds)

Refer to Article 10, Section 3 in this chapter.

88 Dimethipin



2,3-dihydro-5,6-dimethyl-1,4-dithiine 1,1,4,4-tetraoxide

$C_6H_{10}O_4S_2$ MW: 210.27 CAS No.: 55290-64-7

[Summary of dimethipin]

Dimethipin is a plant growth regulator that inhibits the synthesis of proteins of plant epidermal cells. It is used as a defoliant for defoliation of cotton plants, gum trees and vine like plants, as a desiccant for aerial part of potatoes, as well as a promoter of drying of rice, rapeseed, linseed and sunflower seed harvested.

Dimethipin has not been registerd as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.04 ppm

[Method listed in the Analytical Standards of Feeds]

1. Gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 88.1]

A. Reagent Preparation

Dimethipin Standard Solution. Weigh accurately 20 mg of dimethipin standard[$C_6H_{10}O_4S_2$][1], transfer to a 100 mL volmetric flask and dissolve by adding 20 mL of acetone. Further, add 2,2,4-trimethylpentane to the graduation line of the flask to prepare the dimethipin standard stock solution (1 mL of this solution contains 0.2 mg as dimethipin).

Before use, dilute accurately a certain amount of the standard stock solution with 2,2,4-trimethylpentane – acetone (4 : 1) to prepare several dimethipin standard solutions that contain 0.2 – 2 µg of dimethipin in 1 mL.

B. Quantification

Extraction Weigh 10.0 g of an analysis sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 20 mL of acetonitrile – water (3: 1) to moisten and leave to stand for 10 minutes. Further, add it 100 mL of acetonitrile and extract by shaking for 30 minutes.

Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetonitrile sequentially, and filter the wash by suction in the similar way. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 10 mL of cyclohexane – acetone mixture (7 : 3) accurately, transfer the solution to a 10 mL centrifuge tube and centrifuge at 1,500×*g* for 5 minutes. Filter the supernatant through a membrane filter (pore size: 0.5 µm or less) and use the filtrate as sample solution for gel

permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph. Dispense each dimethipin elution fraction into a 100 mL recovery flask, concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue with 2.5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) and use this solution as a sample solution for Column treatment.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column[2] (20 mm in inner diameter, 300 mm in length, particle size 15 µm)

Guard column: Styrene-divinylbenzene copolymer column[2] (20 mm in inner diameter, 100 mm in length, particle size 15 µm)

Eluent: Cyclohexane – acetone (7 : 3)

Flow rate: 5 mL/min

Fraction collection: 85-125 mL

Column treatment. Place the sample solution on a synthetic magnesium silicate minicolumn (100 mg)[*1], and discard the first 1 mL of the eluate. Collect the following 1 mL of the eluate to be a sample solution to be subjected to gas chromatography.

Gas chromatography. Inject 2 µL each of the sample solution and dimethipin standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame photometric detector (Filter for sulfur detection)

Column: Fused silica capillary column (5% diphenyl/95 % dimethylpolysiloxane coating, 0.32 mm in inner diameter, 30 m in length, film thickness 0.25 µm) [3]

Carrier gas: He (1.8 mL/min)

Make up gas: N_2 (30 mL/min)

Hydrogen: 75 mL/min

Dry air: 100 mL/min

Sample injection : Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (2 min) → 10 °C/min → 250 °C → (10 min)

Detector temperature: 280 °C

Calculation. Obtain square roots of the peak height or peak area from the resulting chromatograms[4] to prepare a calibration curve and subsequently calculate the amount of dimethipin in the sample.

* 1. Sep-Pak VAC Florisil Cartridge (Reservoir volume 1 mL, Waters) or equivalents.

«Summary of analysis method»

In this method, dimethipin in samples is extracted with acetonitrile, purified with a GPC and a Florisil minicolumn and quantified by a gas chromatograph with a flame photometric detector (filter for sulfur

detection).

The flow sheet of the analysis method is shown in Figure 6.1.88-1.

10 g sample
- Add 20 mL of acetonitrile-water (3:1), leave to stand for 10 minutes
- Add 100 mL of acetonitrile, shake for 30 minutes
- Filter by suction (Grade No. 5B)
- Wash with 50 mL of acetonitrile
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- Dissolve in 10 mL of cyclohexane-acetone (7:3)
- Centrifuge (1,500×*g*, 5 minutes)
- Membrane filter (pore size 0.5 μm or less)

GPC (Inject 5 mL of sample solution)
- Elution fraction 85-125 mL
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- Dissolve in 2.5 mL of 2,2,4-trimethylpentane-acetone (4:1)

Sep-Pak Vac Florisil cartridge
- Discard the first 1 mL
- Collect the following 0.5~1 mL

GC-FPD(S)

Figure 6.1.88-1. Flow sheet of the analysis method for dimethipin

Reference: Reiko Kazama,Yuji Shirai, Toshiaki Hayakawa: Research Report of Animal Feed, 22, 36 (1997).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
chicken formula feed	250~1,000	3	77.3~90.7	8.1
pig formula feed	250~1,000	3	76.3~82.7	10.4
alfalfa	250~1,000	3	85.7~100.3	4.9

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
growing chick formula feed	4	500	91.6	3.1	2.7	0.15

• Lower limit of quantification: 100 μg/kg in samples

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences, and other manufacturers.

[2] A column packed with styrene-divinylbenzen copolymer hard gel.

[3] For example, DB-5 (Agilent Technologies).

[4] An example of chromatogram is shown in Figure 6.1.88-2.

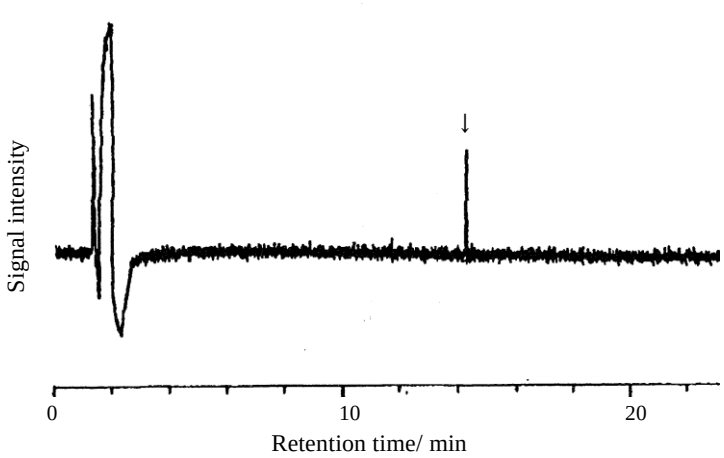


Figure 6.1.88-2. Chromatogram of a formula feed spiked with an amount equivalent to 0.25 mg/kg dimethipin (The arrow indicates the peak of dimethipin)

Measurement Conditions

Detector: Flame photometric detector (Filter for sulfur detection)

Column: J&W Scientific DB-5 (0.32 mm in inner diameter, 30 m in length, film thickness 0.25 μm)

Carrier gas: He (1.8 mL/min, initial flow rate)

Make up gas: N_2 (30 mL/min)

Hydrogen: 75 mL/min

Dry air: 100 mL/min

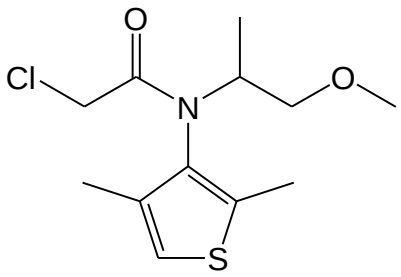
Sample injection : Splitless mode

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (2 min) → 10 °C/min → 250 °C → (10 min)

Detector temperature: 280 °C

89 Dimethenamid



(*RS*)-2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-(2-methoxy-1-methylethyl)acetamide

$C_{12}H_{18}ClNO_2S$ MW: 275.8 CAS No.: 87674-68-8, 163515-14-8 (*S*-isomer (dimethenamid-P))

[Summary of dimethenamid]

Dimethenamid is a non-hormone, absorption-type acid amide herbicide containing a thiophen ring.

Dimethenamid was registered as an agricultural chemical for cabbage, soybean and the like in 1996, in Japan. The trade name is “Fieldstar”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(Dimethenamid includes dimethenamid and dimethenamid-P.)

Barley, rye, corn: 0.1 ppm / Other grains: 0.01 ppm

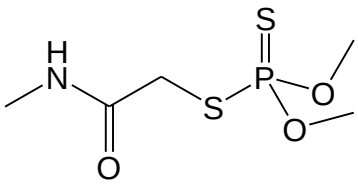
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

90 Dimethoate



O,*O*-dimethyl *S*-methylcarbamoylmethyl phosphorodithioate

$C_5H_{12}NO_3PS_2$ MW: 229.2 CAS No.: 60-51-5

[Summary of dimethoate]

Dimethoate is a systemic organophosphorous insecticide developed by Montecatini (Italy) and American Cyanamid (USA), having relatively low toxicity. It is rapidly-acting against sap-sucking pests and also shows residual efficacy.

Dimethoate was registerd as an agricultural chemical in 1961, in Japan. The trade names are "Dimethoate", "Kamikirin".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Barley: 0.04 ppm / Wheat: 0.05 ppm / Oat, rye and milo: 0.2 ppm / Corn: 1 ppm / Pasture grass: 2 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

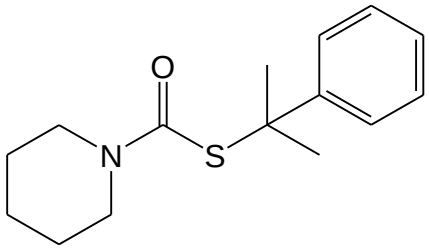
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

91 Dimepiperate



S-1-methyl-1-phenylethyl piperidine-1-carbothioate
$C_{15}H_{21}NOS$ MW: 263.4 CAS No.: 61432-55-1

[Summary of dimepiperate]

Dimepiperate is a thiocarbamate herbicide whose effectiveness against millet has been confirmed.

Dimepiperate has been registered as an agricultural chemical in Japan. However, it was expired in 2004.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

92 Methyl bromide

CH_3Br

methyl bromide
CH_3Br MW: 94.939 CAS No.: 74-83-9

[Summary of methyl bromide]

Methyl bromide is a colorless, odorless (but has a chloroform-like odor at high concentrations) gas that is used as a fumigant to control stored grain insect pests, such as maize weevil and rice weevil, as well as soil insect pests like nematode across a wide range of agricultural sectors. Methyl bromide has a melting point of −94 °C and boiling point of 3.6 °C, dissolves in water (17.5 g/L) and is soluble in organic solvents. It disappears by reaction with alkaline water or SH compounds.

Methyl bromide was registered as an agricultural chemical in 1952, in Japan. The trade names are "Mechiburon" and "Kayahyumu".

«Maximum Residue Limits in grains in the Food Sanitation Law»

(As inorganic bromine)

Wheat, barley and rye: 50 ppm / Corn: 80 ppm / Other grains: 50 ppm

[Method listed in the Analytical Standards of Feeds]

1. Gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 92.1]

A. Reagent Preparation

1) *O*,*O*-diethyl *S*-methyl dithiophosphate standard solution. Weigh accurately 25 mg of *O*,*O*-diethyl *S*-methyl dithiophosphate[$C_5H_{13}PS_2$][1], transfer to a 100 mL volmetric flask and dissolve by adding hexane. Further, add the same solvent to the graduation line of the flask to prepare the *O*,*O*-diethyl *S*-methyl dithiophosphate standard stock solution (1 mL of this solution contains 0.25 mg as *O*,*O*-diethyl *S*-methyl dithiophosphate).

Before use, dilute accurately a certain amount of the standard stock solution with hexane to prepare several *O*,*O*-diethyl *S*-methyl dithiophosphate standard solutions that contain 0.1 – 1.5 μg of *O*,*O*-diethyl *S*-methyl dithiophosphate in 1 mL.

2) Collecting solution. Dissolve 10 mg of *O*,*O*-diethyl *S*-ammonium dithiophosphate[2] in 60 mL of *N*,*N*-dimethylformamide (Prepare at the point of use.).

B. Quantification

Separation, collection and dithiophosphate esterification[3]. Weigh 25.0 g of an analysis sample and transfer it to a 500 mL two-neck flask. Connect the flask air-tightly to a collecting apparatus (two amber gas washing bottles each containing 30 mL of the collecting solution that are connected in series and immersed in a water bath at 60 °C). Add 200 mL of water adjusted to a pH of 2 with sulfuric acid and 1 mL of butanol to the two-neck flaks, to which a gas inlet tube is attached immediately. Inject nitrogen gas into the two-neck flaks in the water bath at 60 °C at flow rate of 60 – 80 mL/min[4] for 60 minutes

for separation, collection and dithiophosphate esterification[5] of methyl bromide to obtain a sample solution to be subjected to purification.

Purification. Transfer 150 mL of the sample solution prepared with sodium chloride solution (5 w/v%) to a 300 mL separating funnel A, add 50 mL of hexane, shake for 3 minutes and leave to stand. Transfer the water layer (lower layer) to a 300 mL separating funnel B, and the hexane layer (upper layer) to a Erlenmeyer flask, respectively. Add 50 mL of hexane to the separating funnel B, treat in the similar way and combine the hexane layer with the content of the Erlenmeyer flask above. Dehydrate the hexane layer with a suitable amount of sodium sulfate (anhydrous) and filter through a filter paper (No. 5A) into a 200 mL recovery flask. Wash the Erlenmeyer flask above and the filter paper with a small amout of hexane sequentially, filter the washeings through this filter paper and combine the filtrates. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to ca. 2 mL*1 [6], transfer it with a small amount of hexane to 10 mL volmetric flask, add the same solvent up to the graduation line of the flask to prepare a sample solution to be subjected to gas chromatography.

Gas chromatography. Inject 1 µL each of the sample solution and respective *O*,*O*-diethyl *S*-methyl dithiophosphate standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame photometric detector (Filter for phosphorus detection) [7]

Column: Fused silica capillary column (5% diphenyl/ 95 % dimethyl-polysiloxane coating, 0.32 mm in inner diameter, 30 m in length, film thickness 0.25 µm) [8]

Carrier gas: He (1.5 mL/min)

Make up gas: N_2 (30 mL/min)

Hydrogen: 75 mL/min

Dry air: 100 mL/min

Sample injection : Splitless mode (60 s) [9]

Injection port temperature: 230 °C

Column oven temperature: Initial temperature 60 °C (hold 0.5 min) → ramp 20 °C/min → 80 °C (hold 0.5 min) → ramp 10 °C/min → 160 °C (hold 0.5 min)

Detector temperature: 240 °C

Calculation. Obtain the peak area from the resulting chromatograms[10] to prepare a calibration curve, and calculate the amount of methyl bromide[CH_3Br]in the sample using the following formula.

The amount of methyl bromide in the sample (µg/kg) = $A \times 400 \times 0.474$[11]

A: The weight (ng) of *O*,*O*-diethyl *S*-methyl dithiophosphate in 1 µL of the sample solution obtained from the calibration curve.

* 1. Do not dry up.

«Summary of analysis method»

In this method, methyl bromide in feeds is separated by aeration under sulfuric acid condition, collected in *O*,*O*-diethyl *S*-ammonium dithiophosphate · *N*,*N*-dimethylformamide solution (DPA · DMF solution) for dithiophosphate estrification, fractionated by liquid-liquid partition using hexane and

quantified by a gas chromatograph with a flame photometric detector (filter for phosphorus detection).

The flow sheet of the analysis method is shown in Figure 6.1.92-1.

25 g sample
- Add 200 mL of distilled water (adjusted to pH 2 with surfuric acid)
- Add 1 mL of butanol

Aeration, derivatization
- In a water bath at 60 °C for 60 minutes
- Collect into DPA · DMF solution, derivatize

Fractionate using hexane
- Add 150 mL 5 % sodium chloride solution
- Add 50 mL hexane ×2

Hexane layer
- Dehydrate with sodium sulfate (anhydrous)
- Concentrate under vacuum (to 2~3 mL, not dry up)
- Dilute with hexane to volume of 10 mL

GC-FPD(P)

Figure 6.1.92-1. Flow sheet of the analysis method for methyl bromide

Reference: Toshiaki Hayakawa, Shinji Kawaguchi: Research Report of Animal Feed, 18, 13 (1993).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
chicken formula feed	125~500	3	92.6~97.8	9.5
pig formula feed	125~500	3	90.4~100.9	5.9
cattle formula feed	125~500	3	91.8~100.6	7.7

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
dairy cattle formula feed	5	250	92.4	6.1	6.2	0.31

• Lower limit of quantification: 5 μg/kg in samples

«Notes and precautions»

[1] It is most likely unavailable now. At the time this method was investigated, *O,O*-diethyl *S*-methyl dithiophosphate (5 g) was available from Koso Chemical.

[2] Diethylammonium dithiophosphate (10 g) is available from Kanto Chemical. If peaks of blanks or impurities are detected with the use of this product, dissolve this product in acetone, reprecipitate, filter, wash with benzene and air dry before use.

[3] The apparatus for collection and derivatization of methyl bromide is shown in Figure 6.1.92-2.

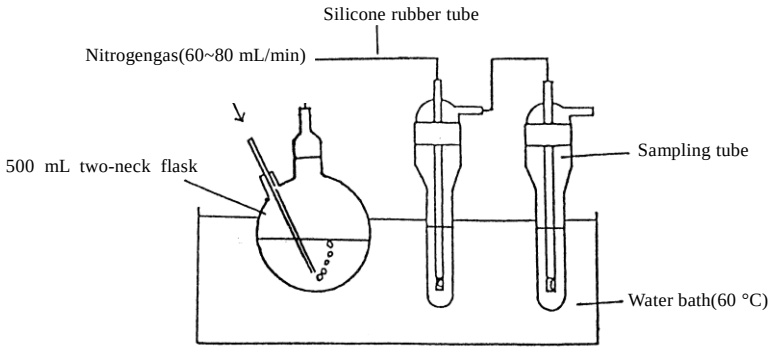
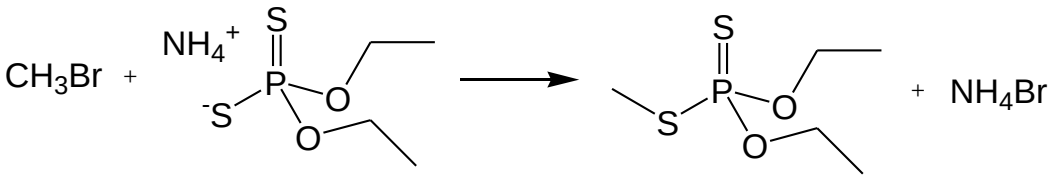


Figure 6.1.92-2. Apparatus for collection & derivatization of methyl bromide

[4] Inadequate flow rate of nitrogen gas can lead to a dispersion and decrease in the recovery caused by insufficient bubbling.

[5] Dithiophosphate esterification reaction formula of methyl bromide



[6] Excessive concentration can lead to the loss of *O*,*O*-diethyl *S*-methyl dithiophosphate.

[7] NPD may be used.

[8] For example, DB-5 (Agilent Technologies).

[9] Sample injection mode can be selected from split, splitlees or cool-on modes.

[10] An example of chromatogram is shown in Figure 6.1.92-3.

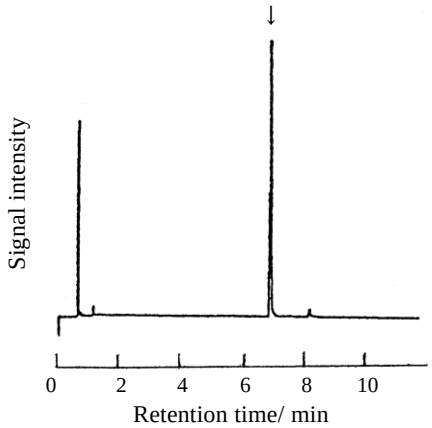


Figure 6.1.92-3. Chromatogram of a formula feed spiked with an amount equivalent to 0.25 mg/kg methyl bromide (The arrow indicates the peak of *O*,*O*-diethyl *S*-methyl dithiophosphate.)

Measurement conditions

Detector: Flame photometric detector (Filter for phosphorus detection)

Column: J&W Scientific DB-5 (0.53 mm in inner diameter, 15 m in length, film thickness 1.5 μm)

Carrier gas: He (10 mL/min, initial flow rate)

Make up gas: N_2 (30 mL/min)

Hydrogen: 75 mL/min

Dry air: 100 mL/min

Sample injection : Cool on mode

Injection port temperature: 230 °C

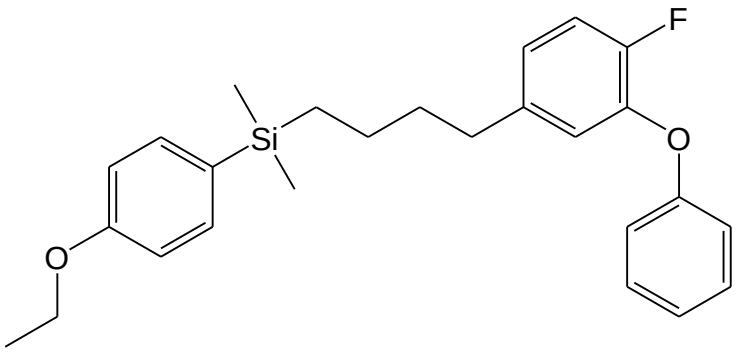
Column oven temperature: Initial temperature 60 °C (hold 0.5 min) → ramp 20 °C/min → 80 °C → (hold 0.5 min) → ramp 10 °C/min → 160 °C (hold 0.5 min) → ramp 30 °C/min → 230 °C (hold 2 min)

Detector temperature: 250 °C

[11] Conversion factor

$$0.474 = \frac{\text{Molecular weight of methyl bromide (94.95)}}{\text{Molecular weight of } O,O\text{-diethyl } S\text{-methyl dithiophosphate (200.27)}}$$

93 Silafluofen



(4-ethoxyphenyl)[3-(4-fluoro-3-phenoxyphenyl)propyl](dimethyl)silane

$C_{25}H_{29}FO_2Si$ MW: 408.6 CAS No.: 105024-66-6

[Summary of silafluofen]

Silafluofen is a pyrethroid insecticide containing a silicon atom, developed by Dainihon Jochugiku in 1984 and Hoechst (Germany, now Bayer CropScience) in 1985 independnetly. It acts by altering the nerve membrane permeability to sodium ion and eventually inhibiting the nerve conduction in insects.

Silafluofen was registered as an agricultural chemical for control insect pests in rice, soybean and the like in 1995, in Japan. The trade name is "MR. Joker".

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.05 ppm

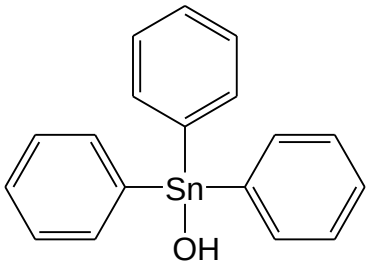
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

94 Fentin hydroxide (TPTH)



triphenyltin hydroxide

$C_{18}H_{16}OSn$ MW: 367.03 CAS No.: 76-87-9

[Summary of Fentin hydroxide]

Fentin hydroxide is an organotin fungicide, which had been used as a magic bullet for Cercospora leaf spot of beet, because it had been regarded as being not so harmful due to its low vapor pressure and stabile against ultraviolet irradiation. Fentin hydroxide registration as an agricultural chemical was expired in 1990.

Fentin hydroxide is now regulated as a class II specified chemical substance under the Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture etc. However, it has been widely used as a insecticide and an antifungal ship bottom paint for a long time, marine pollution and contamination of fish and shellfish have now become a serious problem.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(As fentin (including triphenyltin hydroxide, triphenyltin acetate and triphenyltin chloride converted into fentin content respectively.)

Wheat, barley, rye, corn and othergrains: 0.05 ppm

[Method listed in the Analytical Standards of Feeds]

1. Gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 94.1]

A. Reagent Preparation

Triphenyltin chloride Standard Stock Solution. Weigh accurately 20 mg of triphenyltin chloride [$C_{18}H_{15}ClSn$][1], transfer to a 100 mL volmetric flask and dissolve with hexane. Further, add the same solvent to the graduation line of the flask to prepare triphenyltin chloride stock solution (1 mL of this solution contains 0.2 mg as triphenyltin chloride).

B. Quantification

Extraction. Weigh accurately 5 – 10 g of an analysis sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 10 mL of water to moisten and leave to stand for 5 minutes. Further, add it 70 mL of methanol – hydrochloric acid (9: 1) and extract by shaking for 30 minutes.

Place a 200 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 10 mL of methanol – hydrochloric

acid (9 : 1) sequentially, and filter the washings by suction in the similar way. Use the filtrate as a sample solution for purification.

Purification. Transfer sample solution to a 500 mL separating funnel A already containing 50 mL of sodium chloride solution (10 w/v%) and 50 mL of ethyl acetate – hexane (3 : 2), wash the Erlenmeyer flask that has contained the sample solution with 50 mL of ethyl acetate and add the wash to the separating funnel A. Shake the separating funnel A vigorously for 5 minutes and leave to stand. Transfer the water layer (lower layer) to a 500 mL separating funnel B. Add 50 mL of ethyl acetate – hexane (3 : 2) to the separating funnel B, shake vigorously for 5 minutes and leave to stand. Combine the ethyl acetate – hexane layer (upper layer) with the content of the separating funnel A above. Further, add 150 mL of hexane to the separating funnel A, shake gently and leave to stand for 30 minutes. Discard the water layer and transfer the ethyl acetate – hexane layer to the Erlenmeyer flask. Dehydrate the ethyl acetate – hexane layer with a suitable amount of sodium sulfate (anhydrous) and filter through a filter paper (No. 2S) into a 500 mL recovery flask. Wash the Erlenmeyer flask above and the residue with a small amout of hexane sequentially, filter the washes through this filter paper and combine the filtrates. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to almost dryness. Further, add 10 mL of hexane each and concentrate the filtrate under reduced pressure twice in the similar way to remove hydrochloric acid, and dry up by nitrogen gas flow. Dissolve the residue by adding 10 mL of hexane accurately to obtain a sample solution to be subjected to propylation.

Propylation reaction. Transfer 1 – 2 mL of the sample solution accurately to a 50 mL test tube, add 1 mL of *n*-propylmagnesium bromide solution[2] and leave to stand for 30 minutes. Further, add 7 mL of sulfuric acid (0.5 mol/L) bit by bit[3] to decompose excessive *n*-propylmagnesium bromide. After add 7 mL of methanol to this test tube, transfer the whole content to a 100 mL separating funnel C. Wash the test tube above with 25 mL of water and 20 mL of hexane, and combine the washes sequentially with the content of the separating funnel C. Shake the separating funnel C for 5 minutes and leave to stand. Transfer the water layer (lower layer) to a 100 mL separating funnel D and the hexane layer (upper layer) to an Erlenmeyer flask, respectively. Add 20 mL of hexane to the separating funnel D, shake for 5 minutes, leave to stand and transfer the hexane layer to the Erlenmeyer flask above. Dehydrate the hexane layer with a suitable amount of sodium sulfate (anhydrous) and filter through a filter paper (No. 2S) into a 100 mL recovery flask. Wash the Erlenmeyer flask above and the filter paper with a small amout of hexane sequentially, filter the washes through this filter paper and combine the filtrates. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and dry up by nitrogen gas flow.

Dissolve the residue by adding 10 mL of hexane to obtain a sample solution to be subjected to column treatment.

Column treatment Wash a synthetic magnesium silicate minicolumn (910 mg) with 10 mL of hexane. Place a 100 mL recovery flask under the minicolumn and place the sample solution on the minicolumn. Wash the recovery flask that has contained the sample solution with a small amount of hexane, add the washings to the minicolumn and elute propylated triphenyltin chloride flow out by natural flow[4]. Further, add 10 mL of hexane – diethyl ether (99: 1) to the column to obtain an eluate in the similar way.

Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 2 mL of hexane accurately to obtain a sample solution to be subjected to gas chromatography.

Propylation of standard solution Transfer 1 mL of triphenyltin chloride standard stock solution accurately to a 50 mL test tube and propylate under the same conditions as described in the section "Propylation reaction".

Dissolve the residue by adding 10 mL of hexane accurately, dilute a certain amount of this solution accurately with hexane to prepare several standard solutions that contain 0.025 – 1.5 μg of triphenyltin chloride in 1 mL.

Gas chromatography. Inject 2 μL each of the sample solution and standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame photometric detector (Filter for tin detection)

Column: Fused silica capillary column (100 % dimethylpolysiloxane coating, 0.32 mm in inner diameter, 30 m in length, film thickness 0.25 μm) [5]

Carrier gas: He (4 mL/min)

Make up gas: N_2 (50 mL/min)

Hydrogen: 75 mL/min

Dry air: 100 mL/min

Sample injection : Splitless mode (60 s)

Injection port temperature: 280 °C

Column oven temperature: Initial temperature: 100 °C (hold 2 min) → ramp 25 °C/min → 270 °C (hold 5 min)

Detector temperature: 280 °C

Calculation. Obtain the peak area from the resulting chromatograms[6] to prepare a calibration curve, calculate the amount of triphenyltin chloride, and further determine the amount of triphenyltin hydroxide in the sample by multiplying 0.952.

«Summary of analysis method»

In this method, extract triphenyltin hydroxide as triphenyltin chloride with hydrochloric acid-added methanol, purified by liquid-liquid partition, propylated, further purified with the use of a Florisil minicolumn and quantified by a gas chromatograph with a flame photometric detector (filter for tin detection).

The flow sheet of the analysis method is shown in Figure 6.1.94-1.

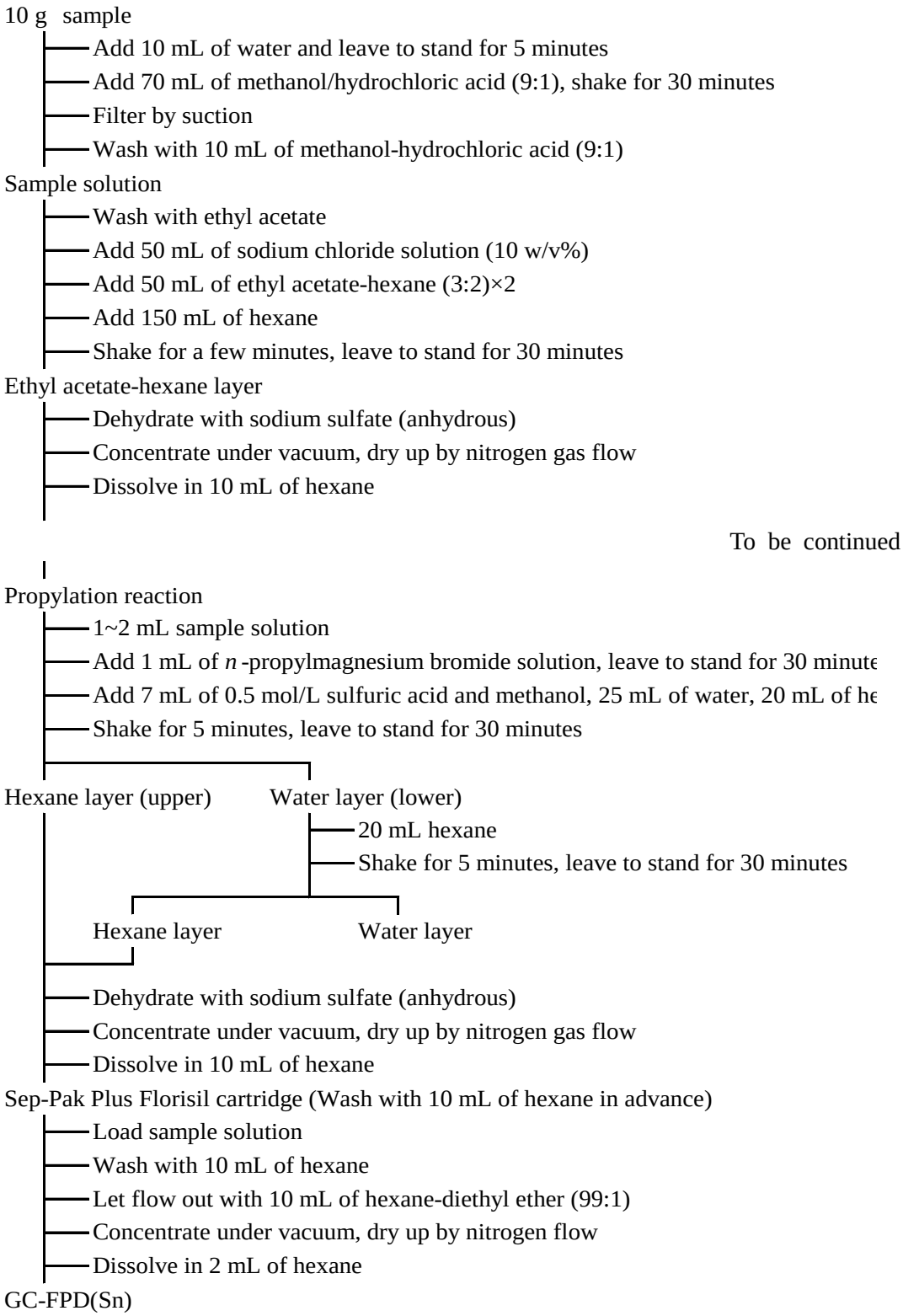


Figure 6.1.94-1. Flow sheet of the analysis method for triphenyltin hydroxide

Reference: Susumu Yoshinaga: Research Report of Animal Feed, 23, 33(1998).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Chicken formula feed	500~2,000	3	86.5~94.0	13.9
Pig formula feed	500~2,000	3	81.0~87.3	4.3
Dairy cattle formula feed	500~2,000	3	80.3~82.3	2.7
Fish meal	500~2,000	3	82.5~91.6	4.9
Timothy hay	500~2,000	3	74.2~79.2	4.0

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
piglet formula feed	6	1,000	88.0	5.8	8.3	0.51

• Lower limit of quantification: 50 μg/kg in samples

«Notes and precautions»

[1] Available from Wako Pure Chemical Industries as triphenyltin chloride.

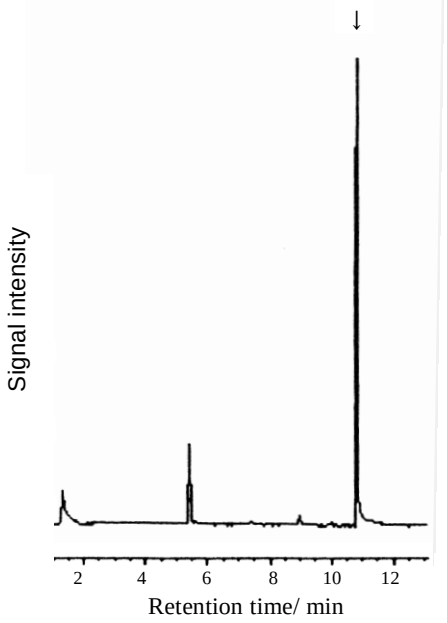
[2] Because of possible sudden boiling, add *n*-propylmagnesium bromide solution bit by bit.

[3] Because of the risk of heat generation and sudden boiling, add sulfuric acid bit by bit and, if necessary, cool in ice.

[4] Let flow naturally.

[5] For example, DB-1 (Agilent Technologies).

[6] An example of chromatogram is shown in Figure 6.1.94-2.



Measurement conditions

Detector: Flame photometric detector (Filter for tin detection)

Column: J&W scientific DB-1 (0.32 mm in inner diameter, 30 m in length, film thickness 0.25 μm)

Carrier gas: He (4 mL/min)

Make up gas: N_2 (50 mL/min)

Hydrogen: 75 mL/min

Dry air: 100 mL/min

Sample injection : Splitless mode

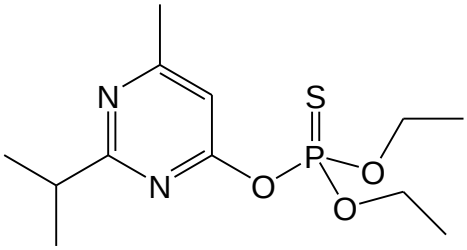
Injection port temperature: 280 °C

Column oven temperature: Initial temperature: 100 °C (2 min) → 25 °C/min → 270 °C (5 min)

Detector temperature: 280 °C

Figure 6.1.94-2. Chromatogram of a pig formula feed spiked with triphenyltin hydroxide (The arrow indicates the peak of propyltriphenyltin)

95 Diazinon



O,*O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate

$C_{12}H_{21}N_2O_3PS$ MW: 304.3 CAS No.: 333-41-5

[Summary of diazinon]

Diazinon, a colorless oily liquid, is an organophosphorous insecticide developed by Ciba-Geigy (Switzerland).

It is used for control of a wide range of insect pests as well as for expelling of hygiene pests in home. It is rapid-acting, has permeability into plants, but little persistence.

Diazinon was registereted as an agricultural chemical in 1960, in Japan. The trade names are "Dianzinon" and "Exozinon".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

Corn: 0.02 ppm / Oat, barley, wheat, milo and rye: 0.1 ppm / Pasture grass: 10 ppm

Rice plant silage: 1 ppm / Rice straw: 2 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

3. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (2)

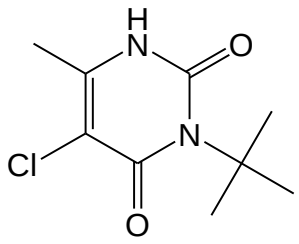
Target Analytes:

Group A: EPN, diazinon, parathion, fenitrothion, fenthion, phenthoate, phosalone and malathion (8 compounds).

Group B: Dichlorvos (1 compound)

Refer to Article 3, Section 2 in this chapter.

96 Terbacil



3-*tert*-butyl-5-chloro-6-methyluracil

$C_9H_{13}ClN_2O_2$ MW: 216.7 CAS No.: 5902-51-2

[Summary of terbacil]

Terbacil is an urasil herbicide developed by DuPont (USA) in the 1960's.

Terbacil was registered as an agricultural chemical for citrus in 1970, in Japan. The trade name is "Sinbar".

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

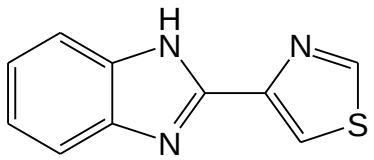
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

97 Thiabendazol (TBZ)



2-(thiazol-4-yl)benzimidazole

$C_{10}H_7N_3S$ MW: 201.25 CAS No.: 148-79-8

[Summary of thiabendazol (TBZ)]

Thiabendazol, a benzimidazole fungicide developed by Sankyo Corporation, is a white powder. It is not only registered as an agricultural chemical, but also is a specified food additive as a preservative for use in citrus and banana. In the USA, it is also used as a post-harvest agricultural chemical.

Thiabendazol was registeted as an agricultural chemical in 1972, in Japan. The trade names are "BioGuard" and "UniTect".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Oat, barley, corn, milo and rye: 0.05 ppm / heat: 0.5 ppm / Pasture grass: 10 ppm

[Method listed in the Analytical Standards of Feeds]

1. Liquid chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 97.1]

A. Reagent Preparation

1) Thiabendazol standard solution. Weigh accurately 20 mg of thiabendazol[$C_{10}H_7N_3S$] [1], transfer to a 100 mL brown volmetric flask and dissolve by adding methanol. Further, add the same solvent up to the graduation line of the flask to prepare the thiabendazol standard stock solution (1 mL of this solution contains 0.2 mg as thiabendazol).

Before use, dilute accurately a certain amount of the standard stock solution with methanol to prepare several thiabendazol standard solutions that contain 0.05 – 0.5 µg of thiabendazol in 1 mL.

2) Sodium acetate buffer. Dissolve 33 g of sodium acetate (anhydrous) and 200 g of sodium chloride with water to make 1 L.

B. Quantification

Extraction[2]. Weigh 5 g of an analysis sample accurately, transfer it to a 300 mL separating funnel, add 30 mL of sodium acetate buffer and 100 mL of ethyl acetate and extract by shaking for 30 minutes. Place a tall beaker under a Büchner funnel and filter the extract through a filter paper (No. 5B) [3] by suction. Then, wash the separating funnel above and the residue with 30 mL of ethyl acetate and 10 mL of water sequentially, and filter the washings by suction in the similar way. Use this filtrate as a sample solution for purification.

Purification. Transfer sample solution to a 300 mL separating funnel A, add 30 mL of sodium hydroxide

(1 mol/L), shake for 5 minutes and leave to stand. Discard the water layer (lower layer). Add 30 mL of water to the separating funnel A and treat in the similar way. Add 50 mL of hydrochloric acid (0.1 mol/L) to the separating funnel A, shake for 5 minutes[4] and leave to stand. Transfer the water layer to a 500 mL separating funnel B. Add 50 mL of hydrochloric acid (0.1 mol/L) to the separating funnel A and treat in the similar way. Add 30 mL of sodium hydroxide (1 mol/L) and 100 mL of ethyl acetate to separating funnel B, shake for 5 minutes and leave to stand. Transfer the water layer to a 500 mL separating funnel C. Add 100 mL of ethyl acetate to the separating funnel C, treat in the similar way and combine the ethyl acetate layer (upper layer) with the content of the separating funnel B. Add 30 mL of water to the separating funnel B, shake and leave to stand. Transfer the ethyl acetate layer to an Erlenmeyer flask. Dehydrate the ethyl acetate layer with a suitable amount of sodium sulfate (anhydrous) and filter through a filter paper (No. 5A) into a 500 mL recovery flask. Wash the Erlenmeyer flask above and the filter paper with a small amout of ethyl acetate, and combine the washings through this filter paper. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and dry up by nitrogen gas flow.

Dissolve the residue by adding 5 mL of water to obtain a sample solution to be subjected to column treatment.

Column treatment. Wash an octadecylsilylated silica minicolumn (360 mg) with 10 mL of methanol and 10 mL of water sequentially.

Place the sample solution on the minicolumn and let flow out. Wash the recovery flask that has contained the sample solution with 5 mL of water, add the washing to the column to let flow out. Further, add 5 mL of water – methanol (7 : 3) to the minicolumn to wash. Place a 50 mL recovery flask under the column and add water – methanol (1 : 1) to the minicolumn to elute thiabendazol. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and dry up by nitrogen gas flow.

Dissolve the residue by adding 5 mL of methanol accurately and filter through a membrane filter (pore size: 0.5μm or less) to obtain a sample solution to be subjected to liquid chromatography.

Liquid chromatography. Inject 10 μL each of the sample solution and respective thiabendazol standard solutions into a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Fluorescence detector (300 nm excitation wavelength, 355 nm emission wavelength)

Column: Octadecylsilylated silica gel column (4.6 mm in inner diameter, 250 mm in length, particle size 5 μm)[*1] [5]

Eluent: Methanol – potassium dihydrogenphosphate solution[*2] (1 : 1)

Flow rate: 0.8 mL/min

Column oven temperature: 40 °C

Calculation. Obtain the peak height or peak area from the resulting chromatograms[6] to prepare a calibration curve and subsequently calculate the amount of thiabendazol in the sample.

* 1. Finepak SIL C_{18} T-5 (JASCO) or equivalents.

2. Dissolve 1.36 g of potassium dihydrogenphosphate in water to make 1 L.

«Summary of analysis method»

In this method, by taking advantage of high solubility in ethyl acetate and water solubility of thiabendazol when being rendered faintly alkaline and being acidified respectively, thiabendazol is extracted with ethyl acetate, purified with the use of liquid-liquid partition as well as a C_{18} minicolumn and quantified by a liquid chromatograph with a fluorescence detector.

The flow sheet of the analysis method is shown in Figure 6.1.97-1.

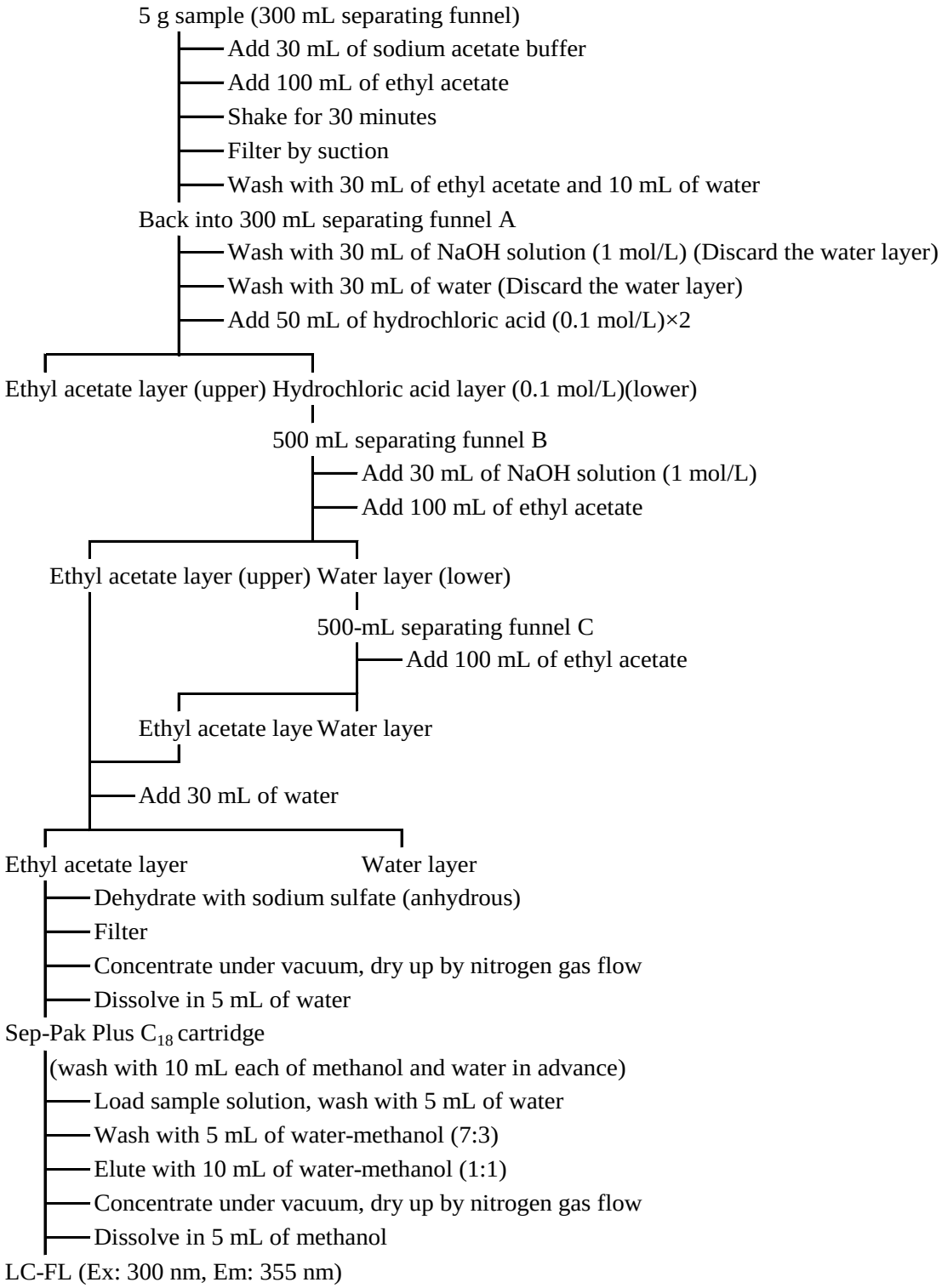


Figure 6.1.97-1. Flow sheet of the analysis method for thiabendazol

Reference: Atsushi Noguchi: Research Report of Animal Feed, 17, 42(1992).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Corn	50~500	3	82.9~87.2	4.2
starting chick formula feed	50~500	3	85.5~90.1	6.1
finishing beef cattle formula feed	50~500	3	82.8~84.3	7.0

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
growing chick formula feed	6	100	81.7	5.1	7.1	0.32

• Lower limit of quantification: 10 μg/kg in samples

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical and other manufacturers.

[2] As an extraction solvent, acetone – water (7 : 3) can also be used. In that case, the extraction method is as follows.

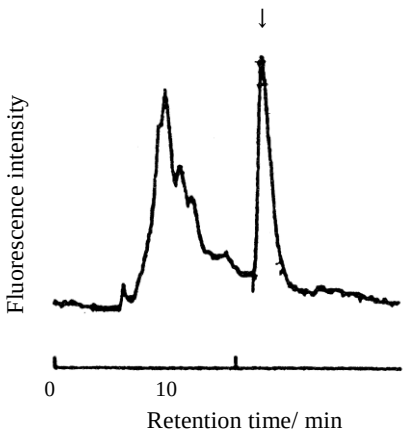
Transfer 20 g of an analysis sample (10 g of grass hay) to a 500 mL separating funnel, add 30 mL of water and leave to stand for 30 minutes. Further, add 70 mL of acetone, shake for 30 minutes to extract. Place a 200 mL volmetric flask under a a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the separating funnel above and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way. Then, add acetone up to the graduation line of the volmetric flask. Transfer 50 mL (100 mL for grass hay) of this solution to a 200 mL recovery flask and concentrate in a water bath at 40 °C or lower to ca. 10 mL under reduced pressure. Transfer the concentrate with ethyl acetate to a 300 mL separating funnel, add 50 mL of sodium acetate buffer and 50 mL of ethyl acetate and shake for 5 minutes. Collect the ethyl acetate layer to a 300 mL separating funnel A. Add 50 mL of ethyl acetate to the water layer, treat in the similar way. The subsequent procedures are same as those described in "B. Quantification" after the sentence "Add 50 mL of hydrochloric acid (0.1 mol/L) to the separating funnel A".

[3] A filter aid, such as Celite 545 may be used. If it is clogged in the middle of filtering, scraping the surface of the filter aid will work well.

[4] Caution is demanded, because emulsion is formed easily. Adding water allows easier separation of the emulsion formed.

[5] Any columns packed with an endcapped packing material equivalent to this one may be used.

[6] An example of chromatogram is shown in Figure 6.1.97-2.



Measurement conditions

Detector: excitation wavelength 300 nm, emission wavelength 355 nm

Column: Finepak SIL C18 T-5 (4.6 mm in inner diameter, 250 mm in length, particle size 5 μm)

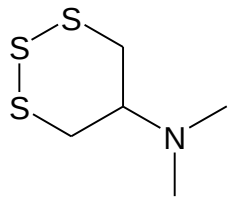
Eluent: Methanol – potassium dihydrogenphosphate solution (Dissolve 1.36 g of potassium dihydrogenphosphate in water to make 1L) (1: 1)

Flow rate: 0.8 mL/min

Column oven temperature: 40 °C

Figure 6.1.97-2. Chromatogram of a formula feed spiked with an amount equivalent to 0.1 mg/kg thiabendazol (The arrow indicates the peak of thiabendazol.)

98 Thiocyclam



N,*N*-dimethyl-1,2,3-trithian-5-ylamine

Thiocyclam	$C_5H_{11}NS_3$	MW: 181.34	CAS No.: 31895-21-3
Thiocyclam oxalate	$C_7H_{13}NO_4S_3$	MW: 271.38	CAS No.: 31895-22-4

[Summary of thiocyclam]

Thiocyclam is a nereistoxin insecticide that inhibits acetylcholine stimulus transmission in insects.

Thiocyclam was registered as an agricultural chemical (as thiocyclam oxalate) to be used on rice, vegetable and the like in 1981, in Japan. The trade name is "Evisect".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(The sum of cartap, thiocyclam converted into cartap content and bensultap converted into cartap content)

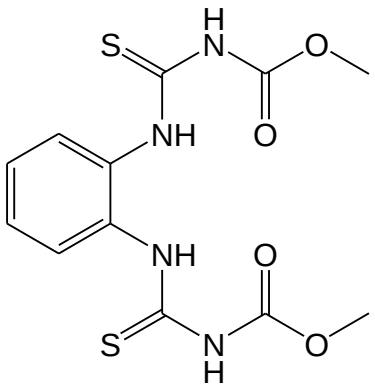
Oat, barley, wheat, corn, milo and rye: 0.2 ppm / Pasture grass: 0.7 ppm

[Method listed in the Analytical Standards of Feeds]

1. Analysis method for cartap, thiocyclam and bensultap by liquid chromatograph-mass spectrometer

   Refer to Article 45.1 in this section.

99 Thiophanate-methyl



dimethyl 4,4′-(*o*-phenylene)bis(3-thioallophanate)

$C_{12}H_{14}N_4O_4S_2$ MW: 342.39 CAS No.: 23564-05-8

[Summary of thiophanate-methyl]

Thiophanate-methyl is a benzimidazole fungicide developed by Nippon Soda. It acts by being hydrolyzed in the metabolic process to form carbendazim.

Thiophanate-methyl was registereted as an agricultural chemical in 1971, in Japan. The trade names are "Topjin M", "Topgrass Dry", etc.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

(The sum of carbendazim, benomyl converted into carbendazim content, thiophanate converted into carbendazim content and thiophanate methyl converted into carbendazim content)

Oat, barley, wheat, milo and rye: 0.6 ppm / Corn: 0.7 ppm / Pasture grass: 10 ppm

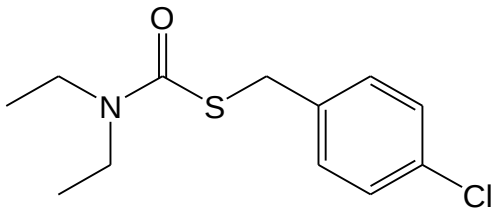
Rice plant silage: 0.1 ppm / Rice straw: 0.3 ppm

[Method listed in the Analytical Standards of Feeds]

1. Analysis method for carbendazim, thiophanate-methyl and benomyl by liquid chromatograph-mass spectrometer

Refer to Article 48.1 in this section.

100 Thiobencarb (Benthiocarb)



S-4-chlorobenzyl *N*,*N*-diethyl thiocarbamate
$C_{12}H_{16}ClNOS$ MW: 257.8 CAS No.: 28249-77-6

[Summary of thiobencarb]

Thiobencarb (benthiocarb) is a thiocarbamate herbicide developed by Kumiai Chemical Industry. Its mechanism of action is inhibition of cell growth by inhibition of protein synthesis at the growing points of plants.

Thiobencarb was registretes as an agricultural chemical in 1970, in Japan. Registered name is benthiocarb. The trade name is “Saturn”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Administrative Guidelines for Hazardous Substances in Feeds]

Rice straw: 0.1 ppm

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye and corn: 0.1 ppm

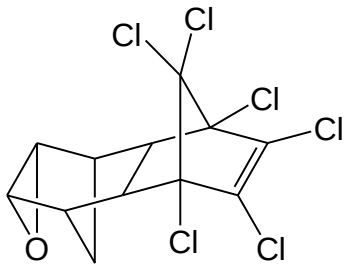
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

101 Dieldrin (HEOD)



(1a*R*,2*R*,2a*S*,3*S*,6*R*,6a*R*,7*S*,7a*S*)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7: 3,6-dimethanonaphtho[2,3-*b*]oxirene

$C_{12}H_8Cl_6O$ MW: 380.9 CAS No.: 60-57-1

[Summary of dieldrin (HEOD)]

Dieldrin (HEOD), which is an epoxide of aldrin, is an organochlorine insecticide. It is acid- and alkali-stable, and has aldrin-like properties.

Because dieldrin decomposes in the environment more slowly than aldrin and is absorbed by crops, soil pollution and crop contamination persist for prolonged periods.

Dieldrin had also been used for termite control, but now, its use is banned totally.

Although aldrin and dieldrin are listed separately in the Analytical Standards of Feeds, the Maximum Residue Limits established in the Law Concerning Safety Assurance and Quality Improvement of Feeds is the sum of their contents.

Dieldrin had been registered as an agricultural chemical in 1954, in Japan. However, it was expired in 1975. In 1981, its manufacture, sale and use for all purpose were prohibited.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(The sum of aldrin and dieldrin)

Pasture grass: 0.02 ppm

Pig feed, chicken and quail feed, as well as cattle, sheep, goat and deer feed: 0.02 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

Group A: α-BHC, β-BHC, γ-BHC, δ-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-

DDT, aldrin, α-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, β-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

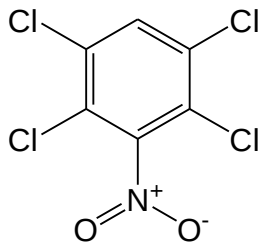
Refer to Article 1, Section 2 in this chapter.

3. Simultaneous analysis method of organochlorine agricultural chemicals by gas chromatography

Target Analytes: α-BHC, β-BHC, γ-BHC, δ-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, endrin, dieldrin, heptachlor and heptachlor epoxide (15 compounds)

Refer to Article 2, Section 3 in this chapter.

102 Tecnazene



1,2,4,5-tetrachloro-3-nitrobenzene

$C_6HCl_4NO_2$ MW: 260.9 CAS No.: 117-18-0

[Summary of tecnazene]

Tecnazene is an aromatic insecticide.

Tecnazene has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.05 ppm

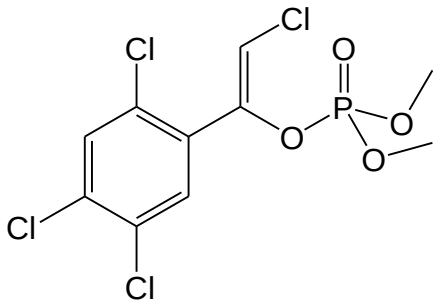
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

103　Tetrachlorvinphos (CVMP)



(*Z*)-2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate

$C_{10}H_9Cl_4O_4P$　MW: 366.0　CAS No.: 22248-79-9

[Summary of tetrachlorvinphos (CVMP)]

Tetrachlorvinphos (CVMP) is an organophosphorous insecticide developed by Shell Chemicals (USA).

Tetrachlorvinphos was registeret as an agricultural chemical in 1971, in Japan. Registered name had been CVMP. However, it was expired in 2003.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limitUniform limit)

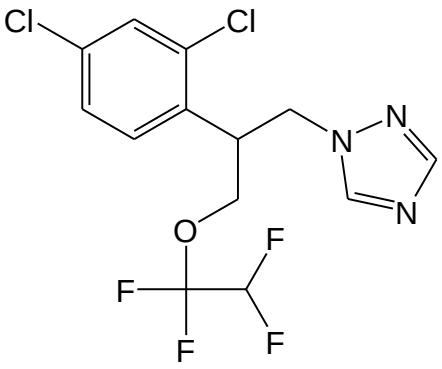
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

104 Tetraconazole



(*RS*)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether

$C_{13}H_{11}Cl_2F_4N_3O$ MW: 372.1 CAS No.: 112281-77-3

[Summary of tetraconazole]

Tetraconazole is a triazole fungicide.

Tetraconazole was registered as an agricultural chemical to be used on fruit trees and vegetables in 1998, in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat: 0.05 ppm / Barley: 0.2 ppm / other grains: 0.1 ppm

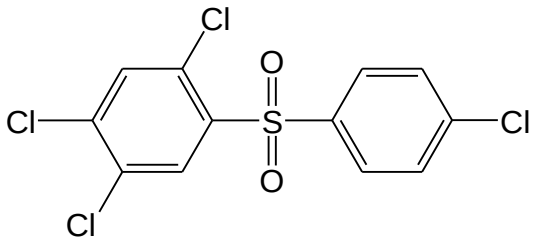
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

105 Tetradifon



4-chlorophenyl 2,4,5-trichlorophenyl sulfone

$C_{12}H_6Cl_4O_2S$ MW: 356.1 CAS No.: 116-29-0

[Summary of Tetradifon]

Tetradifon is a diphenylsulfone miticide, developed by Philips-Duphar GmbH (Netherlands) in 1954.

Tetradifon was registered as an agricultural chemical to be used on fruit trees and vegetables in 1973, in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn: 5 ppm

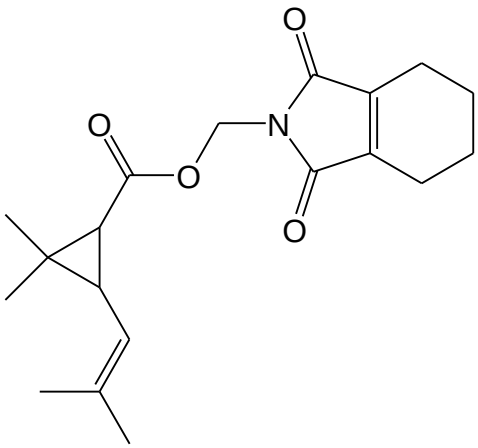
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

106 Tetramethrin



cyclohex-1-ene-1,2-dicarboximidomethyl (1*RS*)-*cis*-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate

$C_{19}H_{25}NO_4$ MW: 331.41 CAS No.: 7696-12-0

[Summary of tetramethrin]

Tetramethrin is a synthetic pyrethroid insecticide.

Tetramethrin was registered as an agricultural chemical in 1968, in Japan. Registered name had been phthalthrin. However, it was expired in 1979.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

[Method listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

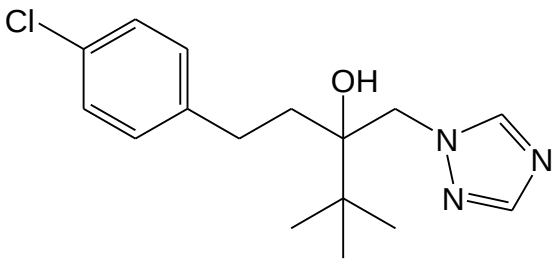
Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

Refer to Article 4, Section 2 in this chapter.

107 Tebuconazole



(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol
$C_{16}H_{22}ClN_3O$ MW: 307.8 CAS No.: 107534-96-3

[Summary of tebuconazole]

Tebuconazole is a triazole fungicide, which inhibits mycelial growth by inhibiting biosynthesis of lipids (sterol) in various fungi.

Tebuconazole was registered as an agricultural chemical in 1995, in Japan. The tradename is "Silvacur".

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn: 0.1 ppm / Rye: 0.2 ppm / Wheat: 2 ppm / Barley: 3 ppm / Other grains: 0.2 ppm

[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Simultaneous analysis method for tebuconazole and fenarimol by gas chromatography

Target Analytes: Tebuconazole and fenarimol (2 compounds)

Refer to Article 16, Section 3 in this chapter.

3. Analysis method for tebuconazole by gas chromatograph-mass spectrometer [Analytical Standards of Feeds, Chapter 6, Section 1, Article 107.3]

Scope of application: Grass hay[*1]

A Reagent Preparation

1) Tebuconazole standard solution. Weigh accurately 25 mg of tebuconazole[$C_{16}H_{22}ClN_3O$][1], transfer to a 50 mL brown volmetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the tebuconazole standard stock solution (1 mL of this solution contains 0.5 mg as tebuconazole).

Before use, dilute accurately a certain amount of the tebuconazole standard stock solution with a diluent solvent to prepare several tebuconazole standard solutions that contain 0.001 – 1.5 μg of

tebuconazole in 1 mL.

2) Diluent solvent. Add 50 μL of polyethylene glycol to 100 mL of 2,2,4-trimethylpentane – acetone (4 : 1) to prepare the diluent solbent.

B Quantification

Extraction. Weigh 10.0 g of an analysis sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 20 mL of acetonitrile – water (3 : 1) and leave to stand for 10 minutes. Further, add 100 mL of acetonitrile and shake for 30 minutes to extract.

Place a 200 mL volmetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask above and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Further, add acetonitrile up to the graduation line of the volmetric flask, transfer 10 mL of this dolution accurately to a 50 mL recovery flask, concentrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and add 20 mL of water to prepare a sample solution to be subjected to column treatment I.

Column treatment I. Place the sample solution on the porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that has contained the sample solution three times with 20 mL each of hexane, add the washings to the column in order of precedence and elute tebuconazole by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 60 mL of hexane to the column and elute in the similar way. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow. Dissolve the residue by adding 10 mL of hexane accurately to prepare a sample solution to be subjected to column treatment II.

Column treatment II. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane.

Transfer 2 mL of the sample solution accurately to the minicolumn and let flow out by natural flow until the liquid level reaches the upper end of the column packing material. Wash the minicolumn with 4 mL of hexane and let the wash flow out in the similar way. Further, add 10 mL of hexane – acetone (19 : 1) and let it flow out in the similar way. Place a 50 mL recovery flask under the column, add 15 mL of hexane – acetone (7 : 3) to the minicolumn to elute tebuconazole. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 10 mL of the diluent solvent accurately to prepare a sample solution to be subjected to measurement by a gas chromatograph mass spectrometer.

Measurement by a gas chromatograph mass spectrometer. Inject 2 μL each of the sample solution and respective standard solutions into a gas chromatograph mass spectrometer to obtain selected ion monitoring chromatograms.

Example of measurememt conditions

Column: Fused silica capillary column (5 % diphenyl/95 % dimethylpolysiloxane chemically bonded, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)[2]

Carrier gas: He (1.0 mL/min, initial flow rate)

Sample injection : Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 70 °C (hold 2 min) → ramp 20 °C/min → 280 °C (hold 10 min)

Interface temperature: 280 °C

Detector: Quadrupole mass spectrometer*2

Ion source temperature: 200 °C

Ionization method: Electron ionization (EI) method

Ionizing voltage: 70 eV

Monitor ion: Target ion *m/z* 250, reference ion *m/z* 125

Calculation. Obtain the peak area from the resulting selected ion monitoroing chromatograms[3] to prepare a calibration curve and subsequently calculate the amount of tebuconazole present in the sample.

* 1. This method is applied when the content of tebuconazole in the sample is over ca. 5 mg/kg.

2. The measurement conditions for GCMS-QP2010 (Shimadzu Corporation).

«Summary of analysis method»

In this method, a part of the procedures (GPC) of the Simultaneous Analysis of Tebuconazole and Fenarimol by Gas Chromatograph Mass Spectrometer [Analytical Standards of Feeds, Chapter 6, Section 1 107.2] is skipped, because the recovery tended to decrease, when grass hay was spiked with high concentrations of tebuconazole.

The flow sheet of the analysis method is shown in Figure 6.1.107-1.

10 g sample
- 20 mL acetonitrile/water (3:1), leave to stand for 10 minutes
- 100 mL acetonitrile, shake for 30 minutes
- Filter by suction (Grade No. 5B)
- Wash with 50 mL of acetonitrile
- Dilute with acetonitrile to 200 mL

10 mL sample solution
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas
- 20 mL water

Chem Elut cartridge (for 20 mL retention)
- Load sample solution, leave to stand for 5 minutes
- Wash with 20 mL of hexane (×3)
- Elute with 60 mL of hexane
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas
- 10 mL hexane

Sep-Pak Plus Florisil cartridge (wash with 5 mL of hexane in advance)
- Load 2 mL of sample solution
- Wash with 4 mL of hexane
- Wash with 10 mL of hexane-acetone (19:1)
- Elute with 15 mL of hexane-+acetone (7:3)
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas
- 10 mL diluent solvent

GC-MS

Figure 6.1.107-1. Flow sheet of the single-component analysis method for tebuconazole

Reference: Masayo Nomura: Research Report of Animal Feed, 33, 52(2008).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (mg/kg)	Replicate	Average recovery (%)	Repeatability RSD (% or less)
oat hay	0.5~30	3	93.3~98.8	2.3
alfalfa	0.5~30	3	88.8~93.5	5.2
timothy	0.5~30	3	93.5~94.3	8.2
Bermuda straw	0.5~30	3	87.7~99.4	5.4

• Collaborative study

Sample type	No. of labs	Spike concentration (mg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
oat hay	10	20	91.5	5.8	9.7	0.94
timothy hay	9	20	93.5	3.1	9.0	0.87

• Lower limit of quantification: 0.5 µg/kg (*SN* ratio)

• Lower limit of detection: 0.2 µg/kg (*SN* ratio)

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industriesand other manufacturers.

[2] For example, HP-5MS (Agilent Technologies).

[3] An example of selected ion chromatogram is shown in Figure 6.1.107-2.

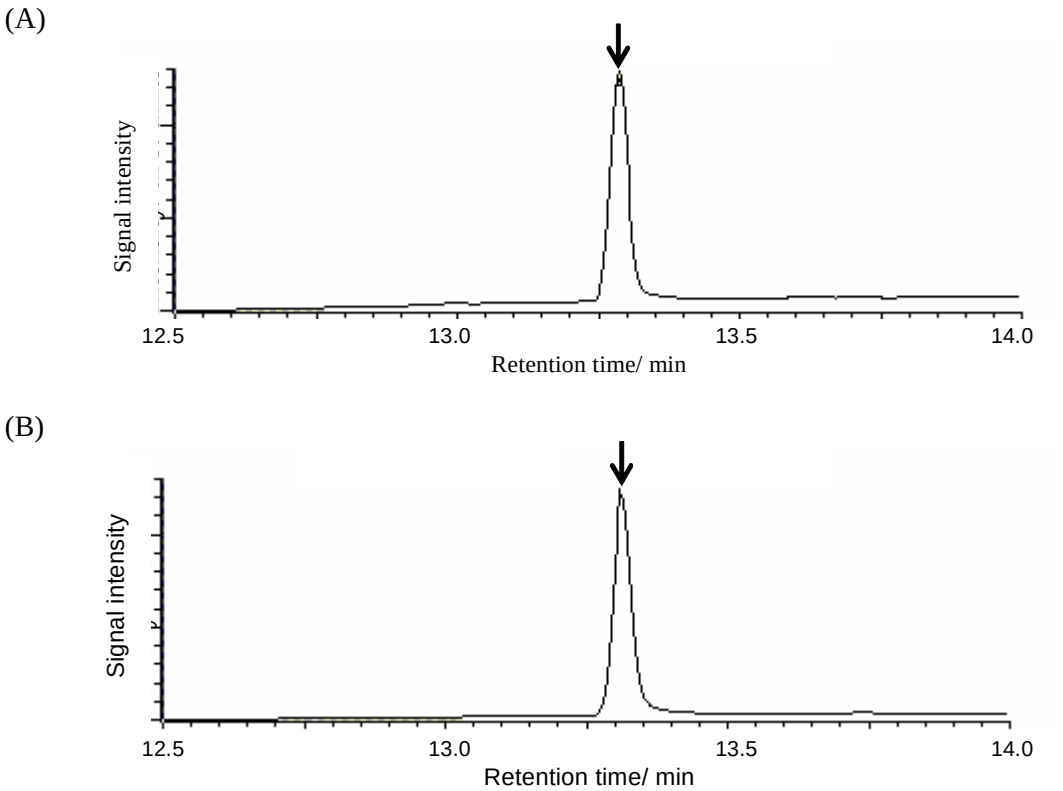


Figure 6.1.107-2. SIM chromatogram of the standard solution and sample solution (The arrow indicates the peak of tebuconazole.)

(A) Tebuconazole standard solution (0.3 ng)

(B) Timothy sample solution (spiked with tebuconazole equivalent to 30 mg/kg)

Measurement conditions

Detector: Quadrupole mass spectrometer

Column: Agilent Technologies HP-5MS (0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)

Carrier gas: He (1.0 mL/min, initial flow rate)

Sample injection: Splitless mode

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 70 °C (2 min)→20 °C/min→280 °C (10 min)

Interface temperature: 280 °C

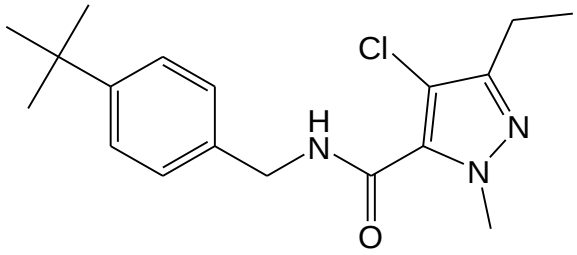
Ion source temperature: 200 °C

Ionization method: Electron impact (EI) method

Ionizing voltage: 70 eV

Monitor ion: Target ion *m*/*z* 250, reference ion *m*/*z* 125

108 Tebufenpyrad



N-(4-*tert*-butylbenzyl)-4-chloro-3-ethyl-1-methylpyrazole-5-carboxamide

$C_{18}H_{24}ClN_3O$ MW: 333.9 CAS No.: 119168-77-3

[Summary of tebufenpyrad]

Tebufenpyrad is a pyrazole miticide invented by Mitsubishi Chemical in 1987.

Tebufenpyrad was registered as an agricultural chemical for use on fruit trees in 1993, in Japan. The trade name is “Pyranica”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

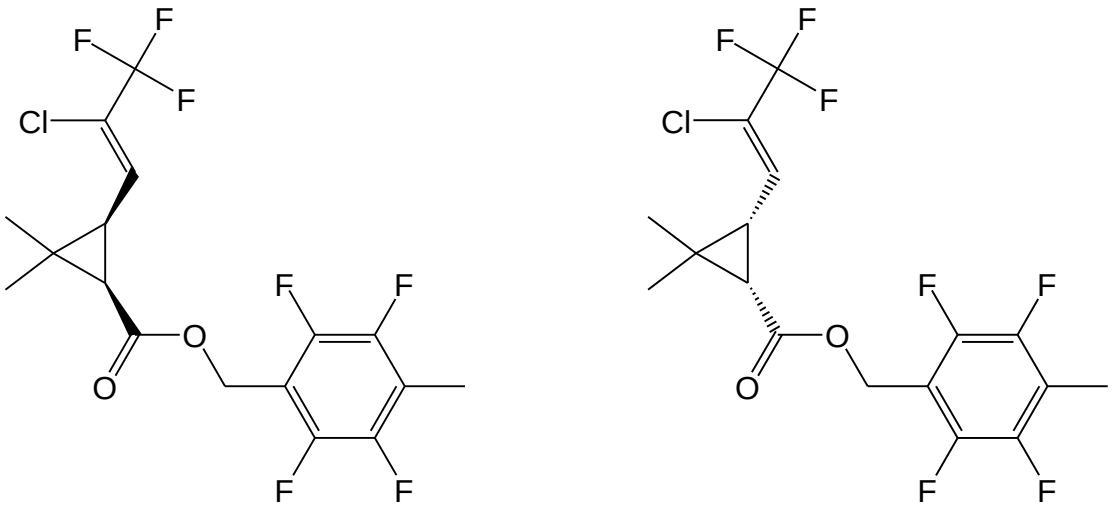
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

109 Tefluthrin



2,3,5,6-tetrafluoro-4-methylbenzyl (1*RS*)-*cis*-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropanecarboxylate

$C_{17}H_{14}ClF_7O_2$ MW: 418.7 CAS No.: 79538-32-2

[Summary of tefluthrin]

Tefluthrin is a synthetic pyrethroid insecticide developed by ICI (UK).

Tefluthrin was registered as an agricultural chemical in 1993, in Japan. The trade name is “Force”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn: 0.1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

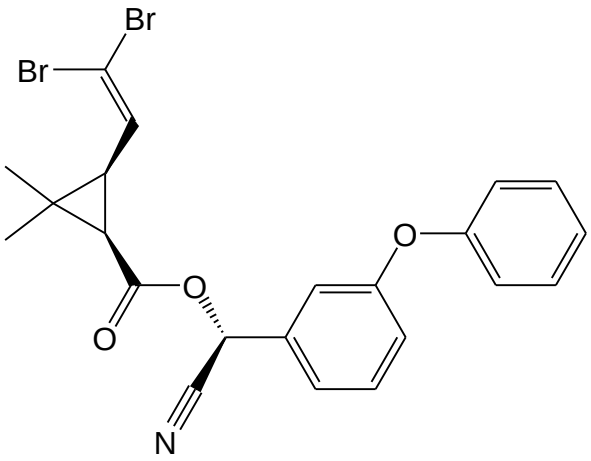
Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

Refer to Article 4, Section 2 in this chapter.

110 Deltamethrin



(*S*)-*α*-cyano-3-phenoxybenzyl (1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

$C_{22}H_{19}Br_2NO_3$ MW: 505.2 CAS No.: 52918-63-5

[Summary of deltamethrin]

Deltamethrin is a synthetic pyrethroid insecticide developed by Roussel-Uclaf (France), effective against a wide range of insect pests on fruit trees, vegetables and the like.

Deltamethrin has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(The sum of deltamethrin and tralomethrin)

Oat, barley, wheat, corn, milo and rye: 1 ppm / Pasture grass: 5 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

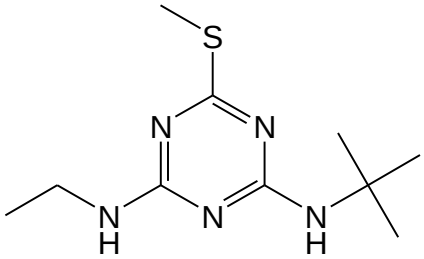
Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

Refer to Article 4, Section 2 in this chapter.

111 Terbutryn



N^2-*tert*-butyl-N^4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine

$C_{10}H_{19}N_5S$ MW: 241.4 CAS No.: 886-50-0

[Summary of terbutryn]

Terbutryn is a triazine herbicide.

Terbutryn has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.1 ppm

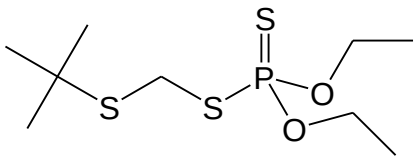
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

112 Terbufos



S-tert-butylthiomethyl *O*,*O*-diethyl phosphorodithioate

$C_9H_{21}O_2PS_3$ MW: 288.4 CAS No.: 13071-79-9

[Summary of terbufos]

Terbufos, a pale yellow liquid, is an organophosphorous insecticide developed by American Cyanamid (USA).

It is effective against soil insect pests, aphid, fruit fly, thrips, etc.

Terbufos has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Rye: 0.005 ppm / Barley, wheat and corn: 0.01 ppm / Oat and milo: 0.05 ppm / Pasture grass: 1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

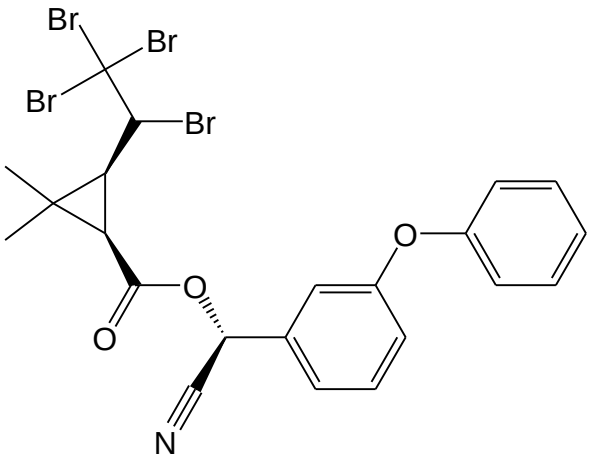
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

113 Tralomethrin



(*S*)-*α*-cyano-3-phenoxybenzyl (1*R*)-*cis*-2,2-dimethyl-3-[(*RS*)-1,2,2,2-tetrabromoethyl]cyclopropanecarboxylate

$C_{22}H_{19}Br_4NO_3$ MW: 665.0 CAS No.: 66841-25-6

[Summary of tralomethrin]

Tralomethrin is a synthetic pyrethroid insecticide developed by Roussel-Uclaf (France).

Tralomethrin was registered as an agricultural chemical for use on fruit trees in 1987, in Japan. The trade name is "Scout".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(The sum of deltamethrin and tralomethrin)

Oat, barley, wheat, corn, milo and rye: 1 ppm / Pasture grass: 5 ppm

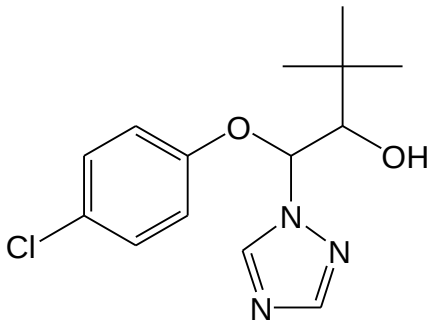
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

114　Triadimenol



(1*RS*,2*RS*;1*RS*,2*SR*)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol

$C_{14}H_{18}ClN_3O_2$　　MW: 295.8　　CAS No.: 55219-65-3

[Summary of triadimenol]

Triadimenol is a triazole fungicide developed by Bayer AG (Germany), which is formed by the metabolism of triadimefon, a member of the same fungicide family, with in plant or fungus body. CODEX has established the Maximum Residue Limit for triadimenol including triadimefon (Pulse: 0.1 ppm / fruits and vegetables: 0.1-3 ppm).

Triadimenol has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(Including the residue due to the use of triadimefon.)

Corn: 0.1 ppm / Wheat, barley, rye and other grains: 0.5 ppm

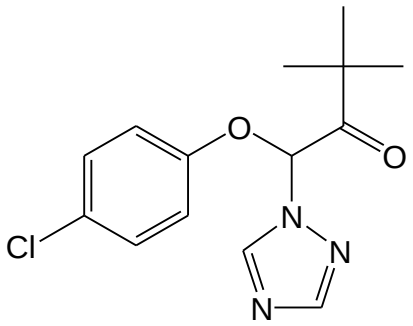
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for triazole agricultural chemicals by gas chromatography

Target Analytes: Triadimenol, triadimefon and propiconazole (3 compounds)

Refer to Article 6, Section 3 in this chapter.

115 Triadimefon



(*RS*)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one

$C_{14}H_{16}ClN_3O_2$ MW: 293.8 CAS No.: 43121-43-3

[Summary of triadimefon]

Triadimefon is a triazole fungicide developed by Bayer AG (Germany), which exerts fungicidal activity against germs by inhibiting specifically the biosynthesis system of ergosterol, a component of cell membranes, to induce cellular morphological aberrations. Codex has established the Maximum Residue Limit for triadimefon including triadimenol (Pulse: 0.1 ppm / fruits and vegetables: 0.1-3 ppm).

It is effective against powdery mildew, rust, frogeye etc. of fruit trees, vegetables as well as wheat, barley, etc.

Triadimefon was registered as an agricultural chemical in 1983, in Japan. The trade name is “Bayleton”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, rye and corn: 0.1 ppm / Barley: 0.5 ppm / Other grains: 0.1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

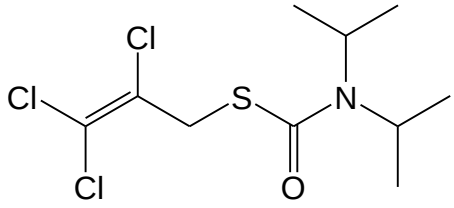
Refer to Article 1, Section 3 in this chapter.

2. Simultaneous analysis method for triazole agricultural chemicals by gas chromatography

Target Analytes: Triadimenol, triadimefon and propiconazole (3 compounds)

Refer to Article 6, Section 3 in this chapter.

116 Tri-allate



S-2,3,3-trichloroallyl diisopropylthiocarbamate

$C_{10}H_{16}Cl_3NOS_3$ MW: 304.7 CAS No.: 2303-17-5

[Summary of Tri-allate]

Tri-allate is a thiocarbamate herbicide.

Tri-allate has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.05 ppm

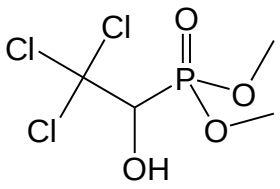
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

117　Trichlorfon (DEP)



dimethyl (*RS*)-2,2,2-trichloro-1-hydroxyethylphosphonate

$C_4H_8Cl_3O_4P$　MW: 257.44　CAS No.: 52-68-6

[Summary of trichlorfon (DEP)]

Trichlorfon (DEP) is a low-toxic organophosphorous insecticide developed by Bayer AG (Germany). It is converted into dichlorvos within plant body and thermally decomposed into dichlorvos and dimethyl phosphate (DMP).

Trichlorfon was registered as an agricultural chemical in 1957, in Japan. Registered name is DEP. The trade name is “Dipterex”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Administrative Guidelines for Hazardous Substances in Feeds]

Rice straw and paddy rice: 2 ppm

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.10 ppm.

[Method listed in the Analytical Standards of Feeds]

1. Gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 117.1]

A.　Reagent Preparation

Trichlorfon standard stock solution.　Weigh accurately 20 mg of trichlorfon[$C_4H_8Cl_3O_4P$][1], transfer to a 100mL volmetric flask and dissolve by adding acetone. Further, add the same solvent to the graduation line of the flask (1 mL of this solution contains 0.2 mg as trichlorfon). Then, dilute a certain amount of this solution with acetone accurately to prepare trichlorfon standard stock solution that contains 20 μg of trichlorfon in 1 mL.

B.　Quantification

Extraction.　Weigh 10.0 g of an analysis sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 15 mL of water to moisten and leave to stand for 30 minutes. Further, add 80 mL of acetone and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate in a water bath at 40 °C or lower to ca. 15 mL under reduced pressure, add 5 g of sodium chloride, and use this solution as a sample solution for column treatment I.

Column treatment I. Load the sample solution on the porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 200 mL recovery flask under the column, wash the recovery flask that has contained the sample solution three times with 10 mL each of ethyl acetate, add the washings to the column in order of precedence. Let the washings flow out by natural flow until the liquid level reaches the upper end of the column packing material to elute trichlorfon. Further, add 70 mL of the same solvent to the column, elute in the similar way. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 10 mL of cyclohexane – acetone (4 : 1) accurately, filter the solution through a membrane filter (pore size: 0.5 μm or less), and use the filtrate as a sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph. Dispense each elution fraction into a 100 mL recovery flask, concentrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 2 mL of hexane – acetone (9 : 1) accurately and use this solution as a sample solution for Column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column[2] (20 mm in inner diameter, 300 mm in length, particle size 15 μm)

Guard column: Styrene-divinylbenzene copolymer column[2] (20 mm in inner diameter, 100 mm in length, particle size 15 μm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 85-115 mL

Column treatment II. Wash a silica gel minicolumn (690 mg) with 5 mL of hexane.

Load the sample solution on the minicolumn. Wash the recovery flask that has contained the sample solution twice with 2 mL each of hexane – acetone (9 : 1), add the washings to the silica gel column in order of precedence. Further, add 4 mL of the same solvent to the minicolumn to wash it[*1].

Place a 50 mL pear shaped flask under the minicolumn and add 30 mL of hexane – acetone (7 : 3) to the minicolumn to elute trichlorfon[*1]. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue with 1 mL of acetone to prepare a sample solution to be subjected to acetylation reaction.

Acetylation reaction. Add 1 mL of acetic anhydride[3] to the sample solution and leave to stand at room temperature for 16 hours. Transfer the sample solution to a 5 mL volmetric flask. Wash the pear shaped flask that has contained the sample solution with a small amount of acetone and combine the washings with the content of the volmetric flask. Further, add acetone up to the graduation line of the flask to prepare a sample solution to be subjected to gas chromatography[*2].

Acetylation of standard stock solution. Transfer 1 mL of the trichlorfon standard solution accurately to 50 mL pear shaped flask, add 1 mL of acetic anhydride and leave to stand at room temperature for 16 hours.

Dilute the acetylated standard stock solution with acetone accurately to prepare several standard solutions that contain 0.02 – 2.0 μg of trichlorfon in 1 mL.

Gas chromatography. Inject 1 μL each of the sample solution and respective standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame photometric detector (Filter for phosphorus detection)

Column: Fused silica capillary column (50 % trifluoropropyl methyl/50 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)[4]

Carrier gas: He (1.5 mL/min)

Make up gas: He (30 mL/min)

Hydrogen: 75 mL/min

Dry air: 100 mL/min

Sample injection : Splitless mode (60 s)

Injection port temperature: 150 °C

Column oven temperature: Initial temperature 80 °C (hold 1 min) → ramp 20 °C/min → 280 °C (hold 5 min)

Detector temperature: 250 °C

Calculation. Obtain the peak area from the resulting chromatograms[5] to prepare a calibration curve and subsequently calculate the amount of trichlorfon present in the sample.

* 1. Flow rate 2 - 3 mL/min.

2. If necessary, transfer the sample solution to a plastic centrifugal precipitation tube (1.5 mL), centrifuge at 5,000×*g* for 5 minutes to obtain a supernatant to be subjected to gas chromatography.

«Summary of analysis method»

In this method, trichlorfon in samples is extracted with aqueous acetone, purified with a porous diatomite column, a GPC and a magnesium silicate column, acetylated and quantified by a gas chromatograph with a flame photometric detector (filter for phosphorus detection).

The flow sheet of the analysis method is shown in Figure 6.1.117-1.

10 g sample
- 15 mL water, leave to stand for 30 minutes
- 80 mL acetone, shake for 30 minutes
- Filter by suction (Grade No. 5B)
- Wash with 50 mL of acetone
- Concentrate under vacuum (to 15 mL)
- 5 g sodium chloride

Chem Elut cartridge (20 mL retention)
- Load sample solution, leave to stand for 5 minutes
- Wash with 100 mL ethyl acetate
- Concentrate under vacuum, dry up by nitrogen gas flow
- 8 mL cyclohexane-acetone (4:1)
- Membrane filter (0.5 μm)

GPC（Load 4 mL of sample solution)
- Elution fraction 85 - 115 mL
- Concentrate under vacuum, dry up by nitrogen gas flow
- 2 mL hexane-acetone (9:1)

Sep-Pak Plus Silica cartridge (wash with 5 mL of hexane in advance)
- Load sample solution
- Wash with 2 mL of hexane-acetone (9:1) (×2)
- Wash with 4 mL of hexane-acetone (9:1)
- Elute with 30 mL of hexane-acetone (7:3)
- Concentrate under vacuum, dry up with nitrogen gas flow

Acetylation
- 1 mL acetone
- 1 mL ahhydrous acetone
- Leave to stand for 16 hours
- Dilute with acetone to 5 mL

GC-FPD(P)

Figure 6.1.117-1 Flow sheet of the analysis method for trichlorfon

Reference: Akira Furukawa, Takeshi Uchiyama: Research Report of Animal Feed, 27, 28(2002).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
adult hen formula feed	50~250	3	83.4~92.4	7.8
dairy cattle formula feed	50~250	3	78.9~90.9	8.3
alfalfa hay cube	50~250	3	85.9~98.9	8.0
oat hay	50~250	3	101.9~102.5	8.8

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
adult hen formula feed	7	100	94.4	5.0	9.0	0.41
alfalfa hay cube	7	100	97.9	7.1	9.7	0.44

• Lower limit of quantification: 20 µg/kg in samples

«Notes and precautions»

[1] Available from Wako Pure Chemical Industries and Kanto Chemical.

[2] Column packed with styrene-divinylbenzene copolymer hard gel.

[3] Stir thoroughly.

[4] For example, Rtx-200 (Restek).

[5] An example of chromatogram of acetylated trichlorfon is shown in Figure 6.1.117-2.

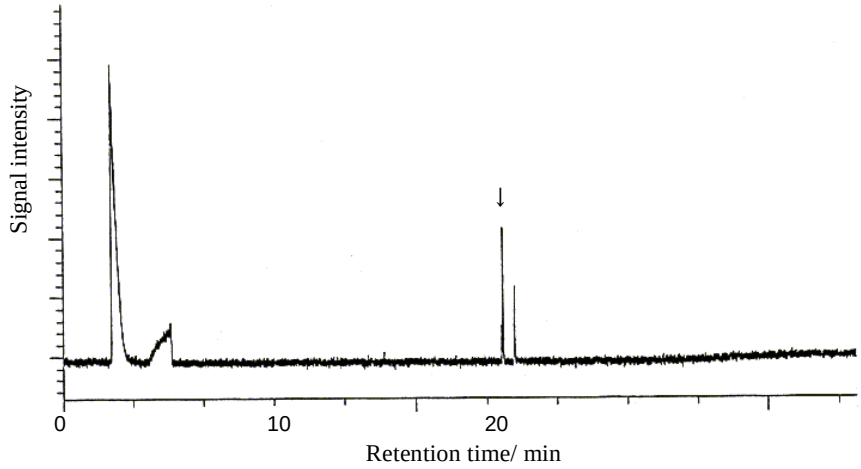


Figure 6.1.117-2 Chromatogram of a formula feed spiked with an amount equivalent to 100 µg/kg trichlorfon (The arrow indicates the peak of acetylated trichlorfon.)

Measurement conditions

Detector: Flame photometric detector (Filter for phosphorus detection)

Column: Restek Rtx-200 (0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)

Carrier gas: He (1.5 mL/min, initial flow rate)

Make up gas: He (30 mL/min)

Hydrogen: 75 mL/minn

Dry air: 100 mL/min

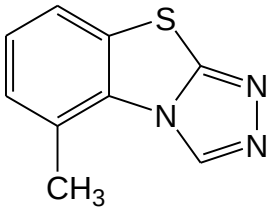
Sample injection : Splitless mode

Injection port temperature: 150 °C

Column oven temperature: Initial temperature 80 °C (hold 1 min) → ramp 20 °C/min → 280 °C (hold 5 min)

Detector temperature: 250 °C

118 Tricyclazole



5-methyl-1,2,4-triazolo[3,4-*b*][1,3]benzothiazole
$C_9H_7N_3S$ MW: 189.2 CAS No.: 41814-78-2

[Summary of tricyclazole]

Tricyclazole is a benzothiazole fungicide and effective against rice blast.

Tricyclazole was registered as an agrochmilcal for use on rice in 1881, in Japan. The trade name is "Beam".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Oat, barley, wheat, corn, milo and rye: 0.02 ppm / Pasture grass: 5 ppm

[Method listed in the Analytical Standards of Feeds]

1 Analysis method for tricyclazole by gas chromatograph-mass spectrometer [Analytical Standards of Feeds, Chapter 6, Section 1, Article 118.1]

A. Reagent Preparation

Tricyclazole standard solution[1]. Weigh accurately 50 mg of tricyclazole[$C_9H_7N_3S$], transfer to a 100 mL volmetric flask and dissolve by adding acetone. Further, add acetone up to the graduation line of the flask to prepare the tricyclazole standard stock solution (1 mL of this solution contains 0.50 mg as tricyclazole).

Before use, dilute accurately a certain amount of the tebuconazole standard stock solution with acetone to prepare several tricyclazole standard solutions that contain 0.002 – 0.2 μg of tricyclazole in 1 mL.

B. Quantification

Extraction

1) Grass hay. Weigh 10.0 g of an analysis sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 100 mL of acetonitrile – water (13 : 7), shake for 30 minutes to extract. Place a 200 mL volmetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask above and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Further, add acetonitrile up to the graduation line of the volmetric flask[*1]. Transfer 20 mL of this solution accurately to a 100 mL recovery flask, concentrate under reduced pressure in a water bath at 40 °C or lower to ca. 5 mL to prepare a sample solution to be subjected to column treatment I.

2) Other feeds. Weigh 10.0 g of an analysis sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 50mL of acetonitrile – water (13 : 7), shake for 30 minutes to extract. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask above and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to ca. 15 mL to prepare a sample solution to be subjected to column treatment I.

Column treatment I. Load the sample solution on the porous diatomite column (for 20 mL retention). Wash the recovery flask that has contained the sample solution with 5 mL of water, add the washing to thecolumn and leave to stand for 5 minutes. Place a 200 mL recovery flask under the column. Wash the recovery flask that has contained the sample solution three times with 20 mL each of hexane – ethyl acetate (1 : 1), add the washings to the column in order of precedence and let flow out by natural flow until the liquid level reaches the upper end of the column packing material to elute tricyclazole. Further, add 40 mL of hexane – ethyl acetate (1 : 1) to the column and elute in the simlar way. Add 1 mL of acetone – diethylene glycol (49 : 1) to the eluate.

Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and dry up by nitrogen gas flow. Dissolve the residue by adding10 mL of cyclohexane – acetone (4 : 1) accurately. Transfer this solution to a 10 mL centrifuge tube and centrifuge at 1,000×g for 5 minutes. Filter the supernatant through a membrane filter (pore size 0.5 µm or less) to prepare a sample solution to be subjected to gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph. Dispense each elution fraction into a 100 mL recovery flask, concentrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 2 mL of hexane – acetone (1 : 1) and use this solution as a sample solution for Column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column[2] (20 mm in inner diameter, 300 mm in length, particle size 15 µm)

Guard column: Styrene-divinylbenzene copolymer column[2] (20 mm in inner diameter, 100 mm in length, particle size 15 µm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 150-190 mL[3]

Column treatment II. Wash a silica gel minicolumn (690 mg) with 5 mL of acetone and 5 mL of hexane sequentially. Place a 50 mL pear shaped flask under the minicolumn, load the sample solution on the minicolumn and elute tricyclazole by natural flow until the liquid level reaches the upper end of the column packing material[*2]. Wash the recovery flask that has contained the sample solution twice with 2 mL each of hexane – acetone (1 : 1), add the washings to the minicolumn sequentially and elute in the similar way[*2]. Further, add 14 mL of hexane – acetone (1: 1) to the minicolumn and elute in the similar

way[*2].

Concentrate the eluent under reduced pressure in a water bath at 40 °C or lower to almost dryness and dry up by nitrogen gas flow. Dissolve the residue with 2 mL of acetone accurately to prepare a sample solution to be subjected to measurement by a gas chromatograph mass spectrometer.

Measurement by a gas chromatograph mass spectrometer. Inject 1 µL each of the sample solution and respective tricyclazole standard solutions into a gas chromatograph mass spectrometer to obtain selected ion monitoring chromatograms.

Example of measurememt conditions

Column: Fused silica capillary column (5 % diphenyl/95 % dimethyl-polysiloxane chemically bonded, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)[4]

Carrier gas: He (1.0 mL/min, initial flow rate)

Sample injection : Splitless mode (60 s)

Injection port temperature: 280 °C

Column oven temperature: Initial temperature 70 °C (1 min) → ramp 25 °C/min → 200 °C → ramp 8 °C/min → 280 °C (10 min)

Detector: Quadrupole mass spectrometer[*3]

Interface temperature: 250 °C

Ion source temperature: 230 °C

Ionizing voltage: 70 eV

Ionization method: Electron ionization (EI) method

Monitor ion: Target ion *m*/*z* 189, reference ion *m*/*z* 162 and 161

Calculation. Obtain respective the peak height or peak area from the resulting selected ion monitoring chromatograms[5] to prepare a calibration curve and subsequently calculate the amount of tricyclazole in the sample.

* 1. When the sample has a high tricyclazole content, dilute the extract with acetonitrile before the following procedures.

2. Flow rate shall be 2 - 3 mL/min. If necessary, use an vacuum manifold.

3. The measurement conditions for GCMS-QP2010 (Shimadzu Corporation).

«Summary of analysis method»

In this method, tricyclazole in samples is extracted with aqueous acetonitrile, purified with a porous diatomite column, a GPC and a silica gel column and quantified by a gas chromatograph mass spectrometer.

The flow sheet of the analysis method is shown in Figure 6.1.118-1.

10 g sample
- 50 mL (for grass hay 100 mL) acetonitrile-water (13:7), shake for 30 minutes
- Filter by suction (Grade No. 5B)
- Wash with 50 mL of acetonitrile

Grass hay→
- Dilute with acetonitrile to 200 mL

20 mL extract
- Concentrate the extract under vacuum to 15 mL (grass hay 5 mL)(40 °C or below)

Chem Elut cartridge (for 20 mL retention)
- Load the sample solution, wash with 5 mL of water, leave to stand for 5 minutes
- Wash with 20 mL of hexane/ethyl acetate (1:1) (×3)
- Elute with 40 mL of hexane/ethyl acetate (1:1)
- 1 mL acetone-diethyl glycol (49:1)
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- 10 mL cyclohexane/acetone (4:1)
- Centrifuge (1,000×*g* , for 5minutes)
- Membrane filter (pore size 0.5 µm or less)

GPC (inject 5 mL of sample solution)
- Elution fraction 150 - 190 mL
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- 2 mL hexane-acetone (1:1)

Sep-Pak Plus Silica cartridge (wash with 5 mL each of acetone and hexane in advance)
- Wash with 2 mL of hexane-acetone (1:1)×2
- Elute with 14 mL of hexane-acetone (1:1)
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- 2 mL acetone

GC-MS

Figure 6.1.118-1. Flow sheet of the analysis method for tricyclazole

Reference: Tomoharu Nozaki, Toshiaki Yamata: Research Report of Animal Feed, 32, 45 (2007).

Japan Food Research Laboratories: The projects on the development of analysis methods to establish Maximum Residue Limits of harmful chemical substances etc. and on the research on their transfer to livestock commissioned by MAFF in 2006. The development of analysis methods to establish Maximum Residue Limits of harmful chemical substances etc., 119 (2007)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (µg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
corn	4~20	3	116.3~116.8	1.6
ryegrass	400~5,000	3	81.4~98.1	2.3

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
corn	7	20	100.7	7.1	15.7	0.72
timothy	7	5,000	101.8	6.8	16.8	1.34

• Lower limit of quantification: 4 μg/kg (recovery and relative standard deviation)

• Lower limit of detection: 1 μg/kg (*SN* ratio)

«Notes and precautions»

[1] The standards are available from Kanto Chemical, Wako Pure Chemical Industries, and other manufacturers.

[2] Column packed with Styrene-divinylbenzene copolymer hard gel with the eluate. The column and the guard column spedified in Appendix 2 of the Analytical Standards of Feeds is Shodex CLNpak EV-2000 AC and Shodex CLNpak EV-G AC manufactured by Showa Denko respectively.

[3] Because elution fraction may vary among lots of column, depending on frequency of use, etc., it requires careful check in advance in each laboratory.

[4] For example, Rtx-5MS (Restek).

[5] An example of selected ion monitoring chromatogram (SIM) is shown in Figure 6.1.118-2.

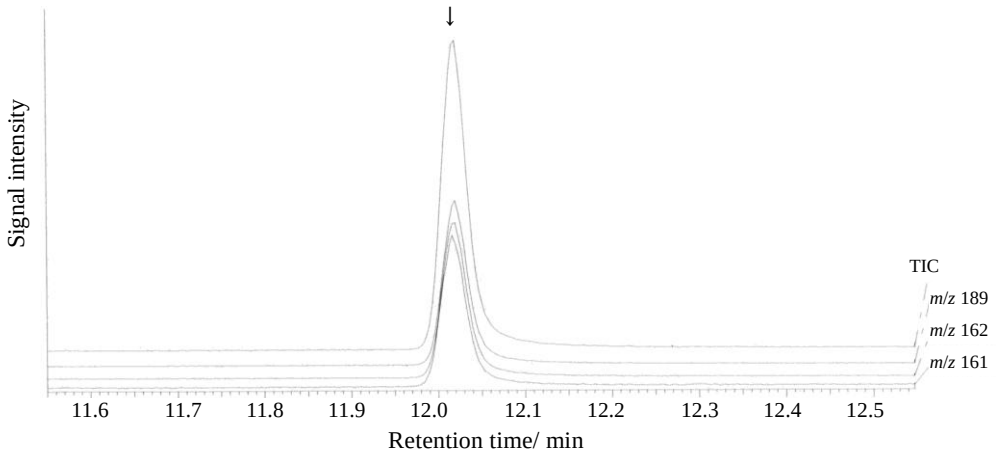
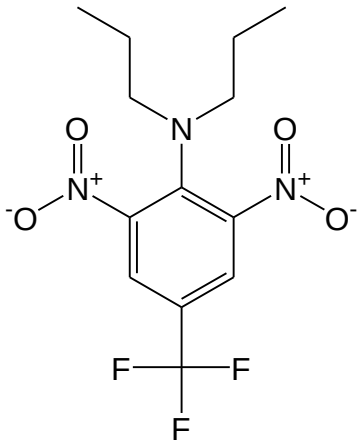


Figure 6.1.118-2. SIM chromatogram of the tricyclazole standard solution (100 μg/mL)
(The arrow indicates the peak of tricyclazole.)

119 Trifluralin



α,*α*,*α*-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine

$C_{13}H_{16}F_3N_3O_4$ MW: 335.3 CAS No.: 1582-09-8

[Summary of trifluralin]

Trifluralin is a non-selective, soil-active dinitroaniline herbicide developed by Eli Lilly (USA), which works on annual gramineous weeds, broad leaf weeds, etc.

Trifluralin was registered as an agricultural chemical in 1966, in Japan. The trade name is “Trefanocide”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn: 0.05 ppm / Wheat, barley, rye and other grains: 0.1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

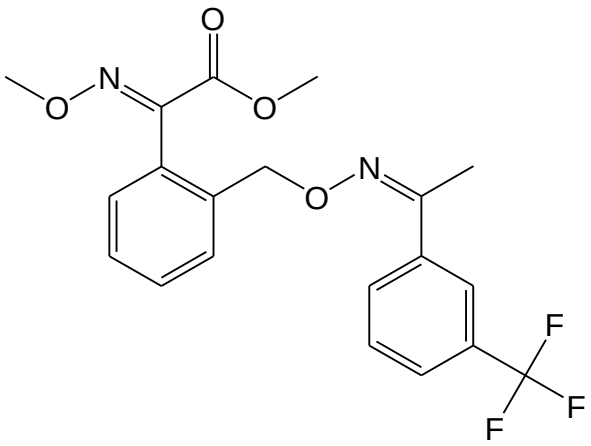
Refer to Article 1, Section 3 in this chapter.

2. Simultaneous analysis method for dicofol and trifluralin by gas chromatography

Target Analytes: Dicofol and trifluralin (2 compounds)

Refer to Article 15, Section 3 in this chapter.

120 Trifloxystrobin



methyl (*E*)-methoxyimino-{(*E*)-*α*-[1-(*α*,*α*,*α*-trifluoro-*m*-tolyl)ethylideneaminooxy]-*o*-tolyl}acetate

$C_{20}H_{19}F_3N_2O_4$ MW: 408.4 CAS No.: 141517-21-7

[Summary of trifloxystrobin]

Trifloxystrobin is a strobilurin fungicide developed originally by Novartis (Switzerland), later by Bayer AG (Germany). It has been confirmed that trifloxystrobin inhibits spore germination and post-germination host invasion through its inhibitory effect on mitochondrial electron transport system of germs.

Trifloxystrobin was registered as an agricultural chemical on sugar beet, grape, etc. in 2001, in Japan. The trade name is “Flint”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn: 0.05 ppm / Wheat: 0.2 ppm / Barley: 0.5 ppm

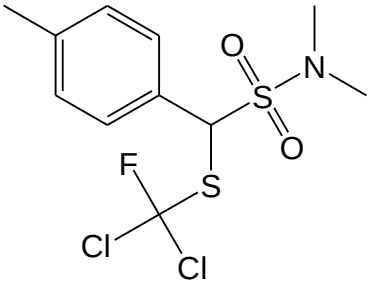
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

121 Tolylfluanid



N-dichlorofluoromethylthio-*N*',*N*'-dimethyl-*N*-*p*-tolylsulfamide

$C_{10}H_{13}Cl_2FN_2O_2S_2$ MW: 347.2 CAS No.: 731-27-1

[Summary of tolylfluanid]

Tolylfluanid is a phenylsulfamide fungicide developed by Bayer AG (Germany), which exerts fungicidal effect on a wide range of fungi by interfering various enzymes by reaction with -SH bond.

Tolylfluanid has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

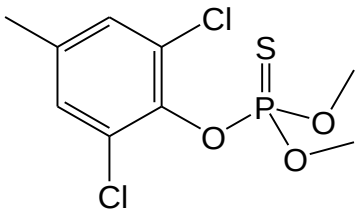
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

122 Tolclofos-methyl



O-2,6-dichloro-*p*-tolyl *O*,*O*-dimethyl phosphorothioate
$C_9H_{11}Cl_2O_3PS$ MW: 301.13 CAS No.: 57018-04-9

[Summary of tolclofos-methyl]

Tolclofos-methyl, a colorless powder, is an organophosphorous fingicide developed by Sumitomo Chemical, which has a potent fungicidal activity against *Rhizoctonia* and also exerts fungicidal activity against the germs of the white root-rot and the southern blight.

Tolclofos-methyl was registered as an agricultural chemical in 1984, in Japan. The trade names are "Rizorex" and "Glancer".

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.1 ppm

[Method listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

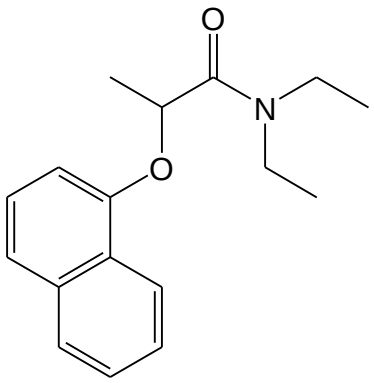
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

123 Napropamide



(*RS*)-*N*,*N*-diethyl-2-(1-naphthyloxy)propionamide

$C_{17}H_{21}NO_2$ MW: 271.4 CAS No.: 15299-99-7

[Summary of napropamide]

Napropamide is an acid amide (naphthoxy-acid amide) herbicide developed by Stauffer Chemical (USA), which is mainly used as a soil-active pre-germination herbicide.

Napropamide was registered as an agricultural chemical for use on lawn grass in 1975, in Japan. The trade name is “Kusaless”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

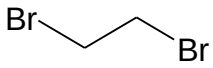
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

124　Ethylene dibromide (EDB)



1,2-dibromoethane

$C_2H_4Br_2$　MW: 187.861　CAS No.: 106-93-4

[Summary of ethylene dibromide (EDB)]

Ethylene dibromide (EDB) is widely used in particular as a soil-active nematocide against root-knot nematode, etc., as well as a fumigant for use on fruits and grains (Its use as a fumigant on grains, etc. was banned in 1984.).

Ethylene dibromide was registered as an agricultural chemical in 1956, in Japan. Registered name had been EDB. However, it was expired in1990.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Oat, barley, corn, milo and rye: 0.01 ppm / Wheat: 0.1 ppm

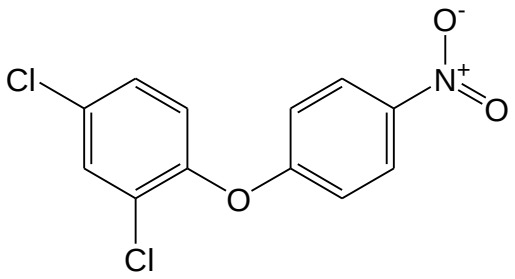
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for EPTC and ethylene dibromide by gas chromatograph-mass spectrometer

Target Analytes: EPTC and ethylene dibromide (2 compounds)

Refer to Article 8, Section 3 in this chapter.

125 Nitrofen (NIP)



2,4-dichlorophenyl 4-nitrophenyl ether

$C_{12}H_7Cl_2NO_3$ MW: 284.1 CAS No.: 1836-75-5

[Summary of nitrofen (NIP)]

Nitrofen (NIP), a diphenyl ether herbicide developed by Rohm and Haas (USA), was used to inhibit germination of annual weeds in rice and vegetable fields.

Nitrofen was registered as an agricultural chemical in 1963, in Japan. Registered name had been NIP. However, it was expired in 1982.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

[Method listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

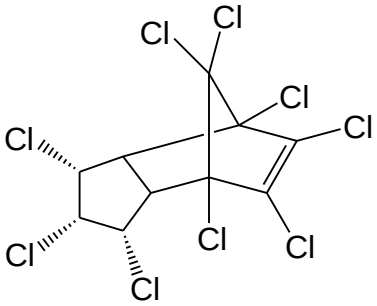
Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

126 Nonachlor (*cis*-nonachlor and *trans*-nonachlor)

cis-Nonachlor

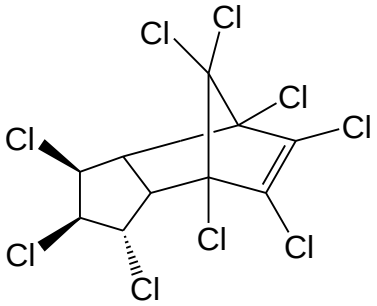


1*β*,2*β*,3*β*,4*α*,5,6,7*α*,8,8-nonachloro-(2,3,3a*β*,4,7,7a*β*-hexahydro-4,7-methano-1*H*-indene

$C_{10}H_5Cl_9$ MW: 444.22

CAS No.: 5103-73-1

trans-Nonachlor



(1*S*,7*R*)-1*α*,2*β*,3*β*,4,5,6,7,8,8-nonachloro-2,3,3a*α*,4,7,7a*α*-hexahydro-4,7-methano-1*H*-indene

$C_{10}H_5Cl_9$ MW: 444.22

CAS No.: 39765-80-5

[Summary of nonachlor]

Nonachlor, a derivative of chlordane, is an organochlorine insecticide.

Nonachlor has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

[Method listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

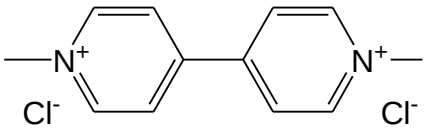
Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

127 Paraquat (paraquat dichloride)



1,1′-dimethyl-4,4′-bipyridinium dichloride

Paraquat	$C_{12}H_{14}N_2$	MW: 186.25	CAS No.: 4685-14-7
Paraquat dichloride	$C_{12}H_{14}Cl_2N_2$	MW: 257.16	CAS No.: 1910-42-5

[Summary of paraquat]

Paraquat is a non-hormone, contact quarternary ammonium herbicide developed by ICI (UK). Because of its potent herbicidal activity by foliage application and immediate inactication in the soil,, it had been widely used for control of undergrowth in fruit orchards, pasture renewal and weed control in non-agricaltural lands.

In Japan, paraquat is used only in a formulation mixed with diquat in low concentrations, because of its high toxicity to man and animals.

Paraquat was registered as an agricultural chemical (as paraquat dichloride) in 1965, in Japan. The trade name is “Gramoxone”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

Barley, wheat and rye: 0.05 ppm / Corn: 0.1 ppm / Oat and milo: 0.5 ppm / Pasture grass: 5 ppm

Rice straw: 0.3 ppm

[Method listed in the Analytical Standards of Feeds]

1. Liquid chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 127.1]

A. Reagent Preparation

1) Paraquat standard stock solution. Weigh accurately 20 mg of paraquat [$C_{12}H_{14}N_2Cl_2$][1] transfer to a 100 mL volmetric flask and dissolve by adding hydrochloric acid (0.01 mol/L). Further, add the same solvent up to the graduation line of the flask to prepare the paraquat standard stock solution (1 mL of this solution contains 0.2 mg as paraquat).

2) Cation-exchange resin (Na^+). Weigh 100 g of strongly acidic cation-exchange resin[*1], transfer to a 500 mL Erlenmeyer flask, add 300 mL of water, stir and discard the supernatant. Repeat these procedures until the pH of the supernatant rises to 6.8 – 7.2, add 300 mL of water to the resin and leave to stand overnight. Then, add 200 mL of sodium hydrochloride solution (2 mol/L) to this resin, stir and discard the supernatant. Repeat these procedures until the pH of the supernatant rises to 12, add 200 mL of sodium hydrochloride solution (2 mol/L) and leave to stand overnight. The, add 300 mL of water to this resin, stir and discard the supernatant. Repeat these procedures until the pH of the supernatant decreases

to 6.8 – 7.2, add 300 mL of water to the resin and store in water.

B. Quantification

Extraction. Weigh 10.0 g of an analysis sample, transfer it to a 500 mL recovery flask, add 90 mL of sulfuric acid (1+2) solution, three or four boiling stones and two or three drops of silicone oil. Connect a reflux condenser to the recovery flask and heat for 5 hours to extract[2].

Place a 500 mL beaker under a Büchner funnel and filter the extract through a glass fiber filter*2 by suction. Then, wash the recovery flask above and the residue with 50 mL of water sequentially, filter the washings by suction in the similar way and add water to the filtrate to make ca. 200 mL. Adjust the pH of this solution to 8.9 – 9.1 with sodium hydrochloride solution (12 mol/L) [3], filter through a glass fiber filter*2. Wash the beaker above and the filter paper with a small amount of water sequentially, filter the washings through the filter above and combine the filtrate to prepare a sample solution to be subjected to column treatment.

Column treatment*3. Pour the cation-exchange resin (Na^+) to a column tube[4] (15 mm in inner diameter) to the height of 6 cm, add 20 mL of water and let flow out by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare the column.

Load the sample solution. Wash the container that has contained the sample solution with a small amout of water and add the washings to the column. Let flow out by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Add 100 mL of water to the column and let flow out in the similar way to wash the column. In the similar way, add 50 mL of hydrochloric acid (2 mol/L), 100 mL of water, 50 mL of ammonium chloride solution (5 w/v%) and 100 mL of water to the column to wash the column sequentially.

Add 50 mL of ammonium chloride solution (5 mol/L) to the column, discard the the first 5 mL of the outflow. Then, place a 50 mL volmetric flask under the column to elute paraquat. Add ammonium chloride solution (5 mol/L) to the graduation line of the volmetric flask to prepare a sample solution to be subjected to fluorescence derivatization [5].

Fluorescence derivatization [6] Transfer 10 mL of the sample solution accurately to a 100 mL separating funnel, add 10 mL of sodium hydrochloride solution (12 mol/L) and 1 mL of potassium ferricyanide (1 w/v%) solution and shake gently. Further, add 20 mL of chloroform to the separating funnel, shake vigorously for 5 minutes and leave to stand. Transfer the chloroform layer (lower layer) to an Erlenmeyer flask. Add 20 mL of chloroform to the separating funnel above, proceed in the similar way and transfer the chloroform layer into the Erlenmeyer flask above. Dehydrate the chloroform layer with a suitable amount of sodium sulfate (anhydrous) and filter through a filter paper (No. 5A) into a 100 mL recovery flask. Then, wash the Erlenmeyer flask above and the filter paper with a small amoint of chloroform sequentially and combine washings through the filter paper above. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 4 mL of water – acetonitrile (93 : 7) accurately and filter through a membrane filter (pore size 0.5 μm or less) to prepare a sample solution to be subjected to liquid chromatography.

Concurrently, add 1 mL of the paraquat standard stock solution accurately and add 9 mL of ammonium chloride solution (5 mol/L) to a 100 mL separating funnel and derivatize under the same conditions as the sample solution.

Dissolve the residue by adding 4 mL of water – acetonitrile (93 : 7) accurately, filter through a membrane filter (pore size 0.5 µm or less) and dilute accurately with the same solvent to prepare several standard solutions that contain 0.01 – 0.5 µg of paraquat in 1 mL.

Liquid Chromatography. Inject 20 µL each of the sample solution and respective standard solutions into a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Fluorescence detector (330 nm excitation wavelength, 436 nm emission wavelength)

Column: Octadecylsilylated silica gel column (4.6 mm in inner diameter, 250 mm in length, particle size 5 µm)[*4] [7]

Eluent: Water – acetonitrile (93 : 7)

Flow rate: 1.3 mL/min

Column oven temperature: 40 °C

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms [8] to prepare a calibration curve and subsequently calculate the amount of paraquat in the sample.

* 1. AG 50W-X8 H^+(particle size: mesh 200 – 100) (manufactured by Bio-Rad Laboratories) or equivalents.

2. GF/A (Whatman) or equivalents.

3. Flow rate during wash: 10 mL/min; during elution: 10 mL/h.

4. Shodex C18-5B (Showa Denko) or equivalents.

«Summary of analysis method»

In this method, paraquat in samples is extracted by boiling under acidic condition with sulfuric acid, purified with the use of an ion-exchange column, fluorescencey derivatized and quantified by a liquid chromatograph with fluorescence detector.

The flow sheet of the analysis method is shown in Figure 6.1.127-1.

10 g sample
- 90 mL sulfuric acid (1+2) solution, boiling stone, silicone oil
- Connect a reflux condenser, heat by a mantle heater for 5 hours
- Filter by suction with a glass fiber filter paper
- Wash the container and the residue with 50 mL of water, combine the filtrate and the wash to make ca. 200 mL
- Adjust the pH of the filtrate with sodium hydroxide solution (12 mol/L) to
- PE funnel, filter with glass fiber filter paper

Cation-exchange resin column
- Load total amount of the sample solution, elute
- Wash with 100 mL of water
- Wash with 50 mL of hydrochloric acid (2 mol/L), 100 mL of water, 50 mL of ammonium chloride solution (5 w/v%) and 100 mL of water
- Add 50 mL of ammonium chloride solution (5 mol/L), discard the initial 5 mL of the outflow
- Elute into a 50 mL volmetric flask
- Dilute with ammonium chloride (5 mol/L)

Fluorescence derivatization (100 mL separating funnel)
- 10 mL sample solution (weigh accurately)
- 10 mL sodium hydroxide solution (12 mol/L)
- Add potassium ferricyanide solution (1 w/v%), swirl gently
- Extract with 20 mL of chloroform twice
- Shake for 5 minutes
- Combine the chloroform layers, dehydrate, filter
- Concentrate under vacuum, dry up
- 4 mL water-acetonitrile (93:7)

LC-FL (Ex: 330 nm, Em: 436 nm)

Figure 6.1.127-1. Flow sheet of the analysis method for paraquat

Reference: Tomoharu Nozaki, Manabu Matsuzaki: Research Report of Animal Feed, 23, 42(1998).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Repeat	Spike recovery (%)	Repeatability RSD (% or less)
adult hen formula feed	100~500	3	76.7~96.3	19.3
piglet formula feed	100~500	3	80.7~92.7	7.7
Sudan grass	100~500	3	79.3~96.0	13.5

• Collaborative study

Sample type	No. of Labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
broiler formula feed	7	200	88.2	6.8	7.5	0.36

• Lower limit of quantification: Formula feed: 50 μg/kg in samples, grass hay: 10 μg/kg in samples

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries and other manufacturers.

[2] Heat just enough to bubble slightly. Overheating could lead to sudden boiling.

[3] The amount of sodium hydroxide solution required for neutralization is ca. 120 mL. Rise in the pH to above 10 could cause decomposition of paraquat, so neutralization shall be performed carefully with the use of a pH meter. In addition, because neutralization produces heat, cooling is required during the neutralization.

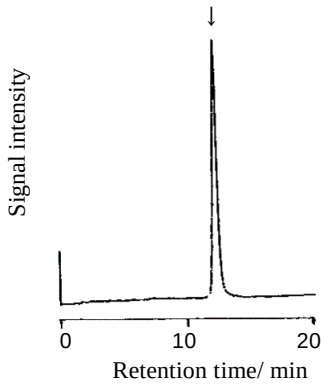
[4] Use a column tube with a fluid reservoir.

[5] Because the drip rate varies depending on the amount of the sample solution loaded, adjust the drip rate to remain constant to the extent possible. Further, too slow drip rate during loading the sample solution colud lead to precipitation of crystalline substances, which can interfere with dripping.

[6] Fluorescence derivatization should be performed quickly.

[7] Any columns packed with an end-capped packing material equivalent to this one may be used.

[8] An example of chromatogram is shown in Figure 6.1.127-2.



Measurement conditions

Detector: 330 nm excitation wavelength, 436 nm emission wavelength

Column: Shodex C18-5B

(4.6 mm in inner diameter, 250 mm in length, particle size 5 μm)

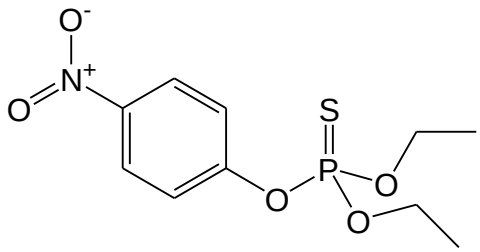
Eluent: Water – acetonitrile (93 : 7)

Flow rate: 1.3 mL/min

Column oven temperature: 40 °C

Figure 6.1.127-2. Chromatogram of a formula feed spiked with an amount equivalent to 0.5 mg/kg paraquat (The arrow indicates the peak of fluorescence-derivatized paraquat.)

128 Parathion (Parathion-ethyl)



O,*O*-diethyl *O*-4-nitrophenyl phosphorothioate
$C_{10}H_{14}NO_5PS$ MW: 291.3 CAS No.: 56-38-2

[Summary of parathion]

Parathion (parathion-ethyl), a yellow liquid, is an organophosphorous insecticide. Because it has been designated as a specified poisonous substance, handle it in accordance with the Poisonous and Deleterious Substances Control Law.

Parathion is effective against a wide range of insect pests including rice stem borer.

Parathion was registered as an agricultural chemical in 1952, in Japan. However, it was banned for use in 1971 because of its high toxicity to man and animals.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Rye: 0.05 ppm / Oat and milo: 0.08 ppm / Wheat and corn: 0.3 ppm / Barley: 0.5 ppm / Pasture grass: 5 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and

monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

3. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (2)

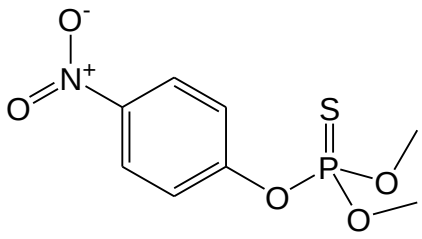
Target Analytes:

Group A: EPN, diazinon, parathion, fenitrothion, fenthion, phenthoate, phosalone and malathion (8 compounds).

Group B: Dichlorvos (1 compound)

Refer to Article 3, Section 2 in this chapter.

129 Parathion-methyl



O,*O*-dimethyl *O*-4-nitrophenyl phosphorothioate

$C_8H_{10}NO_5PS$ MW: 263.2 CAS No.: 298-00-0

[Summary of parathion-methyl]

Parathion-methyl, a white powder, is an organophosphorous insecticide. Because it has been designated as a specified poisonous substance, handle it in accordance with the Poisonous and Deleterious Substances Control Law. Its properties are similar to those of parathion, only it decomposes more easily than parathion.

Parathion-methyl was registered as an agricultural chemical in 1952, in Japan. Registered name had been methyl parathion. However, it was banned for use in 1971 because of its high toxicity.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

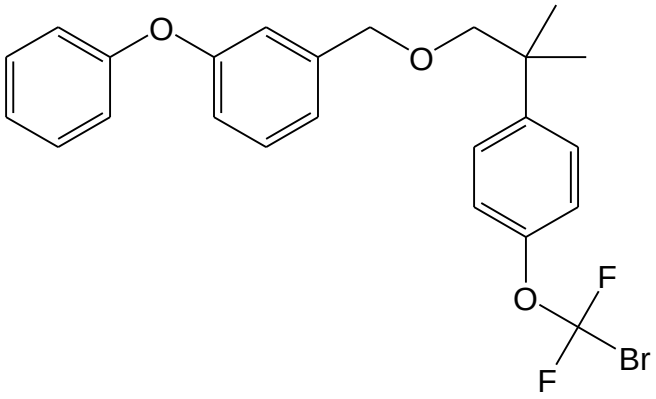
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

130 Halfenprox



2-(4-bromodifluoromethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether

$C_{24}H_{23}BrF_2O_3$ MW: 477.3 CAS No.: 111872-58-3

[Summary of halfenprox]

Halfenprox is a pyrethroid miticide having a chemical structure of phenoxybenzyl propyl ether, developed by Mitsu Toatsu Chemicals in 1986.

Halfenprox was registered as an agricultural chemical for use on fruits etc. in 1994, in Japan. However, it was expired in 2006.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, corn and other grains: 1.0 ppm

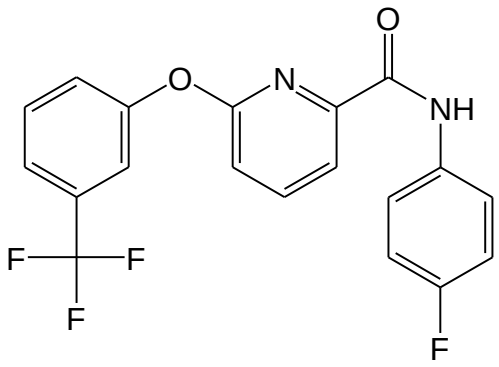
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

131 Picolinafen



N-(4-fluorophenyl)-6-[3-(trifluoromethyl)phenoxy]-2-pyridinecarboxamide

$C_{19}H_{12}F_4N_2O_2$ MW: 376.3 CAS No.: 137641-05-5

[Summary of picolinafen]

Picolinafen, a white fine crystal, is an anilide/pyridine herbicide, developed by BASF (Germany).

Picolinafen has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn and rye: 0.02 ppm / Barley and wheat: 0.04 ppm / Other grains: 0.02 ppm

[Method listed in the Analytical Standards of Feeds]

1. Analysis method for picolinafen by gas chromatograph-mass spectrometer [Analytical Standards of Feeds, Chapter 6, Section 1, Article 131.1]

A. Reagent Preparation

1) Picolinafen standard solution. Weigh accurately 25 mg of picolinafen [$C_{19}H_{12}F_4N_2O_2$][1], transfer to a 50 mL volmetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the picolinafen standard stock solution (1 mL of this solution contains 0.5 mg as picolinafen).

Before use, dilute accurately a certain amount of the standard stock solution with a diluent solvent to prepare several standard solutions that contain 0.002 – 1 µg of picolinafen in 1 mL.

2) Diluent solvent. Add 50 µL of polyethylene glycol (average molecular weight 400) to 100 mL of acetone to prepare the diluent solbent.

B. Quantification

Extraction. Weigh 10.0 g of an analysis sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 20 mL of water (30 mL for grass hay) and leave to stand for 30 minutes. Further, add 100 mL of acetone and shake for 30 minutes to extract.

Place a 200 mL volmetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask above and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way. Further, add acetone up to the

graduation line of the volmetric flask, transfer 40 mL of this solution accurately to a 200 mL recovery flask, concentrate under reduced pressure in a water bath at 40 °C or lower to ca. 4 mL to prepare a sample solution to be subjected to column treatment I.

Column treatment I. Load the sample solution on the porous diatomite column (for 20 mL retention). Wash the recovery flask that has contained the sample solution with 5 mL of water, add the washing to the column and leave to stand for 5 minutes. Place a 200 mL recovery flask under the column. Wash the recovery flask that has contained the sample solution with 10 mL each of hexane three times, add the washings to the column in order of precedence and elute by natural flow until the liquid level reaches the upper end of the column packing material to elute picolinafen. Further, add 50 mL of the same solvent to the column and elute in the similar way. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 10 mL of cyclohexane – acetone (4 : 1) accurately, centrifuge at 1,000×*g* for 5 minutes, filter the supernatant through a membrane filter (pore size: 0.45μm) to prepare a sample solution to be subjected to gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph. Dispense each elution fraction into a 100 mL recovery flask, concentrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 5 mL of hexane and use this solution as a sample solution for Column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column[2] (20 mm in inner diameter, 300 mm in length, particle size 15 μm)

Guard column: Styrene-divinylbenzene copolymer column[2] (20 mm in inner diameter, 100 mm in length, particle size 15 μm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 75-105 mL [3]

Column treatment II. Wash a silica gel minicolumn (910 mg) with 5 mL of hexane.

Load the sample solution on the minicolumn. Wash the recovery flask that has contained the sample solution twice with 5 mL each of hexane, add the washings to the minicolumn in order of precedence and let flow out until the liquid level reaches the upper end of the column packing material.

Place a 50 mL recovery flask under the minicolumn and add 10 mL of hexane – acetone (17 : 3) to the minicolumn to elute picolinafen. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue with 1 mL of the diluent solvent accurately to prepare a sample solution to be subjected to measurement by a gas chromatograph mass spectrometer.

Measurement by a gas chromatograph mass spectrometer. Inject 2 μL each of the sample solution and respective picolinafen standard solutions into a gas chromatograph mass spectrometer to obtain selected ion monitoring chromatograms.

Example of measurememt conditions

Column: Fused silica capillary column (5 % diphenyl/95 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)[4]

Carrier gas: He (1.0 mL/min, initial flow rate)

Sample injection : Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 1 min) → ramp 20 °C/min → 280 °C (hold 10 min) → 300 °C (hold 10 min)

Detector: Quadrupole mass spectrometer*1

Interface temperature: 280 °C

Ion source temperature: 230 °C

Ionizing voltage: 70 eV

Ionization method: Electron ionization (EI) method

Monitor ion: Quantification ion *m*/*z* 376, confirmation ion *m*/*z* 238

Calculation. Obtain respective the peak area from the resulting selected ion monitoring chromatograms[5] to prepare a calibration curve and subsequently calculate the amount of picolinafen in the sample.

* 1. The measurement conditions for GCMS-QP2010 Plus (Shimadzu Corporation).

«Summary of analysis method»

In this method, picolinafen in samples is extracted with aqueous acetone, purified with a porous diatomite column, a GPC and a Florisil minicolumn and quantified by a gas chromatograph mass spectrometer.

The flow sheet of the analysis method is shown in Figure 6.1.131-1.

10 g sample
- 20 mL water (30 mL for grass hay), leave to stand for 30 minutes
- 100 mL acetone, shake for 30 minutes
- Filter by suction (Grade No. 5B)
- Wash with 50 mL of acetone
- Dilute with acetone to 200 mL, dispense 40 mL
- Concentrate under vacuum (40 °C or below, to ca. 4 mL)

Chem Elut cartridge (for 20 mL retention)
- Load the sample solution, wash with 5 mL of water, leave to stand for 5 minutes
- Wash with 10 mL of hexane (×3)
- Elute with 50 mL of hexane
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- 10 mL cyclohexane-acetone (4:1)
- Membrane filter (pore size: 0.5 µm or less)

GPC (inject 5 mL of the sample solution)
- Elution fraction 75 - 105 mL
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- 5 mL hexane

Sep-Pak Plus Florisil cartridge (wash with 5 mL of hexane in advance)
- Load the sample solution
- Wash with 5 mL of hexane (×2)
- Elute with 10 mL of hexane-acetone (17:3)
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- 1 mL acetone containing polyethylene glycol 0.05 v/v%

GC-MS

Figure 6.1.131-1. Flow sheet of the analysis method for picolinafen

Reference: Shingo Matsuo: Research Report of Animal Feed, 34, 15(2009).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (µg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Dairy cattle formula feed	5~100	3	99.4~105	10
pig formula feed	10~100	3	97.2~106	3.1
wheat	10~100	3	91.5~98.2	5.5
corn	10~100	3	90.0~97.7	8.9
ryegrass straw	5~100	3	77.7~91.8	8.7

• Collaborative study

Sample type	No. of labs	Spike concentration (µg/kg)	Spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Ryegrass straw	8	100	88.1	7.5	12	0.54
Dairy cattle formula feed	8	100	97.6	4.2	12	0.53

• Lower limit of quantification: 5 µg/kg in samples (Spike recovery, relative standard deviation)

• Lower limit of detection: 1 µg/kg (*SN* ratio)

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Sigma-Aldrich Japan and other manufacturers.

[2] A column packed with styrene-divinylbenzene copolymer hard gel with the use of eluent. The column and the guard column spedified in Appendix 2 of the Analytical Standards of Feeds is Shodex CLNpak EV-2000 AC and Shodex CLNpak EV-G AC manufactured by Showa Denko. respectively.

[3] Because elution fraction may vary among lots of column, depending on frequency of use, etc., it requires careful check in advance in each laboratory.

[4] For example, Rtx-5MS (Restek).

[5] An example of selected ion monitoring chromatogram (SIM) is shown in Figure 6.1.131-2.

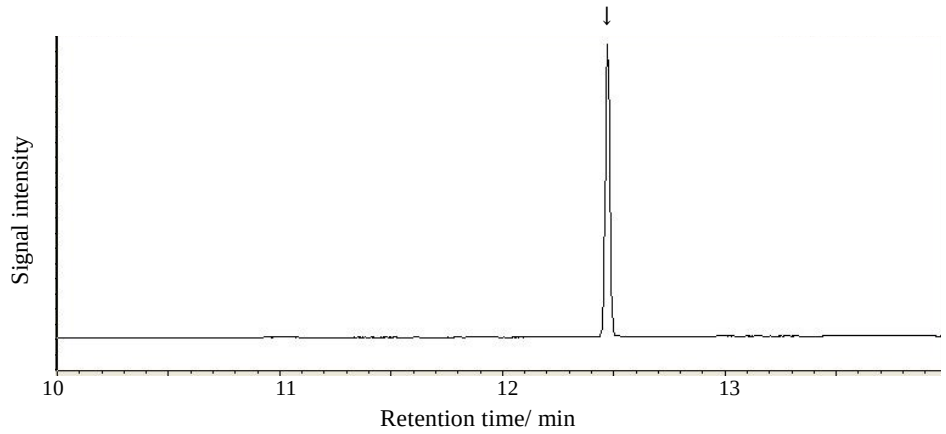
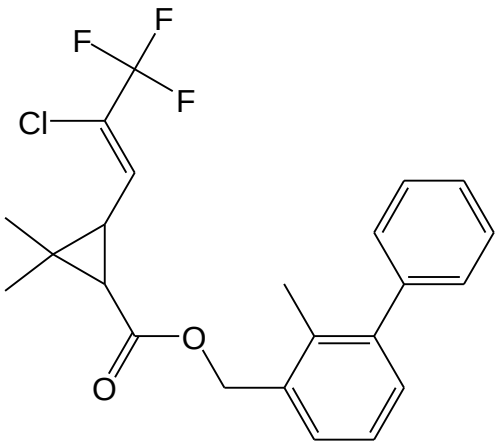


Figure 6.1.131-2. SIM chromatogram of a dairy cattle formula feed spiked with an amount equivalent to 100 mg/kg picolinafen

132 Bifenthrin



2-methylbiphenyl-3-ylmethyl (1*RS*)-*cis*-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropanecarboxylate

$C_{23}H_{22}ClF_3O_2$ MW: 422.9 CAS No.: 82657-04-3

[Summary of bifenthrin]

Bifenthrin is a synthetic pyrethroid insecticide developed by FMC (USA). It has a broad spectrum of activity against insect pests and also exerts a potent insecticidal effect on mite.

Bifenthrin was registered as an agricultural chemical in 1992, in Japan. The trade name is "Terustar".

«Maximum Residue Limits in grains in the Food Sanitation Law»

Barley, rye and corn: 0.05 ppm / Wheat: 0.5 ppm / Other grains: 0.1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

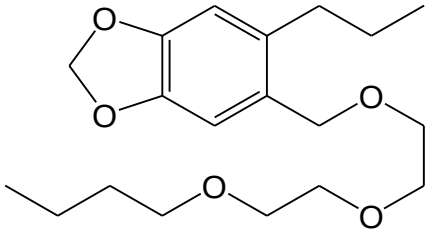
Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

Refer to Article 4, Section 2 in this chapter.

133 Piperonylbutoxide



5-[2-(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole

$C_{19}H_{30}O_5$ MW: 338.44 CAS No.: 51-03-6

[Summary of piperonylbutoxide]

Piperonylbutoxide is used as an agricultural chemical synergist (Agricultural chemical synergists are chemicals that, while not possessing inherent agricultural chemical activity, nonetheless promote or enhance the stability and effectiveness of other agricultural chemical when combined) in combination primarily with pyrethroid insecticides.

Piperonylbutoxide was registered as an insecticide in 1952, in Japan. However, it was expired in 1961. Then it was registered as a plant growth regulator in 1981. However, it was expired in 2004.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Oat, barley, wheat, corn, milo and rye: 24 ppm

[Method listed in the Analytical Standards of Feeds]

1. Liquid chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 133.1]

A. Reagent preparation

1) Piperonylbutoxide standard solution. Weigh accurately 20 mg of piperonylbutoxide[$C_{19}H_{30}O_5$][1], transfer to a 100 mL volumetric flask and dissolve with methanol. Further, add the same solvent up to the graduation line of the flask to prepare the piperonylbutoxide standard stock solution (1 mL of this solution contains 0.2 mg as piperonylbutoxide).

Before use, dilute accurately a certain amount of the standard stock solution with methanol to prepare several piperonylbutoxide standard solutions that contain 0.5 – 5 µg of piperonylbutoxide in 1 mL.

2) Basic alumina. Dry basic alumina for column chromatography (particle size 63 – 200 µm (230 – 70 mesh))[*1] at 105 °C for 1 hour.

3) Magnesium silicate. Dry synthetic magnesium silicate (particle size 74 – 149 µm (200 – 100 mesh))[*2] at 105 °C for 1 hour.

B. Quantification

Extraction. Weigh 40.0 g of an analysis sample[2], transfer to a 300 mL separating funnel, add 100 mL of methanol and shake for 1 hour to extract. Filter the extract through a filter paper (No. 2) to prepare a sample solution to be subjected to purification.

Purification. Transfer 25 mL of the sample solution accurately to a 100 mL recovery flask and concentrate under reduced pressure in a water bath at 50 °C or lower to ca. 2 – 3 mL. Transfer the concentrate to a 100 mL separating funnel A. Wash the recovery flask above with a small amount of methanol and combine the washings with the content of the separating funnel A. Add 50 mL of hexane and 50 mL of sodium chloride solution (10 w/v%), shake vigorously and leave to stand. Transfer the water layer (lower layer) to a 100 mL separating funnel B, and the hexane layer (upper layer) to an Erlenmeyer flask, respectively. Add 50 mL of hexane to the separating funnel B, shake gently and leave to stand. Combine the water layer to the separating funnel A, and the hexane layer to the Erlenmeyer flask above, respectively. Add 50 mL of hexane to the separating funnel A, shake gently and leave to stand. Discard the water layer and transfer the hexane layer to the Erlenmeyer flask above. Dehydrate the hexane layer with a suitable amount of sodium sulfate (anhydrous) and filter through a filter paper (No. 2S) into a 200 mL Erlenmeyer flask. Wash the Erlenmeyer flask above and the filter paper with a small amout of hexane sequentially, filter the washings through this filter paper and combine the filtrates to prepare a sample solution to be subjected to column treatment.

Column treatment. Suspend 2 g of sodium sulfate (anhydrous), 1 g of basic alumina, 1 g of magnesium silicate and 2 g of sodium sulfate (anhydrous) in hexane respectively, pour the suspensions into a column tube (10 mm in inner diameter) sequentially and let flow out by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare the column.

Load the sample solution on the column. Wash the Erlenmeyer flask that has contained the sample solution with a small amout of hexane, add the washings to the column and let flow out by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Place a 100 mL recovery flask under the column and add 50 mL of hexane – chloroform (1 : 1) to the column to elute piperonylbutoxide.

Concentrate the eluate under reduced pressure in a water bath at 50 °C or lower to almost dryness and further dry up by nitrogen gas flow. Dissolve the residue by adding 2 mL of methanol accurately and filter through a membrane filter (pore size: 0.5 µm or less) to prepare a sample solution to be subjected to liquid chromatography.

Liquid chromatography. Inject 20 µL each of the sample solution and respective piperonylbutoxide standard solutions into a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Fluorescence detector (excitation wavelength: 294 nm, emission wavelength: 326 nm)

Column: Octadecylsilylated silica gel column (4.6 mm in inner diameter, 250 mm in length, particle size 5 µm)*3[3]

Eluent: Methanol – water (17 : 3)

Flow rate: 1.0 mL/min

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[4] to prepare a calibration curve and subsequently calculate the amount of piperonylbutoxide in the sample.

* 1. Aluminiumoxid aktiv basisch Art.1076 (Merck) or equivalents.

2. Florisil (Floridin) or wquivalents.

3. UNISIL PACK 5C18-250A (GL Sciences) or equivalents.

«Summary of analysis method»

In this method, piperonylbutoxide in samples is extracted with methanol, purified by liquid-liquid extraction as well as with the use of a synthetic magnesium silicate/basic alumina column and quantified by a liquid chromatograph with a fluorescence detector.

Reference: Norio Saito: Research Report of Animal Feed, 16, 42 (1991)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike Recovery (%)	Repeatability RSD (% or less)
corn	50~1,000	3	80.4~83.9	4.0
milo	50~1,000	3	80.0~84.1	2.5
finishing period broiler formula feed	50~1,000	3	83.0~84.7	7.4

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike Recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
finishing period broiler formula feed	6	500	92.0	2.9	5.8	0.32

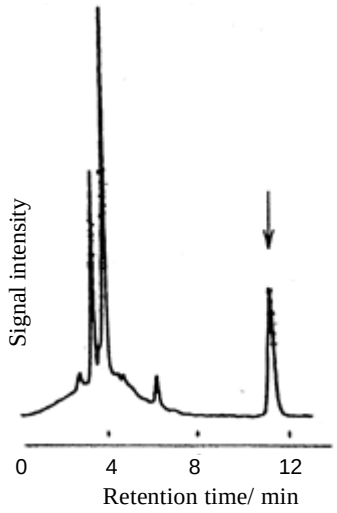
«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers.

[2] It is desirable to adjust the amount of samples to limit the concentration of the piperonylbutoxide in the final sample solution within the concentration range of standard solution described in 1) of section A. In order to determine the concentration roughly, dry under reduced pressure the sample solution described in section B in a water bath at 50 °C or lower, dissolve in 2 mL of methanol and measure the concentration by the liquid chromatograph under the same conditions described in section B. For this determination, a 100 – 10 μg/mL piperonylbutoxide standard solution prepared in the same manner as described in 1) of section A may be used.

[3] Any columns packed with an end-capped packing material equivalent to this one may be used.

[4] An example of chromatogram is shown in Figure 6.1.133-1.



Measurement conditions

Detector: excitation wavelength 294 nm, emission wavelength 326 nm

Column: UNISIL PACK 5C18-250A (4.6 mm in inner diameter, 250 mm in length, particle size 5 μm)

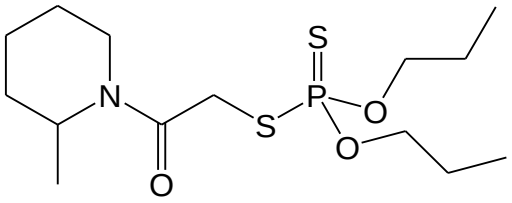
Eluent: Methanol – water (17 : 3)

Flow rate:1.0 mL/min

Column oven temperature: 35 °C

Figure 6.1.133-1. Chromatogram of a formula feed spiked with an amount equivalent to 0.5 mg/kg piperonylbutoxide
(The arrow indicates the peak of piperonylbutoxide.)

134 Piperophos



S-2-methylpiperidinocarbonylmethyl *O*,*O*-dipropyl phosphorodithioate

$C_{14}H_{28}NO_3PS_2$ MW: 353.48 CAS No.: 24151-93-7

[Summary of piperophos]

Piperophos is an organophosphorous herbicide developed by Ciba-Geigy (Switzerland).

Piperophos was registered as an agricultural chemical in 1975, in Japan. However, it was expired in 2003.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

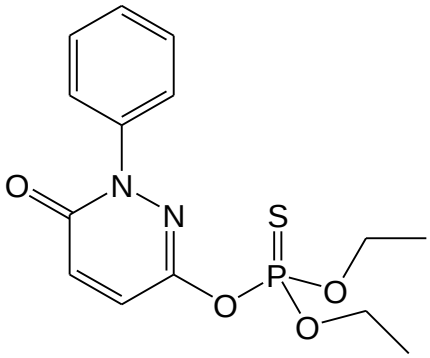
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

135 Pyridaphenthion



O-(1,6-dihydro-6-oxo-1-phenylpyridazin-3-yl) *O*,*O*-diethyl phosphorothioate

$C_{14}H_{17}N_2O_4PS$ MW: 340.3 CAS No.: 119-12-0

[Summary of pyridaphenthion]

Pyridaphenthion is an organophosphorous insecticide developed by Mitsui Toatsu Chemicals in 1968.

Pyridaphenthion was registered as an agricultural chemical in 1973, in Japan. However, it was expired in 2007.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

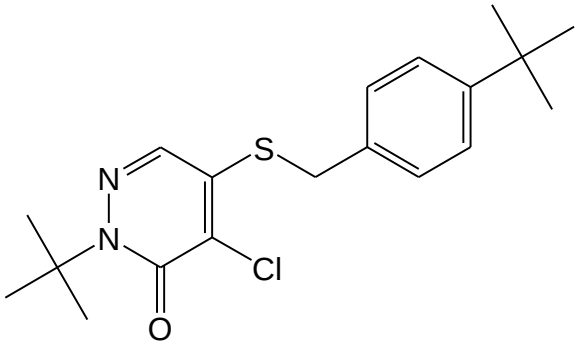
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

136 Pyridaben



2-*tert*-butyl-5-(4-*tert*-butylbenzylthio)-4-chloropyridazin-3(2*H*)-one

$C_{19}H_{25}ClN_2OS$ MW: 364.9 CAS No.: 96489-71-3

[Summary of pyridaben]

Pyridaben is a miticide developed by Nissan Chemical Industries, that has inhibitory effect on electron transport system of mites.

Pyridaben was registered as a pesticide to be used on vegetables, tee trees etc. in 1991, in Japan. The trade name is “Sunmight”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

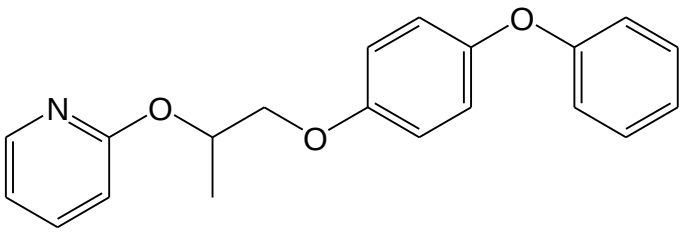
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

137 Pyriproxyfen



4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether

$C_{20}H_{19}NO_3$ MW: 321.4 CAS No.: 95737-68-1

[Summary of pyriproxyfen]

Pyriproxyfen is an insecticide bearing the 4-phenoxyphenoxy skelton, developed by Sumitomo Chemical in 1981. This chemical mimics the action of juvenile hormones and exerts an insecticidal effect on whiteflies, aphids, thrips, etc. by interfering with their metamorphosis into pupae or adults.

Pyriproxyfen was registered as an agricultural chemical in 1995, in Japan. The trade name is "Lano". Pyriproxyfen is also registered as a veterinary drug.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

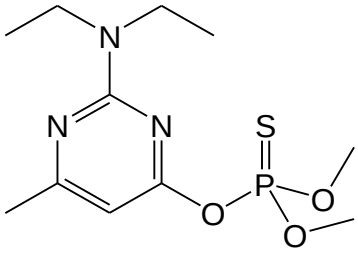
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

138 Pirimiphos-methyl



O-2-diethylamino-6-methylpyrimidin-4-yl *O*,*O*-dimethyl phosphorothioate

$C_{11}H_{20}N_3O_3PS$ MW: 305.3 CAS No.: 29232-93-7

[Summary of pirimiphos-methyl]

Pirimiphos-methyl, a pale yellow liquid, is an organophosphorous insecticide developed by ICI (UK) that is readily soluble in organic solvents and decomposed by acid and alkali. It exerts insecticidal effect on diamondback moth and greenhouse whitefly through contact and fumigation.

Pirimiphos-methyl was registered as an agricultural chemical in 1976, in Japan. The trade name is "Akuterick".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Oat, barley, wheat, corn, milo and rye: 1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

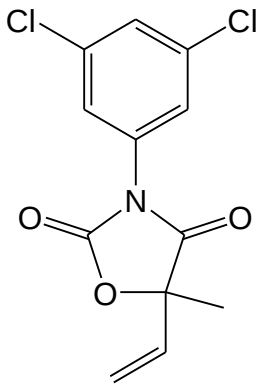
Refer to Article 2, Section 2 in this chapter.

3. Simultaneous analysis method for chlorpyrifos-methyl and pirimiphos-methyl by gas chromatography

Target Analytes: Chlorpyrifos-methyl and pirimiphos-methyl (2 compounds)

Refer to Article 12, Section 3 in this chapter.

139 Vinclozolin



(*RS*)-3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione
$C_{12}H_9Cl_2NO_3$ MW: 286.114 CAS No.: 50471-44-8

[Summary of vinclozolin]

Vinclozolin is a contact oxazole fungicide developed by BASF (Germany), that exerts fungicidal activity against gray mold rot of vegetables and pulses by inhibiting spore formation and mycelial growth.

Vinclozolin was registered as an agricultural chemical in 1981, in Japan. However, it was expired in 1998.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 139.2]

A. Reagent Preparation

1) Vinclozolin Standard Solution. Weigh accurately 20 mg of vinclozolin[$C_{12}H_9Cl_2NO_3$][1], transfer to a 100 mL volumetric flask and dissolve by adding 20 mL of acetone. Further, add 2,2,4-trimethylpentane to the graduation line of the flask to prepare the vinclozolin standard stock solution (1 mL of this solution contains 0.2 mg as vinclozolin).

Before use, dilute accurately a certain amount of the standard stock solution with 2,2,4-trimethylpentane – acetone (4 : 1) to prepare several vinclozolin standard solutions that contain 0.01 – 2 μg of vinclozolin in 1 mL.

2) Magnesium silicate[2]. Dry synthetic magnesium silicate (particle size 149-250 μm (100-60 mesh)) at 130 °C for 16 hours.

B. Quantification

Extraction. Weigh 5 – 10 g of an analysis sample accurately, transfer it to a stoppered 200 mL Erlenmeyer flask, add 15 mL of water to moisten and leave to stand for 30 minutes. Further, add it 80 mL of acetone and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask above and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to ca. 15 mL, add 5 g of sodium chloride to prepare a sample solution to be subjected to column treatment I.

Column treatment I[3]. Load the sample solution on a porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that has contained the sample solution three times with 10 mL each of hexane, add the washings to the column in order of precedence and elute vinclozolin by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 90 mL of hexane to the column and elute in the similar way. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow. Dissolve the residue by adding 10 mL of cyclohexane – acetone (7 : 3) accurately and filter through a membrane filter (pore size: 0.5 µm or less) to prepare a sample solution to be subjected to gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph. Dispense each elution fraction into a 50 mL recovery flask, concentrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 5 mL of hexane – diethyl ether (49 : 1) and to prepare a sample solution to be subjected to column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column[4] (20 mm in inner diameter, 300 mm in length, particle size 15 µm)

Guard column: Styrene-divinylbenzene copolymer column[4] (20 mm in inner diameter, 100 mm in length, particle size 15 µm)

Eluent: Cyclohexane – acetone (7 : 3)

Flow rate: 5 mL/min

Fraction collection: 60-75 mL[5]

Column treatment II. Suspend 10 g of magnesium silicate in hexane, pour into a column (15 mm inner diameter) and let flow out so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Load the sample solution on the column. Wash the recovery flask that has contained the sample solution three times with 5 mL each of hexane – diethyl ether (49 : 1), add the washings to the column in order of precedence and let them flow out by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Further, add 30 mL of hexane – diethyl ether (49 : 1) to the column to obtain an outflow in the similar way.

Place a 200 mL recovery flask under the column and add 80 mL of hexane – diethyl ether (17 : 3) to the column to elute vinclozolin. Concentrate the eluate under reduced pressure in a water bath at 40 °C or

lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately to prepare a sample solution to be subjected to gas chromatography.

Gas chromatography. Inject 2 μL each of the sample solution and respective vinclozolin standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame thermionic detector

Column: Fused silica capillary column (5 % diphenyl/95 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm) [6]

Carrier gas: He (1.5 mL/min)

Make up gas: He (30 mL/min)

Hydrogen: 3 mL/min

Dry air:140 mL/min

Sample injection : Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 1 min) → ramp 20 °C/min → 280 °C (hold 10 min)

Detector temperature: 280 °C

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[7] to prepare a calibration curve and subsequently calculate the amount of vinclozolin present in the sample.

«Summary of analysis method»

In this method, vinclozolin in samples is extracted with aqueous acetone, purified with a porous diatomite column, a GPC and a magnesium silicate column and quantified by a gas chromatograph with an flame thermionic detector.

The flow sheet of the analysis method is shown in Figure 6.1.139-1.

5 g sample
- 5 mL water, leave to stand for 30 minutes
- 80 mL acetone, shake for 30 minutes
- Filter by suction (Grade No. 5B)
- Wash with 50 mL of acetone
- Concentrate under vacuum (to ca.15 mL)
- 5 g sodium chloride

Chem Elut cartridge (20 mL retention)
- Load sample solution, leave to stand for 5 minutes
- Wash with 120 mL of hexane, elute
- Concentrate under vacuum, dry up by nitrogen gas flow
- Filter (5 μm membrane filter)
- Add 10 mL of cyclohexane-acetone (7:3)

GPC (Inject 5 mL of sample solution)
- Elution collection 60-75 mL
- Concentrate under vacuum, dry up by nitrogen gas flow
- 5 mL hexane-diethyl ether (49:1)

Florisil column
- Wash with 45 mL of hexabe-diethyl ether (49:1)
- Elute with 80 mL of hexane-diethyl ether (17:3)
- Concentrate under vacuum, dry up by nitrogen gas flow
- 5 mL 2,2,4-trimethylpentane/acetone (4:1)

GC-FTD

Figure 6.1.139-1. Flow sheet of the analysis method for vinclozolin

Reference: Akira Furukawa, Masato Shibata: Research Report of Animal Feed, 23, 50(1998).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike Recovery (%)	Repeatability RSD (% or less)
starting chick formula feed	250~1,000	3	80.3~91.0	8.3
growing pig formula feed	250~1,000	3	86.0~92.0	6.7
alfalfa hay	250~1,000	3	82.3~86.7	9.7

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike Recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
growing pig formula feed	6	500	85.5	3.3	11.2	0.62

• Lower limit of quantification: 20 μg/kg in samples

«Notes and precautions»

[1] Available from Wako Pure Chemical Industries, Kanto Chemical and Hayashi Pure Chemical.

[2] Prepare just before use and leave to cool in a dry desiccator.

[3] With this method, liquid-liquid extraction can be performed quickly. A sample solution (aqueous solution) injected onto column adsorbs on the surface of porous diatomite as a thin film. By the addition of a water-nonmiscible organic solvent, the target compound is extracted from the water layer

with the solvent running down the column. The advantages of this method are its high extraction efficiency and causing no troubles due to emulsion.

[4] A column packed with styrene-divinylbenzene copolymer hard gel with the eluent.

[5] Because elution fraction may vary among lots of column, depending on frequency of use, etc., it requires confirmation in advance.

[6] For example, DB-5 (Agilent Technologies).

[7] An example of chromatogram is shown in Figure 6.1.139-2.

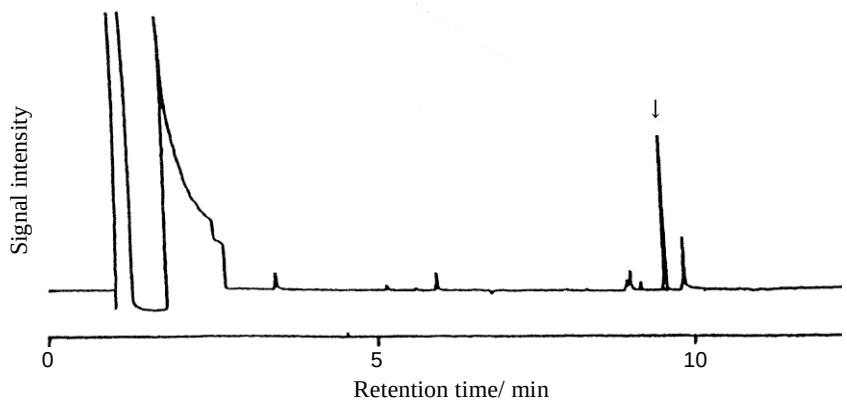


Figure 6.1.139-2. Chromatogram of a chicken formula feed spiked with an amount equivalent to 500 µg/kg vinclozolin
(The arrow indicates the peak of vinclozolin diflubenzuron.)

Measurement conditions

Detector: Flame thermionic detector

Column: J&W scientific DB-5 (0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)

Carrier gas: He (1.5 mL/min, initial flow rate)

Make up gas: He (30 mL/min)

Hydrogen: 3 mL/min

Dry air: 140 mL/min

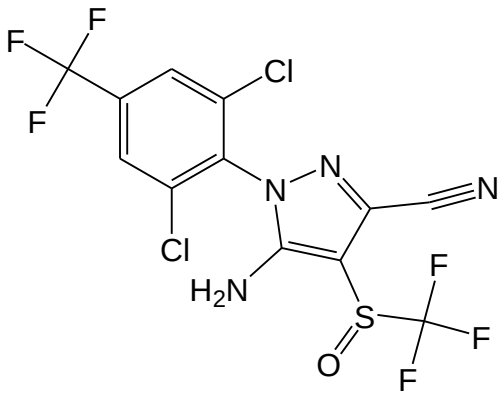
Sample injection: Splitless mode

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 1 min) → ramp 20 °C/min → 280 °C (hold 10 min)

Detector temperature: 280 °C

140 Fipronil



5-amino-1-(2,6-dichloro-*α*,*α*,*α*-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile

$C_{12}H_4Cl_2F_6N_4OS$ MW: 437.1 CAS No.: 120068-37-3

[Summary of fipronil]

Fipronil is a phenylpyrazole insecticide.

In Japan, it acquired the registration as an agricultural chemical for use on paddy rice in 1996. Trade name is "Prince". Fipronil is also used as an epidemic-prevention insecticide as well as a veterinary drug.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

Pasture grass: 2 ppm

Chicken and quail formula feed: 0.01 ppm / Swine feed as well as cattle, sheep, goat and deer feed: 0.02 ppm

Rice plant silage: 0.1 ppm / Rice straw: 0.2 ppm

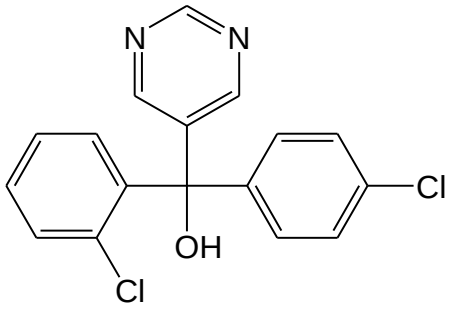
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

141 Fenarimol



(*RS*)-2,4′-dichloro-*α*-(pyrimidin-5-yl)benzhydryl alcohol

$C_{17}H_{12}Cl_2N_2O$ MW: 331.2 CAS No.: 60168-88-9

[Summary of fenarimol]

Fenarimol is a pyrimidine fungicide developed by Eli Lilly (USA). It is effective against powdery mildew of fruit trees and vegetables, apple and pear scab or frogeye leaf spot.

Fenarimol was registerd as an agrichmical in 1987, in Japan. The trade name is “Rubigan”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

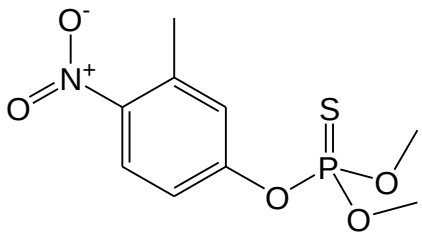
Refer to Article 1, Section 3 in this chapter.

2. Simultaneous analysis method for tebuconazole and fenarimol by gas chromatography

Target Analytes: Tebuconazole and fenarimol (2 compounds)

Refer to Article 16, Section 3 in this chapter.

142 Fenitrothion (MEP)



O,*O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate

$C_9H_{12}NO_5PS$ MW: 277.2 CAS No.: 122-14-5

[Summary of fenitrothion (MEP)]

Fenitrothion (MEP), a pale yellow liquid, is an organophosphorous insecticide developed by Sumitomo Chemical.

Although it has a chemical structure resembling parathion-methyl, its toxicity to warm-blooded animals is mild. Fenitrothion is used as an insecticide for a wide range of agricultural crops.

Fenitrothion was registered as an agricultural chemical in Japan in 1962, in Japan. Registered name is MEP. The trade name is "Smichion".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Oat, corn, milo and rye: 1 ppm / Barley: 5 ppm / Wheat and pasture grass: 10 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

3. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (2)

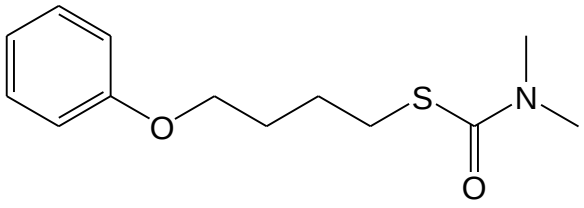
Target Analytes:

Group A: EPN, diazinon, parathion, fenitrothion, fenthion, phenthoate, phosalone and malathion (8 compounds).

Group B: Dichlorvos (1 compound)

Refer to Article 3, Section 2 in this chapter.

143 Fenothiocarb



S-4-phenoxybutyl dimethyl (thiocarbamate)
$C_{13}H_{19}NO_2S$ MW: 253.4 CAS No.: 62850-32-2

[Summary of fenothiocarb]

Fenothiocarb is a thiocarbamate insecticide.

Fenothiocarb was registered as an agricultural chemical for use on “mikan” (mandarine orange) in 1986, in Japan. The trade name is “Panocon”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

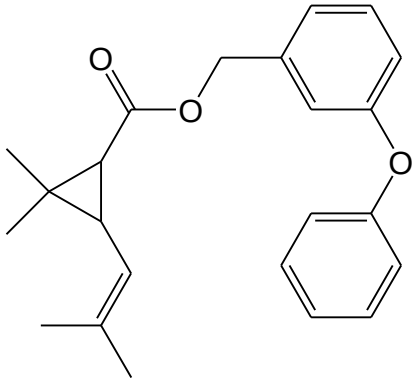
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

144 Phenothrin



3-phenoxybenzyl (1*RS*)-*cis*-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate

$C_{23}H_{26}O_3$ MW: 350.5 CAS No.: 26002-80-2

[Summary of phenothrin]

Phenothrin is a synthetic pyrethroid insecticide developed by Sumitomo Chemical.

Phenothrin has not been registered as an agricultural chemical in Japan. However, phenothrin is used as an insecticide and a pediculicide.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Barley, rye and corn: 0.02 ppm / Wheat: 2 ppm / Other grains: 0.02 ppm

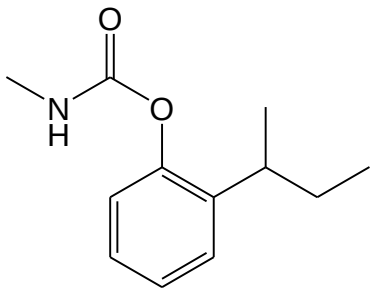
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

145 Fenobucarb (BPMC)



(*RS*)-2-*sec*-butylphenyl methylcarbamate

$C_{12}H_{17}NO_2$ MW: 207.27 CAS No.: 3766-81-2

[Summary of fenobucarb (BPMC)]

Fenobucarb (BPMC), a colorless crystalline solid having a chemical structure similar to that of isoprocarb, is a carbamate insecticide developed by Kumiai Chemical Indystry. It has a low melting point and is unstable in strong acid and alkali.

Diflubenzuron was registered as an agricultural chemical in 1968, in Japan. Registered name is BPMC. The trade names are “BASSA” and “Osbac”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

Oat, barley, wheat, corn, milo and rye: 0.3 ppm

Rice straw and Rice plant silage: 5 ppm / Paddy rice: 3 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)

Target Analytes: XMC, aldicarb (including aldicarb sulfoxide and aldicarb sulfone), isoprocarb, carbaryl, carbofuran, xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb (12 compounds)

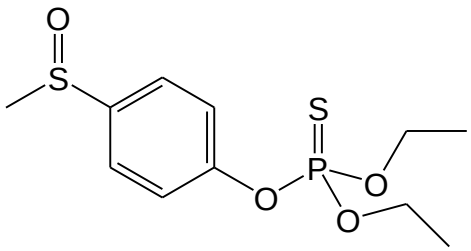
Refer to Article 3, Section 3 in this chapter.

2. Simultaneous analysis method of carbamate agricultural chemicals by gas chromatography

Target Analytes: XMC, isoprocarb, carbaryl, xylylcarb, fenobucarb, propoxur and metolcarb (7 compounds)

Refer to Article 5, Section 3 in this chapter.

146 Fensulfothion (DMSP)



O,*O*-diethyl *O*-4-methylsulfinylphenyl phosphorothioate

$C_{11}H_{17}O_4PS_2$ MW: 308.35 CAS No.: 115-90-2

[Summary of fensulfothion (DMSP)]

Fensulfothion (DMSP), a yellow-brown liquid, is an organophosphorous nematicide that is applied in the soil for control of cyst nematode, root-knob nematode and the like.

Fensulfothion has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn and other grains: 0.1 ppm

[Method listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

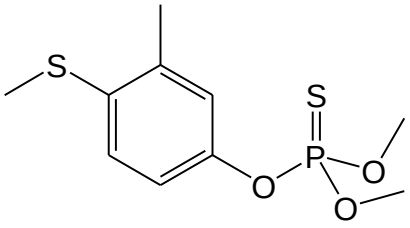
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

147 Fenthion (MPP)



O,*O*-dimethyl *O*-4-methylthio-*m*-tolyl phosphorothioate

$C_{10}H_{15}O_3PS_2$ MW: 278.3 CAS No.: 55-38-9

[Summary of fenthion (MPP)]

Fenthion (MPP), a colorless liquid, is an organophosphorous insecticide, which is used for control of stem borers on rice, ladybird beetles on vegetables and aphids.

Fenthion was registered as an agricultural chemical in 1961, in Japan. Registered name is MPP. The trade name is “Baycid”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

Corn: 5 ppm

Rice plant silage: 0.1 ppm / Rice straw: 2 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

3. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (2)

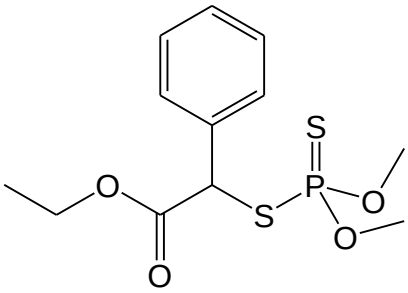
Target Analytes:

Group A: EPN, diazinon, parathion, fenitrothion, fenthion, phenthoate, phosalone and malathion (8 compounds).

Group B: Dichlorvos (1 compound)

Refer to Article 3, Section 2 in this chapter.

148 Phenthoate (PAP)



S-*α*-ethoxycarbonylbenzyl *O*,*O*-dimethyl phosphorodithioate

$C_{12}H_{17}O_4PS_2$ MW: 320.4 CAS No.: 2597-03-7

[Summary of phenthoate (PAP)]

Phenthoate (PAP), a colorless crystalline solid, is an organophosphorous insecticide developed by Montecatini Edison (Italy) and Bayer AG (Germany).

It is mildly toxic and effective against a wide range of insect pests. It is a fast-acting contact insecticide, but works also as a systemic insecticide.

Phenthoate was registered as an agricultural chemical in 1963, in Japan. Registered name is PAP. The trade names are "Papthion" and "Elsan".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

Oat, barley, wheat, corn, milo and rye: 0.4 ppm

Rice plant silage: 1 ppm / Rice straw: 2 ppm / Paddy rice: 0.7 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

3. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (2)

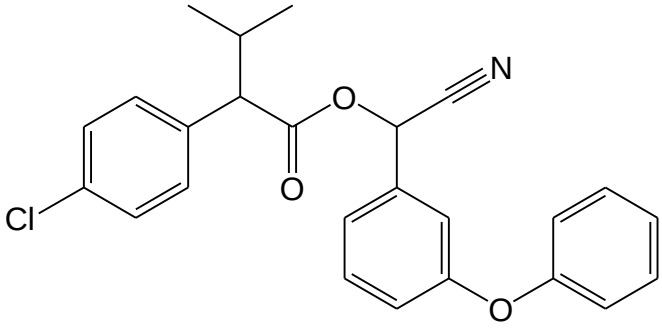
Target Analytes:

Group A: EPN, diazinon, parathion, fenitrothion, fenthion, phenthoate, phosalone and malathion (8 compounds).

Group B: Dichlorvos (1 compound)

Refer to Article 3, Section 2 in this chapter.

149 Fenvalerate



(*RS*)-*α*-cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate
$C_{25}H_{22}ClNO_3$ MW: 419.9 CAS No.: 51630-58-1

Esfenvalerate (CAS No.: 66230-04-4):
(*S*)-*α*-cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-3-methylbutyrate

[Summary of fenvalerate]

Fenvalerate is a synthetic pyrethroid insecticide developed by Sumitomo Chemical. It was synthesized based on the chemical structure of pyrethrin, the main insecticidal component from pyrethrum. Because of its residual efficacy superior to that of pyrethrin, fenvalerate is widely used as a household insecticide.

Fenvalerate was registered as an agricultural chemical in 1983, in Japan. The trade name is "Sumicidin".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(The sum of each isomer. Fenvalerate includes Esfenvalerate.)

Pasture grass: 13 ppm

Chicken and quail feed: 0.5 ppm / Pig feed: 4 ppm / cattle, sheep, goat and deer feed: 8 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

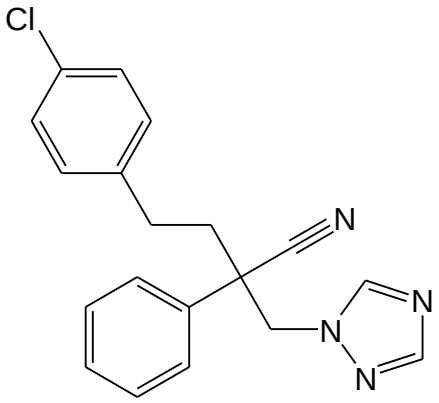
Refer to Article 4, Section 2 in this chapter.

3. Simultaneous analysis method for fenvalerate and permethrin by gas chromatography

Target Analytes: Fenvalerate and permethrin (2 compounds)

Refer to Article 17, Section 3 in this chapter.

150 Fenbuconazole



(*RS*)-4-(4-chlorophenyl)-2-phenyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)butyronitrile

$C_{19}H_{17}ClN_4$ MW: 336.8 CAS No.: 114369-43-6

[Summary of fenbuconazole]

Fenbuconazole is a triazole fungicide developed by Rohm and Haas (USA) in 1978. The mode of action is the inhibition of biosynthesis of ergosterol, a critical component for the integrity of fungal cell membrane.

Fenbuconazole was registered as an agricultural chemical to be used on fruit trees and the like in 2001, in Japan. The trade name is “Indar”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat and rye: 0.1 ppm / Barley: 0.2 ppm

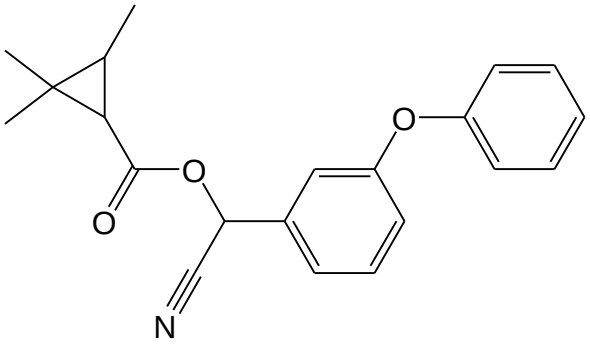
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

151 Fenpropathrin



(*RS*)-*α*-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate

$C_{22}H_{23}NO_3$ MW: 349.4 CAS No.: 39515-41-8, 64257-84-7

[Summary of fenpropathrin]

Fenpropathrin is a synthetic pyrethroid insecticide developed by Sumitomo Chemical. Like other pyrethroid insecticides, fenpropathrin has a broad insecticidal spectrum, and is especially effective against mites.

Fenpropathrin was registered as an agricultural chemical in 1989, in Japan. The trade names are "Rody" and "Danitol".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Pasture grass: 20 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

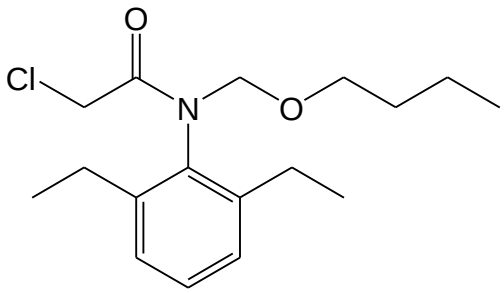
Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

Refer to Article 4, Section 2 in this chapter.

152 Butachlor



N-butoxymethyl-2-chloro-2′,6′-diethylacetanilide

$C_{17}H_{26}ClNO_2$ MW: 311.85 CAS No.: 23184-66-9

[Summary of butachlor]

Butachlor is a non-hormone, acid amide herbicide used for early weed control on paddy fields as a soil treatment. Because of its poor solubility in water and low mobility in soils, butachlor can control weeds for a prolonged period. It is decomposed mainly by microorganisms, and loss through photodecomposition or volatilization is relatively small.

Butachlor was registered as an agricultural chemical in 1973, in Japan. However it was expired in 1997, but was re-registered within the same year. The Trade name is "Machete".

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

[Method listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

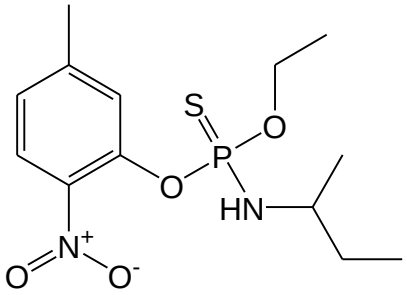
Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

153 Butamifos



(*RS*)-{*O*-ethyl *O*-6-nitro-*m*-tolyl [(*RS*)-*sec*-butyl]phosphoramidothioate}

$C_{13}H_{21}N_2O_4PS$ MW: 332.4 CAS No.: 36335-67-8

[Summary of butamifos]

Butamifos is an organophosphorous herbicide developed by Sumitomo Chemical.

Butamifos was registered as an agricultural chemical to be used on vegetables etc. in 1980, in Japan. The trade names are “Cremart U”, “Tufler” and “Hietop”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

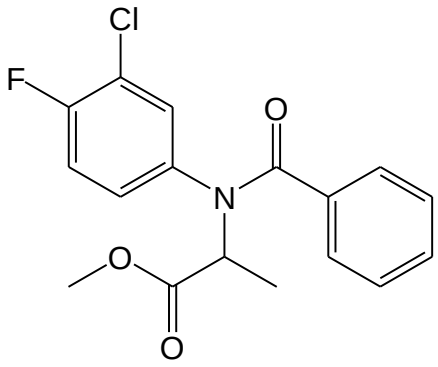
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

154 Flamprop-methyl



Methyl *N*-benzoyl-*N*-(3-chloro-4-fluorophenyl)-DL-alaninate

$C_{17}H_{15}ClFNO_3$ MW: 335.8 CAS No.: 52756-25-9

[Summary of flamprop-methyl]

Flamprop-methyl is an allyl alanine herbicide.

Flamprop-methyl has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat and other grains: 0.05 ppm

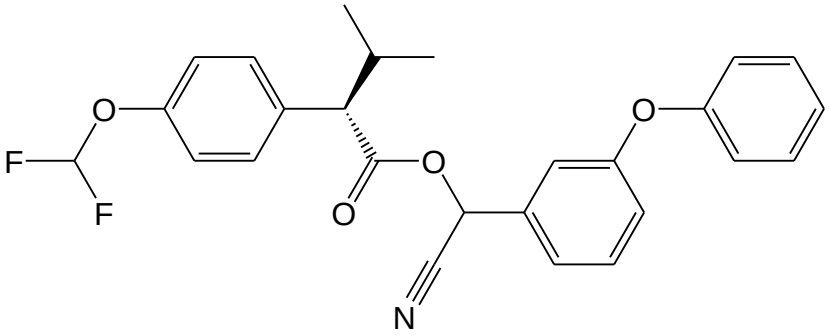
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

155 Flucythrinate



(*RS*)-*α*-cyano-3-phenoxybenzyl (*S*)-2-(4-difluoromethoxyphenyl)-3-methylbutyrate

$C_{26}H_{23}F_2NO_4$ MW: 451.5 CAS No.: 70124-77-5

[Summary of flucythrinate]

Flucythrinate is a synthetic pyrethroid insecticide developed by American Cyanamid (USA).

Flucythrinate was registered as an agricultural chemical in 1987, in Japan. The Trade name is “Pay-Off”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(The sum of each isomer)

Rye and corn: 0.05 ppm / Wheat, barley and other grains: 0.20 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

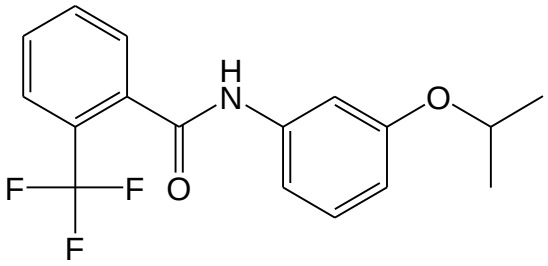
Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

Refer to Article 4, Section 2 in this chapter.

156 Flutolanil



α,*α*,*α*-trifluoro-3'-isopropoxy-*o*-toluanilide

$C_{17}H_{16}F_3NO_2$ MW: 323.3 CAS No.: 66332-96-5

[Summary of flutolanil]

Flutolanil is an amide fungicide developed by Nihon Nohyaku in 1976. This chemical exerts selective fungicidal activity against basidiomycetes through its inhibitory effect on mitochondrial electron transport system (complex II).

Flutolanil was registered as an agricultural chemical for use on rice, wheat, barley, pear, vegetables, etc. in 1985, in Japan. The trade name is “Moncut”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Administrative Guidelines for Hazardous Substances in Feeds]

Rice plant silage: 5 ppm / Rice straw: 20 ppm

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat: 2.0 ppm

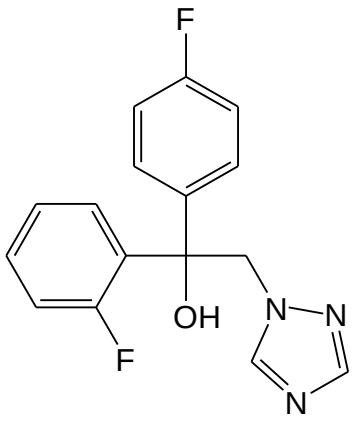
[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

157 Flutriafol



(*RS*)-2,4'-difluoro-*α*-(1*H*-1,2,4-triazol-1-ylmethyl)benzhydryl alcohol

$C_{16}H_{13}F_2N_3O$ MW: 301.3 CAS No.: 76674-21-0

[Summary of flutriafol]

Flutriafol is a triazole fungicide.

Flutriafol has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, rye and corn: 0.02 ppm / Barley: 0.2 ppm / Other grains: 0.02 ppm

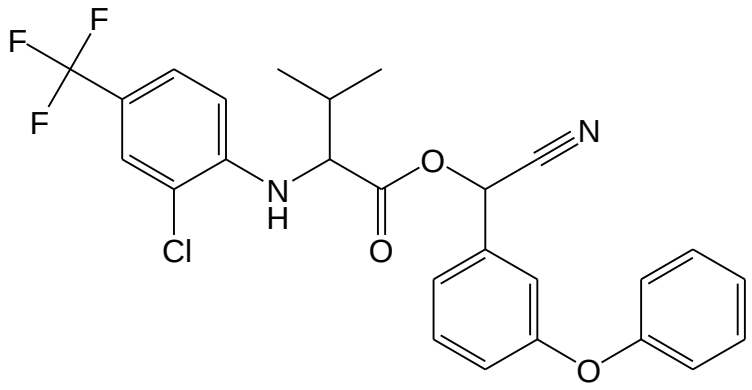
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

158 Fluvalinate



(*RS*)-*α*-cyano-3-phenoxybenzyl *N*-(2-chloro-*α*,*α*,*α*-trifluoro-*p*-tolyl)-DL-valinate

$C_{26}H_{22}ClF_3N_2O_3$ MW: 502.9 CAS No.: 69409-94-5

[Summary of fluvalinate]

Fluvalinate is a synthetic pyrethroid insecticide and miticide developed by Zoecon (USA).

Fluvalinate was registered as an agricultural chemical in 1987, in Japan. The trade name is “Mavrik”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(The sum of each isomer)

Wheat and rye: 0.05 ppm / Barley and other grains: 0.2 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

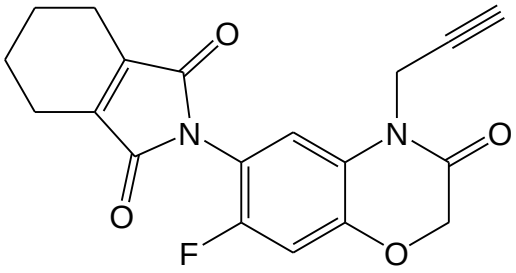
Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

Refer to Article 4, Section 2 in this chapter.

159 Flumioxazin



N-(7-fluoro-3,4-dihydro-3-oxo-4-prop-2-ynyl-2*H*-1,4-benzoxazin-6-yl)cyclohex-1-ene-1,2-dicarboxamide

$C_{19}H_{15}FN_2O_4$ MW: 354.3 CAS No.: 103361-09-7

[Summary of flumioxazin]

Flumioxazin is a photobleaching dicarboximide herbicide developed by Sumitomo Chemical.

Flumioxazin was registered as an agricultural chemical for use on fruit trees, etc. in 2000, in Japan. The trade name is “Dieload”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.05 ppm

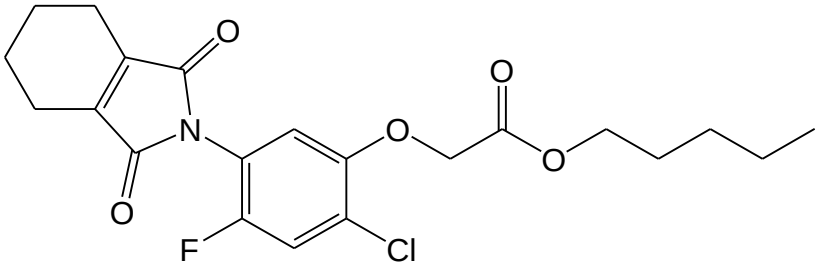
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

160 Flumiclorac-pentyl



pentyl [2-chloro-5-(cyclohex-1-ene-1,2-dicarboximido)-4-fluorophenoxy]acetate

$C_{21}H_{23}ClFNO_5$ MW: 423.9 CAS No.: 87546-18-7

[Summary of flumiclorac-pentyl]

Flumiclorac-pentyl is a dicarboximide herbicide developed by Sumitomo Chemical.

Flumiclorac-pentyl has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn: 0.01 ppm

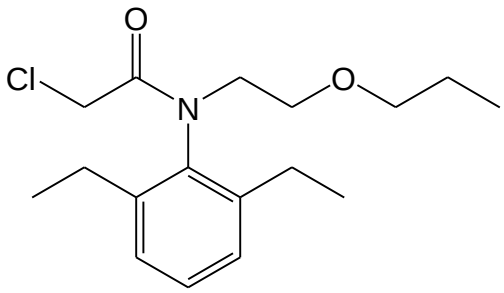
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

161 Pretilachlor



2-chloro-2′,6′-diethyl-*N*-(2-propoxyethyl)acetanilide
$C_{17}H_{26}ClNO_2$ MW: 311.85 CAS No.: 51218-49-6

[Summary of pretilachlor]

Pretilachlor is an anilide herbicide being used as a non-hormone, translocating, soil-applied herbicide, developed by Ciba-Geigy (Switzerland).

Pretilachlor was registered as an agricultural chemical in 1984, in Japan. The trade name is "Solnet".

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

[Method listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

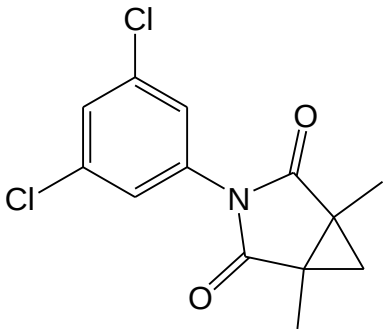
Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

162 Procymidone



N-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide

$C_{13}H_{11}Cl_2NO_2$ MW: 284.1 CAS No.: 32809-16-8

[Summary of procymidone]

Procymidone is a dicarboximide fungicide developed by Sumitomo Chemical.

Procymidone was registered as an agricultural chemical for use on vegetables, citrus, etc. in 1981, in Japan. The trade name is "Sumilex".

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.02 ppm

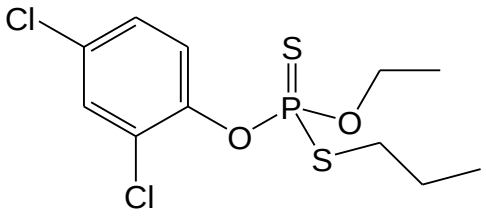
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

163 Prothiofos



(*RS*)-(*O*-2,4-dichlorophenyl *O*-ethyl *S*-propyl phosphorodithioate)

$C_{11}H_{15}Cl_2O_2PS_2$ MW: 345.25 CAS No.: 34643-46-4

[Summary of prothiofos]

Prothiofos, a colorless liquid, is an organophosphorous insecticide developed by Nihon Bayer Agrochem. It is effective against lepidopteran pests and aphids.

Prothiofos was registered as an agricultural chemical in 1975, in Japan. The trade name is “Tokuthion”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

[Method listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

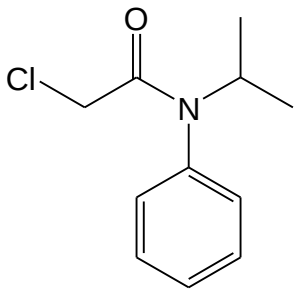
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

164 Propachlor



(*RS*)-2,4′-dichloro-*α*-(pyrimidin-5-yl)benzhydryl alcohol

$C_{11}H_{14}ClNO$ MW: 211.7 CAS No.: 1918-16-7

[Summary of propachlor]

Propachlor is a chloroacetanilide herbicide.

Propachlor has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley and rye: 0.05 ppm / Corn: 0.08 ppm / Other grains: 0.2 ppm

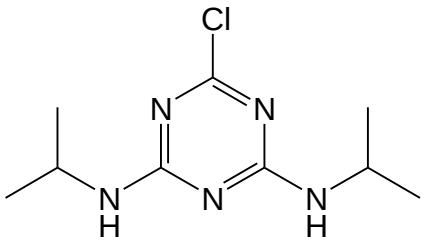
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

165 Propazine



6-chloro-N^2,N^4-diisopropyl-1,3,5-triazine-2,4-diamine

$C_9H_{16}ClN_5$ MW: 229.7 CAS No.: 139-40-2

[Summary of propazine]

Propazine is a triazine herbicide that is used as a non-hormone, translocating soil treatment.

Propazine was registered as an agricultural chemical in 1964, in Japan. Registered name has been propazine. However, it was expired in 1991.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Grains: 0.3 ppm

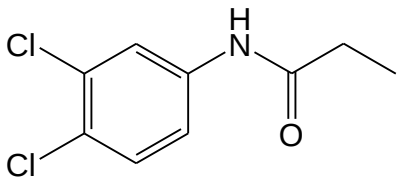
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

166 Propanil (DCPA)



3',4'-dichloropropionanilide

$C_9H_9Cl_2NO$ MW: 218.1 CAS No.: 709-98-8

[Summary of propanil (DCPA)]

Propanil (DCPA) is an acid amide herbicide and used as a contact foliage-applied herbicide.

Propanil was registered as an agricultural chemical in 1961, in Japan. Registered name has been DCPA. However, it was expired in 2007.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley and other grains: 0.2 ppm

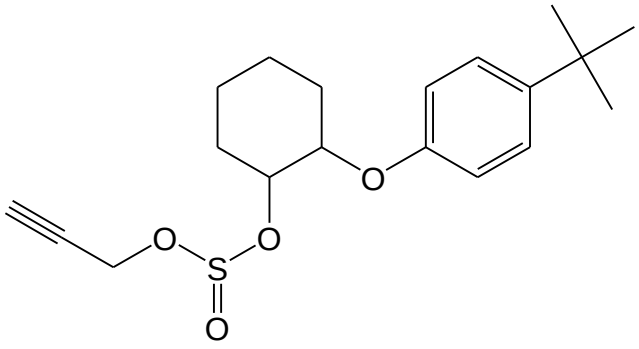
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

167　Propargite (BPPS)



2-(4-*tert*-butylphenoxy)cyclohexyl prop-2-ynyl sulfite

$C_{19}H_{26}O_4S$　MW: 350.5　CAS No.: 2312-35-8

[Summary of propargite (BPPS)]

Propargite (BPPS) is a sulfite ester miticide developed by Naugatuck Chemical (USA).

Propargite was registered as a pesticide to be used on fruit and tee trees in 1967, in Japan. Registered name is BPPS. The trade name is “Omite”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Corn: 0.1 ppm / Other grains: 5 ppm

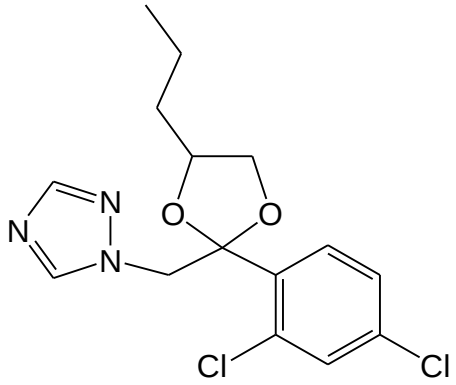
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

168 Propiconazole



(2*RS*,4*RS*;2*RS*,4*SR*)-1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole

$C_{15}H_{17}Cl_2N_3O_2$ MW: 342.2 CAS No.: 60207-90-1

[Summary of propiconazole]

Propiconazole is a triazole fungicide developed by Ciba-Geigy (Switzerland) that exhibits similar mechanism of action to that of triadimefon. It is systemic, has a broad spectrum of activity against fungi. The maximum residue limits established by the Codex Alimentarius Commission are 0.05 – 1 ppm for fruits and 0.05 ppm for seeds, respectively.

Propiconazole was registered as an agricultural chemical in 1991, in Japan. The trade name is "Tilt".

«Maximum Residue Limits in grains in the Food Sanitation Law»

Rye: 0.05 ppm / Wheat, barley and corn: 1.0 ppm / Other grains: 0.05 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

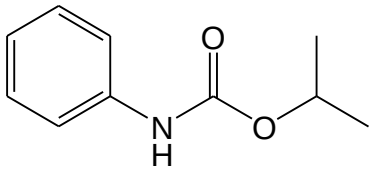
Refer to Article 1, Section 3 in this chapter.

2. Simultaneous analysis method for triazole agricultural chemicals by gas chromatography

Target Analytes: Triadimenol, triadimefon and propiconazole (3 compounds)

Refer to Article 6, Section 3 in this chapter.

169 Propham



isopropyl phenylcarbamate

$C_{10}H_{13}NO_2$ MW: 179.2 CAS No.: 122-42-9

[Summary of propham]

Propham is a carbamate herbicide that is also being used as a plant growth regulator (germination inhibitor in potatoes). Its mechanism of action is thought to be the inhibition of mitosis.

Propham has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Not detected (Limit of detection: 0.01 ppm)

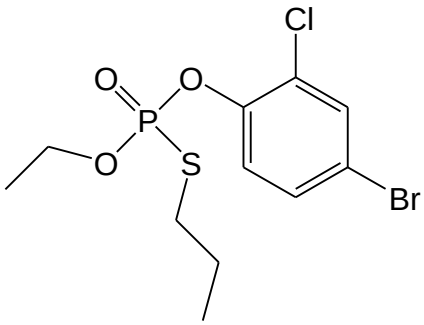
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

170 Profenofos



(*RS*)-(*O*-4-bromo-2-chlorophenyl *O*-ethyl *S*-propyl phosphorothioate)

$C_{11}H_{15}BrClO_3PS$ MW: 373.6 CAS No.: 41198-08-7

[Summary of profenofos]

Profenofos, a clear pale yellow liquid, is an organophosphorous insecticide developed by Ciba-Geigy (Switzerland).

Profenofos was registered as an agricultural chemical to be used on potatoes, tee trees etc. in 1986, in Japan. The trade names are “Encedan” and “Curacron”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.05 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

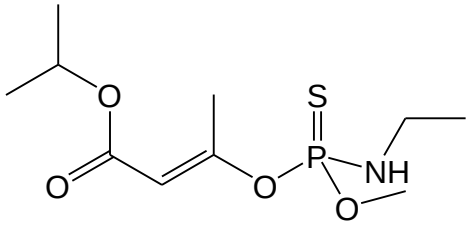
Refer to Article 1, Section 3 in this chapter.

2. Simultaneous analysis method for azinphos-methyl and profenofos by gas chromatography

Target Analytes: Azinphos-methyl and profenofos (2 compounds)

Refer to Article 9, Section 3 in this chapter.

171 Propetamphos



(*RS*)-[(*E*)-*O*-2-isopropoxycarbonyl-1-methylvinyl *O*-methyl ethylphosphoramidothioate]

$C_{10}H_{20}NO_4PS$ MW: 281.3 CAS No.: 31218-83-4

[Summary of propetamphos]

Propetamphos is an organophosphorous insecticide.

Propetamphos has not registered as an agricultural chemical, but is being used for epidemic prevention.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

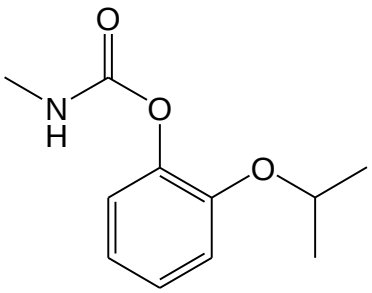
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

172 Propoxur (PHC)



2-isopropoxyphenyl methylcarbamate

$C_{11}H_{15}NO_3$ MW: 209.24 CAS No.: 114-26-1

[Summary of propoxur (PHC)]

Propoxur (PHC), a white crystalline solid, is a carbamate insecticide developed by Bayer AG (Germany). It is decomposed by alkali.

Propoxur was developed as an insect pest control agent against rice planthopper and leafhopper. This insecticide is fast-acting and has contact, ingestion and systemic toxic effects.

Propoxur was registered as an agricultural chemical in 1964, in Japan. Registered name had been PHC. However, it was expired in 2006.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.5 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)

Target Analytes: XMC, aldicarb (including aldicarb sulfoxide and aldicarb sulfone), isoprocarb, carbaryl, carbofuran, xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb (12 compounds)

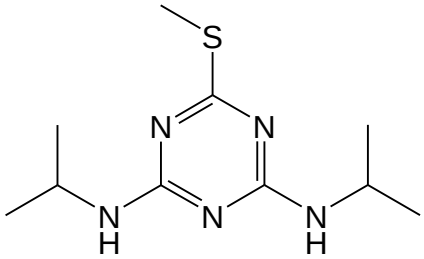
Refer to Article 3, Section 3 in this chapter.

2. Simultaneous analysis method of carbamate agricultural chemicals by gas chromatography

Target Analytes: XMC, isoprocarb, carbaryl, xylylcarb, fenobucarb, propoxur and metolcarb (7 compounds)

Refer to Article 5, Section 3 in this chapter.

173 Prometryn



N^2,*N*^{4}-diisopropyl-6-methylthio-1,3,5-triazine-2,4-diamine

$C_{10}H_{19}N_5S$ MW: 241.4 CAS No.: 7287-19-6

[Summary of prometryn]

Prometryn is a triazine herbicide developed by Ciba-Geigy (Switzerland). It is being used on rice, corn, wheat, barley, soybeans, etc.

Prometryn was registered as an agricultural chemical in 1963, in Japan. The trade name is “Gesagard”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Barley and rye: 0.05 ppm / Wheat: 0.1 ppm / Corn: 0.2 ppm / Other grains: 0.05 ppm

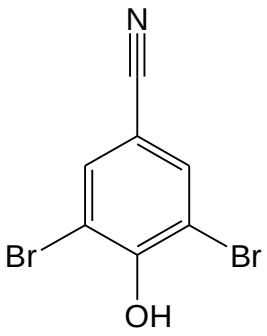
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for ametryn, cyanazine and prometryn by liquid chromatograph-mass spectrometer

Target Analytes: Ametryn, cyanazine and prometryn (3 compounds)

Refer to Article 11, Section 3 in this chapter.

174 Bromoxynil



3,5-dibromo-4-hydroxybenzonitrile
$C_7H_3Br_2NO$ MW: 276.9 CAS No.: 1689-84-5

[Summary of bromoxynil]

Bromoxynil is a non-hormone, contact nitrile herbicide, developed by May & Baker (UK) and Amchem Products (USA) (Both are now affiliated with Rhône-Poulenc (France)).

Bromoxynil has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Pasture grass: 0.1 ppm / Oat, barley, wheat, corn, milo and rye: 0.2 ppm

[Method listed in the Analytical Standards of Feeds]

1. Gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 174.1]

A. Reagent Preparation

Bromoxynil standard solution. Weigh accurately 25 mg of bromoxynil[$C_7H_3Br_2NO$], transfer to a 50 mL volumetric flask and dissolve by adding acetone. Further, add the same solvent to the graduation line of the flask to prepare the bromoxynil standard stock solution (1 mL of this solution contains 0.5 mg as bromoxynil.).

Before use, dilute accurately a certain amount of the standard stock solution with acetone to prepare bromoxynil standard solution that contains 1.0 µg of bromoxynil in 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of an analysis sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 20 mL of water to moisten and 5 mL of hydrochloric acid (1 mol/L) and leave to stand for 30 minutes. Further, add 100 mL of acetone and extract by shaking for 60 minutes. Place a 200 mL volumetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask above and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way. Use the filtrate as a sample solution for purification. Further, add acetone to the graduation line of the flask. Transfer 20 mL of this solution accurately to a 200 mL recovery flask and concentrate under reduced pressure in a water bath at 40 °C or lower to ca. 5 mL[1] to prepare a

sample solution to be subjected to hydrolysis.

Hydrolysis. Add 50 mL of methanol and 10 mL of ammonia water to the sample solution and leave to stand at room temperature for 60 minutes gently shaking occasionally[2]. Concentrate this solution under reduced pressure in a water bath at 40 °C or lower to ca. 10 mL[3] to prepare a sample solution to be subjected to diethyl ether extraction.

Diethyl ether extraction. Transfer the sample solution to a 300 mL separating funnel A, add 100 mL of sodium bicarbonate solution (4 w/v%) and 50 mL of diethyl ether to this separating funnel A, shake for 5 minutes and leave to stand. Transfer the water layer (lower layer) to a 300 mL separating funnel B, add 50 mL of diethyl ether to this separating funnel B, shake for 5 minutes and leave to stand.

Transfer the water layer to a 500 mL separating funnel C, add 15 mL of hydrochloric acid (6 mol/L) little by little to adjust the pH to 2 or lower and leave to stand ca. 20 minutes until bubbling stops[4]. Add 50 mL of diethyl ether to the separating funnel C, shake for 5 minutes and leave to stand. Transfer the water layer to a 500 mL separating funnel D and the diethyl ether layer (upper layer) to a 300 mL Erlenmeyer flask, respectively. Add 50 mL of diethyl ether to the separating funnel D, shake for 5 minutes and leave to stand. Then, discard the water layer and combine the diethyl ether layer with the content of the Erlenmeyer flask. Dehydrate the diethyl ether layer with a suitable amount of sodium sulfate (anhydrous) and filter through a filter paper (No. 5B) into a 200 mL recovery flask. Wash the Erlenmeyer flask above with a small amout of diethyl ether, filter the washings through this filter paper and combine the filtrates.

Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to ca. 1 mL and dry up by nitrogen gas flow[5]. Dissolve the residue by adding 2 mL of acetone to obtain a sample solution to be subjected to methylation.

Methylation. Add 1 mL of methanol and 0.5 mL of trimethylsilyldiazomethane solution[6] to the sample solution and leave to stand at room temperature for 30 minutes gently shaking occasionally[7] to methylate bromoxynil. Add 50 µL of acetone – diethylene glycol (49 : 1) to this solution and dry up by nitrogen gas flow[5]. Dissolve the residue by adding 5 mL of hexane to prepare a sample solution to be subjected to column treatment.

Concurrently, transfer 2 mL of bromoxynil standard solution accurately to a 50 mL pear-shaped flask, methylate in the similar way and dissolve the residue by adding 10 mL of hexane accurately. Dilute accurately a certain amount of this solution with hexane to prepare several bromoxynil standard solutions that contain 2 - 200 ng of bromoxynil in 1 mL.

Column treatment. Wash a synthetic magnesium silicate minicolumn (910 mg) with 10 mL of hexane.

Load the sample solution on the minicolumn and let flow out by natural flow until the liquid level reaches the upper end of the column packing material. Wash the recovery flask that has contained the sample solution twice with 5 mL each of hexane, add the washings to the minicolumn in order of precedence and let flow out in the similar way. Place a 50 mL pear-shaped flask under the minicolumn, add 20 mL of hexane – acetone (99 : 1) to the minicolumn to elute methylated bromoxynil. Add 50 µL of acetone – diethylene glycol (49 : 1) to the eluate, concentrate under reduced pressure in a water bath at 40 °C or lower to ca. 1 mL and further dry up by nitrogen gas flow[5].

Dissolve the residue by adding 2 mL of hexane accurately to prepare a sample solution to be subjected to gas chromatography.

Gas chromatography. Inject 1 µL each of the sample solution and respective standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Electron capture detector

Column: Fused silica capillary column (6 % cyanopropylphenyl/ 94 % dimethyl-polysiloxane coating, 0.32 mm in inner diameter, 30 m in length, film thickness 1.8 µm) [8]

Carrier gas: He (5 mL/min)

Make up gas: N_2 (60 mL/min)

Sample injection : Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 2 min) → ramp 10 °C/min → 260 °C (hold 10 min)

Detector temperature: 300 °C

Calculation. Obtain the peak height from the resulting chromatograms[9] to prepare a calibration curve and subsequently calculate the amount of bromoxynil present in the sample.

«Summary of analysis method»

In this method, bromoxynil in feeds is extracted with acetone acidified with hydrochloric acid, hydrolyzed by alkali, extracted in alkali into the water layer, back-extracted using hydrochloric acid into the diethylether layer, methyl esterified, purified with the use of a Florisil minicolumn and quantified by a gas chromatograph with an electron capture detector.

The flow sheet of the analysis method is shown in Figure 6.1.174-1.

10.0 g sample
- 20 mL water, 5 mL hydrochloric acid (1 mol/L), leave to stand for 30 minutes
- 100 mL acetone, shake for 60 minutes
- Filter by suction
- Wash with 50 mL of acetone, adjust to 200 mL with acetone

Hydrolysis
- 20 mL sample solution
- Concentrate under vacuum (40 °C, to 5 mL)
- 50 mL methanol, 10 mL ammonia water
- Leave to stand for 60 minutes (shake occasionally)
- Concentrate under vacuum (40 °C, to 10 mL)

300 mL separating funnel A
- 100 mL sodium bicarbonate (4 w/v%)
- 50 mL diethyl ether, shake for 5minutes

Water layer (lower layer) | Diethyl ether layer (upper layer)

300 mL separating funnel B
- 50 mL diethyl ether, shake for 5 minutes

Water layer | Diethyl ether layer

500 mL separating funnel C
- 15 mL hydrochloric acid (6 mol/L), leave to stand for 20 minutes
- 50 mL diethyl ether, shake for 5 minutes

To be continued

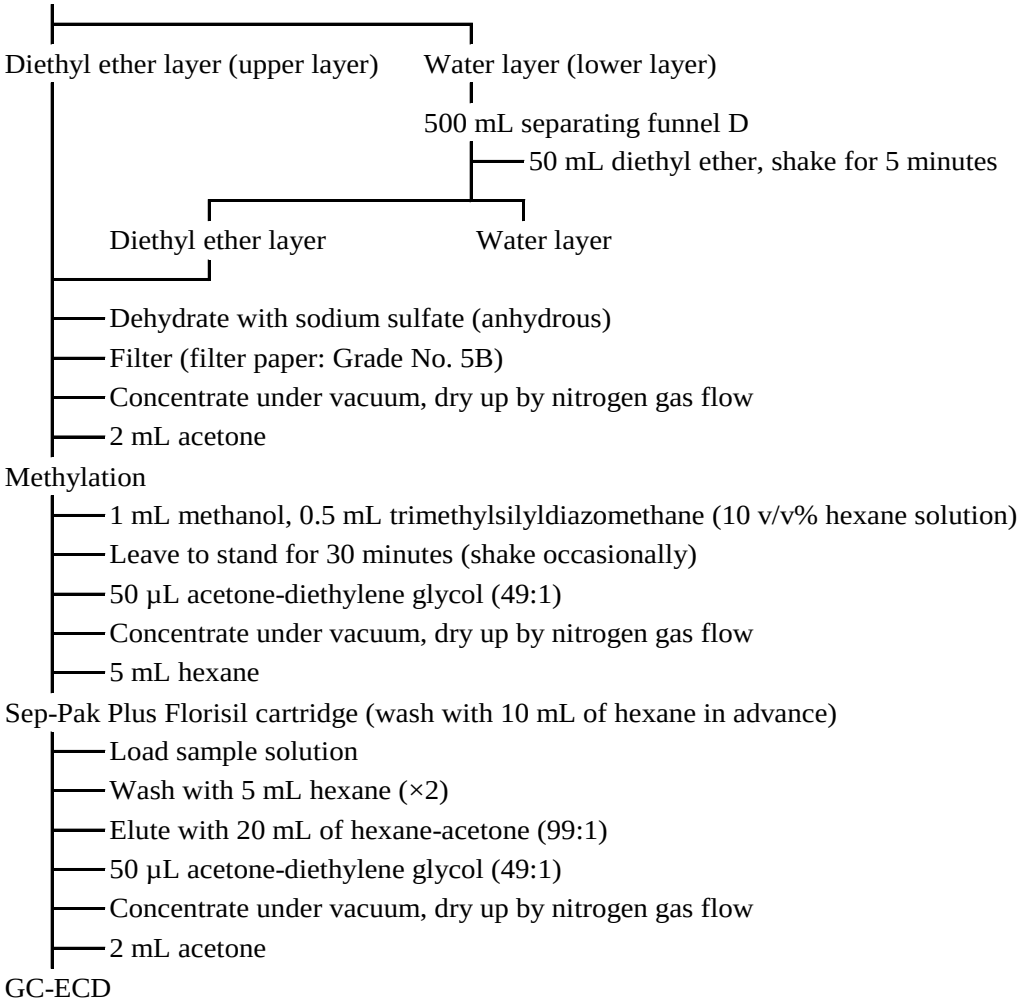


Figure 6.1.174-1. Flow sheet of the analysis method for bromoxynil

Reference: Masakazu Horikiri, Sae Suido, Mayuko Hattori, Yukiko Mitsui: Research Report of Animal Feed, 31, 78(2006).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (µg/kg)	Replicate	Spike Recovery (%)	Repeatability RSD (% or less)
alfalfa	10~100	3	79.8~97.6	9.1
corn	20~200	3	96.5~98.7	7.5
chicken formula feed	20~200	3	104.0~106.8	3.2
cattle formula feed	20~200	3	95.7~98.8	11.8

• Collaborative study

Sample type	No. of labs	Spike concentration (µg/kg)	Spike Recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
timothy	6	100	95.4	4.9	12.3	0.56
corn	6	200	90.7	3.1	7.3	0.35

• Lower limit of quantification: 5 µg/kg (spike recovery and relative standard deviation)

• Lower limit of detection: 2 µg/kg (*SN* ratio)

«Notes and precautions»

[1] Although it takes time because of residual water, concentrate to less than 5 mL when possible, with careful attention to sudden boiling.

[2] Shake gently at ca. 15 minute-intervals.

[3] Concentrate to less than 10 mL when possible.

[4] After 20 minutes of still-standing, shake gently to see if bubbling has stopped.

[5] Because excessive drying up leads to a decrease in recovery, it is better to retain some wetness.

[6] Use new trimethylsilyldiazomethane solution when possible.

[7] Shake gently at ca. 10 minute-intervals.

[8] For example, DB-624 (Agilent Technologies).

[9] An example of chromatogram is shown in Figure 6.1.174-2.

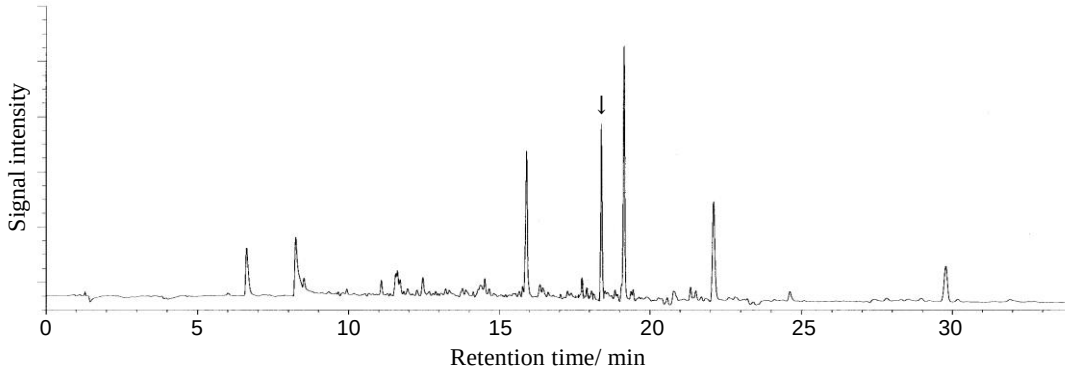
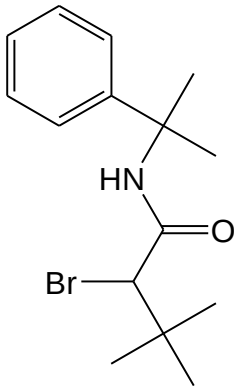


Figure 6.1.174-2. Chromatogram of alfalfa hay spiked with an amount equivalent to 100 µg/kg bromoxynil
(The arrow indicates the peak of bromoxynil derivative.)

175 Bromobutide



(*RS*)-2-bromo-3,3-dimethyl-*N*-(1-methyl-1-phenylethyl)butyramide

$C_{15}H_{22}BrNO$ MW: 312.2 CAS No.: 74712-19-9

[Summary of bromobutide]

Bromobutide is an amide herbicide developed by Sumitomo Chemical., whose mechanism of action is thought to be inhibition of cell division in plants that causes elongation inhibition of roots and eventually leads to killing weeds.

Bromobutide was registered as an agricultural chemical for rice herbicide in 1986, in Japan. It is now available on the market as a herbicide to be used in combination with other pesticides.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Administrative Guidelines for Hazardous Substances in Feeds]

Rice straw: 2 ppm

«Maximum Residue Limits in grains in the Food Sanitation Law»

(In agricultural products, the sum of bromobutide and *N*-(*α*,*α*-dimethylbenzyl)-3,3-dimethylbutylamide (deBr-bromobutide) ; in fish, bromobutide alone. Here, the sum of bromobutide and deBr-bromobutide in agricultural products is that of bromobutide and deBr-bromobutide in bromobutide equivalent.)

0.01 ppm (Uniform limit)

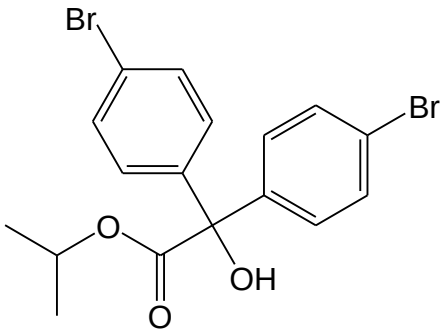
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

176 Bromopropylate



isopropyl 4,4'-dibromobenzilate

$C_{17}H_{16}Br_2O_3$ MW: 428.1 CAS No.: 18181-80-1

[Summary of bromopropylate]

Bromopropylate is a diaryl carbinol miticide.

Bromopropylate was registered as an agricultural chemical in 1968, in Japan. Reginstered name had been phenisobromolate. However, it was expired in 2005. The trade name had been “Acarol”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.05 ppm

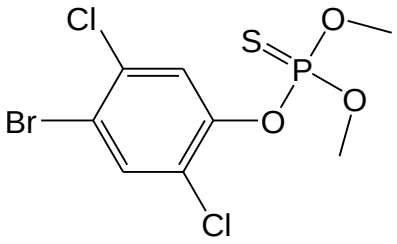
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

177 Bromophos



O-4-bromo-2,5-dichlorophenyl *O*,*O*-dimethyl phosphorothioate

$C_8H_8BrCl_2O_3PS$ MW: 366.0 CAS No.: 2104-96-3

[Summary of bromophos]

Bromophos is an organophosphorous insecticide.

Bromophos has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

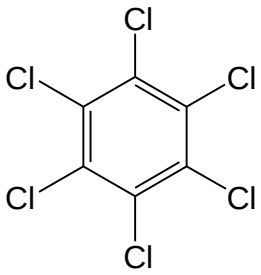
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

178 Hexachlorobenzene (HCB)



hexachlorobenzene

C_6Cl_6 MW: 284.78 CAS No.: 118-74-1

[Summary of hexachlorobenzene (HCB)]

Hexachlorobenzene, also called HCB, is an organochlorine fungicide that is being used for seed treatment against wheat stinking smut and the like.

Hexachlorobenzene has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, rye and corn: 0.03 ppm / Barley: 0.05 ppm / Other grains: 0.03 ppm

[Method listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

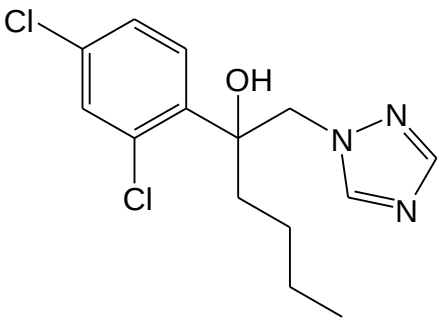
Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

179 Hexaconazole



(*RS*)-2-(2,4-dichlorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)hexan-2-ol

$C_{14}H_{17}Cl_2N_3O$ MW: 314.2 CAS No.: 79983-71-4

[Summary of hexaconazole]

Hexaconazole is a triazole fungicide developed by ICI (UK).

Hexaconazole was registered as an agricultural chemical to be used on fruit trees in 1990, in Japan. The trade name is “Anvil”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Barley and rye: 0.01 ppm / Corn: 0.02 ppm / Wheat: 0.1 ppm / Other grains: 0.01 ppm

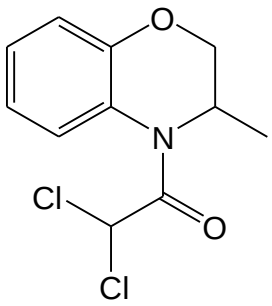
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

180 Benoxacor



(*RS*)-4-dichloroacetyl-3,4-dihydro-3-methyl-2*H*-1,4-benzoxazine

$C_{11}H_{11}Cl_2NO_2$ MW: 260.1 CAS No.: 98730-04-2

[Summary of benoxacor]

Benoxacor is a benzoxadine safener.

Benoxacor has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.01 ppm

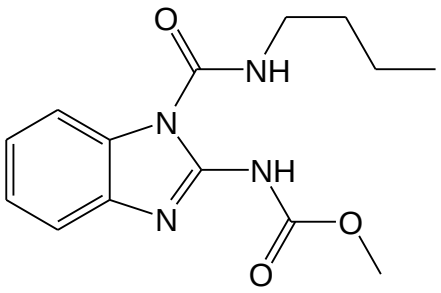
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

181 Benomyl



methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate

$C_{14}H_{18}N_4O_3$ MW: 290.32 CAS No.: 17804-35-2

[Summary of benomyl]

Benomyl is a benzimidazole fungicide developed by DuPont (USA), that exhibits a potent fungicidal effect against gray mold, stem rot, fusarium disease, etc. by foliage application, seed disinfection, soil drench application and the like. When applied, benomyl is hydrolyzed into carbendazim, which remains in plant or soil and exerts fungicidal activity. Its mechanism of action is said to be an inhibitory effect on proteins required for mitosis in pathogenic germs.

Benomyl is a colorless crystalline solid that is insoluble in water, but soluble in acetone and xylene.

Benomyl was registered as an agricultural chemical in 1971, in Japan. The trade name is "Benlate".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

(The sum of carbendazim, benomyl in carbendazim equivalent, thiophanate in carbendazim equivalent and thiophanate-methyl in carbendazim equivalent.)

Oat, barley, wheat, milo and rye: 0.6 ppm / Corn: 0.7 ppm / Pasture grass: 10 ppm

Rice plant silage: 0.1 ppm / Rice straw: 0.3 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Analysis method for carbendazim, thiophanate-methyl and benomyl by liquid chromatograph-mass spectrometer

Refer to Article 48.1 in this section.

2. Liquid chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 181.2]

A. Reagent preparation

Carbendazim standard solution. Weigh accurately 20 mg of carbendazim[$C_9H_9N_3O_2$][1], transfer to a 100 mL volumetric flask and dissolve by adding dichloromethane – methanol (193 : 7). Further, add the same solvent up to the graduation line of the flask to prepare the carbendazim standard stock solution (1 mL of this solution contains 0.2 mg as carbendazim.).

Before use, dilute accurately a certain amount of the standard stock solution with dichloromethane – methanol (193 : 7) to prepare several carbendazim standard solutions that contain 0.25 – 2 μg of carbendazim in 1 mL.

B. Quantification

Extraction. Weigh 10.0 – 20.0 g of an analysis sample[2] accurately, transfer it to a 300 mL separating funnel, add 100 mL of methanol – water (1 : 1) and extract by shaking for 30 minutes (The extract contains benomyl as carbendazim)[*1]. Place a 200 mL volmetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the separating funnel above and the residue twice with 25 mL of methanol – water (1 : 1) sequentially, and filter the washings by suction in the similar way. Further, add methanol to the graduation line of the volumetric flask, transfer 50 – 100 mL of this solution[3] to a 300 mL recovery flask and concentrate under vacuum in a water bath at 50 °C or lower to ca. 10 mL to prepare a sample solution to be subjected to column treatment.

Column treatment. Load the sample solution on a porous diatomite column (for 20 mL retention). Wash the recovery flask that has contained the sample solution with a small amount of water, add the washings to the column and leave to stand for 5 minutes.

Place a 300 mL recovery flask under the column, add 100 mL of dichloromethane – hexane (1 : 1) to the column to elute carbendazim. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow to obtain a residue to be subjected to purification.

Purification[4]. Transfer the residue with 50 mL of dichloromethane to a 100 mL separating funnel A. Then, wash the recovery flask that has contained the residue with 25 mL of hydrochloric acid (0.1 mol/L) and combine the washing with the content of the separating funnel A. Shake the separating funnel A for 5 minutes and leave to stand. Transfer the dichloromethane layer (lower layer) to a 100 mL separating funnel B and the hydrochloric acid layer (upper layer) to a 100 mL tall beaker, respectively. Wash the separating funnel A with a small amount of hydrochloric acid (0.1 mol/L) and combine the washings with the content of the tall beaker. Add 25 mL of hydrochloric acid (0.1 mol/L) to the separating funnel B, shake for 5 minutes and leave to stand. Discard the dichloromethane layer and transfer the hydrochloric acid layer to the tall beaker above.

Adjust the pH of the hydrochloric acid layer with sodium hydroxide solution (1 mol/L) to 6.5 and then transfer to a separating funnel C. Wash the tall beaker above with 50 mL of dichloromethane. Transfer the washings to the separating funnel C, shake for 5 minutes and leave to stand. Transfer the dichloromethane layer (lower layer) to an Erlenmeyer flask. Add 50 mL of dichloromethane to the separating funnel C, proceed in the similar way and transfer the dichloromethane layer to the Erlenmeyer flask. Dehydrate the dichloromethane layer with a suitable amount of sodium sulfate (anhydrous) and filter through a filter paper (No. 2S) into a 300 mL recovery flask. Wash the erlenmeyer flask and the filter paper above with a small amount of dichloromethane sequentially, filter the washings through this filter paper and combine the filtrates. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and dry up by nitrogen gas flow.

Dissolve the residue by adding 2 mL of dichloromethane – methanol (193 : 7) accurately and filter

through a membrane filter (pore size: 0.5μm or less) to obtain a sample solution to be subjected to liquid chromatography.

Liquid chromatography. Inject 20 μL each of the sample solution and respective carbendazim standard solutions into a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Fluorescence detector (excitation wavelength: 285 nm, emission wavelength: 315 nm)

Column: Octadecylsilylated silica gel column (4.6 mm in inner diameter, 250 mm in length, particle size 5 μm)[*2][5]

Eluent: dichloromethane – methanol (193 : 7)

Flow rate: 1.0 mL/min

Column oven temperature: 40 °C

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[6] to prepare a calibration curve and subsequently calculate the amount of benomyl [$C_{14}H_{18}N_4O_3$] in the sample using the following formula.

The amount of benomyl in the sample (μg/kg) = $A \times 1.52$[7] $\times 20$

A : The weight of carbendazim obtained from the calibration curve (ng).

* 1. When this method is applied to a sample containing carbendazim, carbendazim may be extracted just as benomyl and contained within the amount of benomyl in the sample.

2. Nucleosil 50-5 (Macherey-Nagel) or equivalents.

«Summary of analysis method»

In this method, the amount of carbendazim, a decomposition product of benomyl is measured and quantitatively converted to benomyl, because benomyl remains after its decomposition as carbendazim (2-benzimidazole carbamate methyl) and it is very difficult to quantify benomyl itself. Incidentally, thiophanate methyl also is an agricultural chemical decomposedto carbendazium.

The flow sheet of the analysis method is shown in Figure 6.1.181-1.

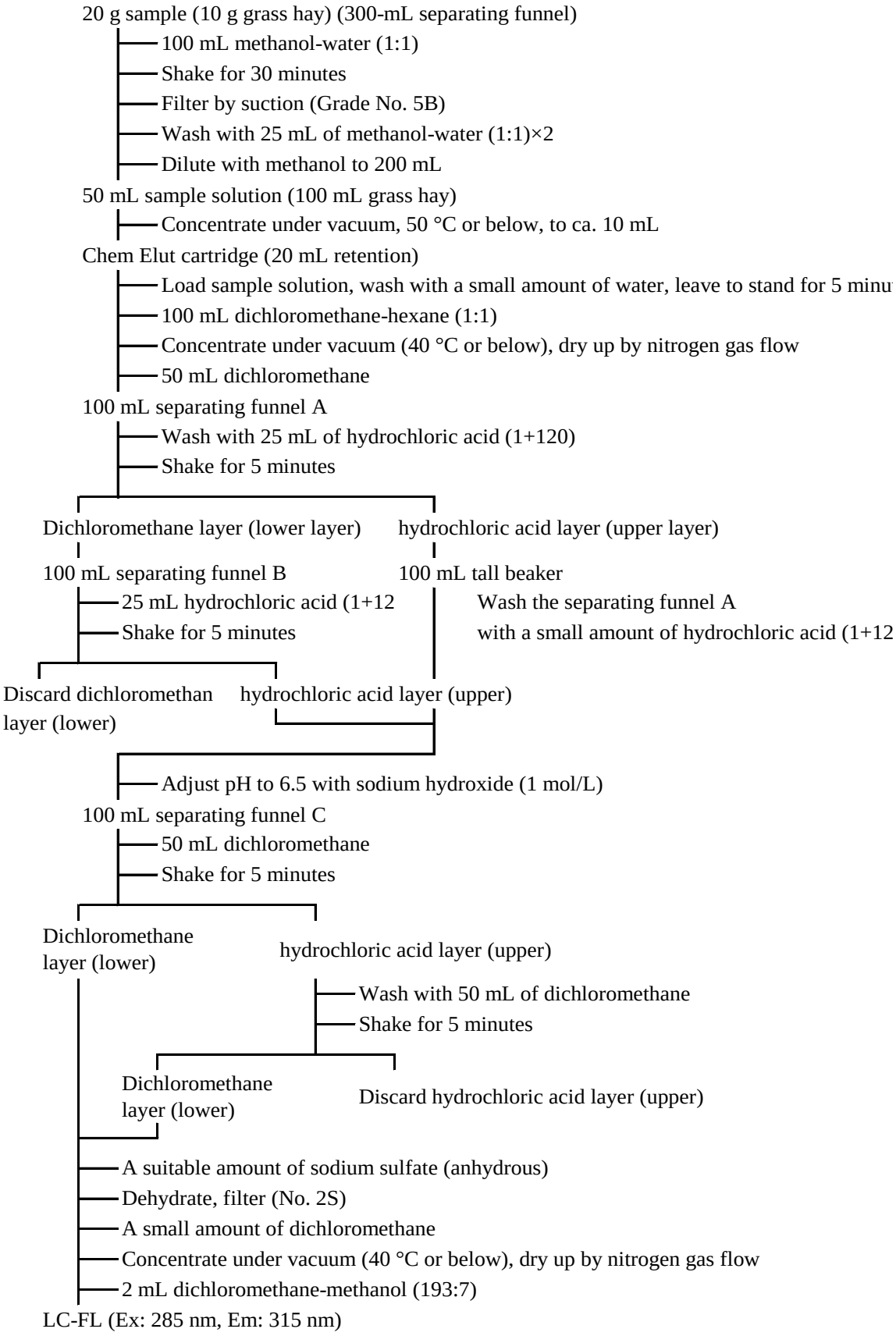


Figure 6.1.181-1. Flow sheet of the analysis method for benomyl

Reference: Norio Saito: Research Report of Animal Feed, 19, 39(1994).

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike Recovery (%)	Repeatability RSD (% or less)
corn	200~1,000	3	98.8~100.6	7.8
finishinh period broiler formula feed	200~1,000	3	92.0~96.7	8.8
Sudan grass	200~1,000	3	90.1~103.7	10.5

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike Recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
finishing period broiler formula feed	6	200	98.0	4.6	7.5	0.37

• Lower limit of quantification: 20 μg/kg in samples

«Notes and precautions»

[1] Carbendazim is a colorless crystalline solid that is soluble in water (acid, alkali), methanol and ethyl acetate. The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers.

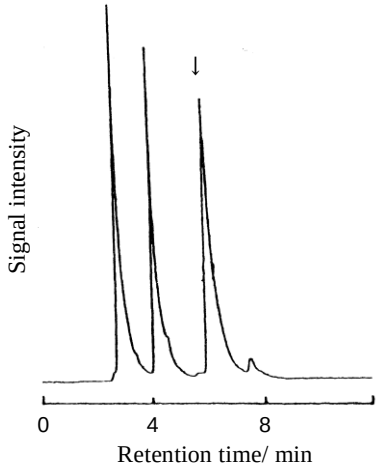
[2] The suitable amount of sample is 20 g for formula feeds and 10 g for grass hay, respectively.

[3] The suitable amount of the extract to be transferred to 300 mL recovery flask is 50 mL for formula feeds and 100 mL for grass hay, respectively.

[4] Carbendazim is soluble in both acidic and alkaline aqueous solutions, while soluble inorganic solvents under near-neutral conditions. In this method, by taking advantage of these properties, carbendazim is purified by transferring from an organic solvent to a hydrochloric acid solution, and further to an organic solvent by neutralizing this hydrochloric acid solution.

[5] Any columns packed with an end-capped packing material equivalent to this one may be used.

[6] An example of chromatogram is shown in Figure 6.1.181-2.



Measurement conditions

Detector: excitation wavelength 285 nm
emission wavelength 315 nm

Column:Nucleosil 50-5 (4.6 mm in inner diameter, 250 mm in length, particle size 5 μm)

Eluent: Dichloromethane – methanol (193 : 7)

Flow rate: 1.0 mL/min

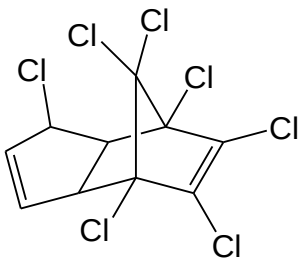
Column oven temperature: 40 °C

Figure 6.1.181-2. Chromatogram of a formula feed spiked with an amount equivalent to 0.5 mg/kg benomyl
(The arrow indicates the peak of carbendazim)

[7] Conversion factor 1.52 = MW of benomyl (290.3) / MW of carbendazim (191.2)

182 Heptachlor
183 Heptachlor epoxide

Heptachlor

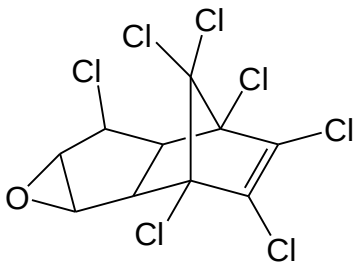


1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene

$C_{10}H_5Cl_7$ MW: 373.3

CAS No.: 76-44-8

Heptachlor epoxide



1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan

$C_{10}H_5Cl_7O$ MW: 389.3

CAS No.: 28044-83-9 (*trans*-, *endo*-, isomer A), 1024-57-3 (*cis*-, *exo*-, isomer B),

[Summary of heptachlor]

Heptachlor, a white crystalline solid with a camphor-like odor, is an organochlorine insecticide, that had been used against soil insect pests.

Heptachlor was registered as an agricultural chemical in 1957, in Japan. However it was expired in 1975.

Heptachlor epoxide is an epoxidized metabolite of heptachlor.

While heptachlor and heptachlor epoxide are listed in the Analytical Standards of Feeds separately, the maximum residue level set in the Ministerial Ordinance is the sum of the both.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(The sum of heptachlor and heptachlor epoxide)

Pasture grass: 0.02 ppm

Pig feed, chicken and quail feed, as well as cattle, sheep, goat and deer feed: 0.02 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen,

cis-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

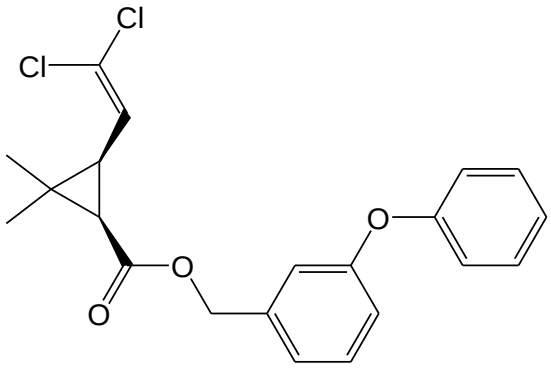
3. Simultaneous analysis method of organochlorine agricultural chemicals by gas chromatography

Target Analytes: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, endrin, dieldrin, heptachlor and heptachlor epoxide (15 compounds)

Refer to Article 2, Section 3 in this chapter.

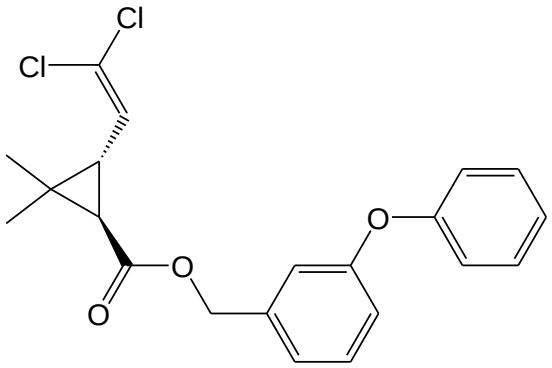
184 Permethrin (*cis*-permethrin and *trans*-permethrin)

cis-permethrin



3-phenoxybenzyl (1*RS*)-*cis*-3-(2,2-dichlorovinyl)-2,2- dimethylcyclopropanecarboxylate

trans-permethrin



3-phenoxybenzyl (1*RS*)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

$C_{21}H_{20}Cl_2O_3$ MW: 391.3

CAS No.: 52341-33-0 (*cis*), 52341-32-9 (*trans*), 52645-53-1 (mixture)

[Summary of permethrin]

Permethrin is synthetic pyrethroid insecticide, synthesized based on the chemical structure of pyrethrin, the main insecticidal component from pyrethrum. Because of its residual efficacy superior to that of pyrethrin, permethrin is widely used as a household insecticide.

Permethrin has theoretically four isomers, however, by gas chromatography, two peaks are produced by the *cis*-isomer and the *trans*-isomer.

Permethrin was registered as an agricultural chemical in 1985, in Japan. The trade name is “Adion”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(The sum of each isomer)

Oat, barley, wheat, corn, milo and rye: 2 ppm / Pasture grass: 55 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography

Target Analytes:

Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

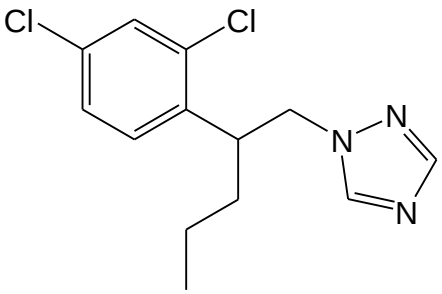
Refer to Article 4, Section 2 in this chapter.

3. Simultaneous analysis method for fenvalerate and permethrin by gas chromatography

Target Analytes: Fenvalerate and permethrin (2 compounds)

Refer to Article 17, Section 3 in this chapter.

185 Penconazole



(*RS*)-1-[2-(2,4-dichlorophenyl)pentyl]-1*H*-1,2,4-triazole

$C_{13}H_{15}Cl_2N_3$ MW: 284.2 CAS No.: 66246-88-6

[Summary of penconazole]

Penconazole is a triazole fungicide.

Penconazole has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.05 ppm

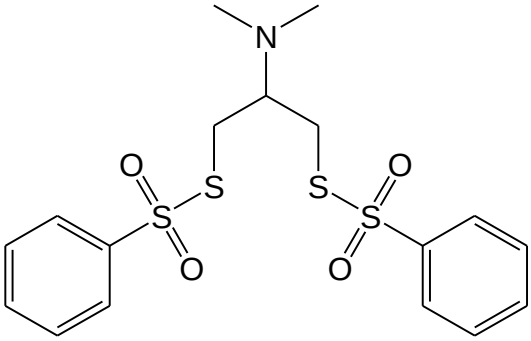
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

186 Bensultap



S,*S*′-2-dimethylaminotrimethylene di(benzenethiosulfonate)

$C_{17}H_{21}NO_4S_4$ MW: 431.61 CAS No.: 17606-31-4

[Summary of bensultap]

Bensultap is a nereistoxin insecticide, which inhibits acetylcholine transmission in insects.

Bensultap was registered as an agricultural chemical to be used on rice, vegetables, etc. in 1986, in Japan. The trade name is “Ruban”.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

(The sum of cartap, thiocyclam in cartap equivalent and bensultap in cartap equivalent)

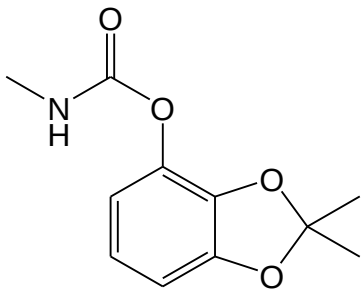
Oat, barley, wheat, corn, milo and rye: 0.2 ppm / Pasture grass: 0.7 ppm

[Method listed in the Analytical Standards of Feeds]

1. Analysis method for cartap, thiocyclam and bensultap by liquid chromatograph-mass spectrometer

Refer to Article 45.1 in this section.

187 Bendiocarb



2,2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate

$C_{11}H_{13}NO_4$ MW: 223.23 CAS No.: 22781-23-3

[Summary of bendiocarb]

Bendiocarb is a systemic carbamate insecticide, developed by Fisons (UK) as an insecticide against sanitary pests (cockroach etc.) and an agricultural insecticide, that has a cholinesterase inhibiting activity and exhibits an insecticidal effect through contact and ingestion.

Bendiocarb was registered as an agricultural chemical in 1984, in Japan. However, it was expired in 2002.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.05 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)

Target Analytes: XMC, aldicarb (including aldicarb sulfoxide and aldicarb sulfone), isoprocarb, carbaryl, carbofuran, xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb (12 compounds)

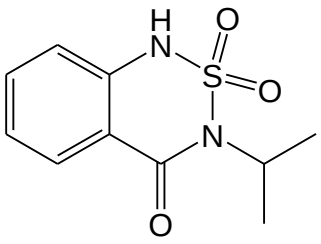
Refer to Article 3, Section 3 in this chapter.

2. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (2)

Target Analytes: Ethiofencarb (including ethiofencarb sulfoxide and ethiofencarb sulfone), bendiocarb and methiocarb (including methiocarb sulfoxide and methiocarb sulfone) (3 compounds)

Refer to Article 4, Section 3 in this chapter.

188 Bentazone (Bentazon)



3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide

Bentazone	$C_{10}H_{12}N_2O_3S$	MW: 240.3	CAS No.: 25057-89-0
Bentazone sodium	$C_{10}H_{12}N_2NaO_3S$	MW: 263.3	CAS No.: 50723-80-3

[Summary of Bentazone]

Bentazone is a non-hormone translocating herbicide developed by BASF (Germany). It is effective against annual weeds in paddy and dry fields.

Bentazone was registered as an agricultural chemical in 1975, in Japan. However, it was expired in 2005. Then, bentazone-sodium was registered as an agricultural chemical in 1985. The trade name is "Basagran".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

(Including bentazone and bentazone-sodium)

Oat, barley, wheat, corn, milo and rye: 0.2 ppm / Pasture grass: 3 ppm

Rice plant silage: 0.1 ppm / Rice straw: 0.3 ppm

[Method listed in the Analytical Standards of Feeds]

1. Gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 188.1]

A. Reagent Preparation

Bentazone standard stock solution. Weigh accurately 25 mg of bentazone[$C_{10}H_{12}N_2O_3S$][1], transfer to a 50 mL brown volumetric flask and dissolve by adding acetone. Further, add the same solvent to the graduation line of the flask to prepare a bentazone standard stock solution (1 mL of this solution contains 0.5 mg as bentazone).

B. Quantification*1

Extraction. Weigh 10.0 g of an analysis sample, transfer it to a stoppered 200 mL brown Erlenmeyer flask, moisten by adding 15 mL of water and leave to stand for 30 minutes. Further, add 100 mL of methanol and extract by shaking for 60 minutes. Place a 200 mL volumetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask above and the residue with 50 mL of methanol sequentially, and filter the washings by suction in the similar way. Further, add methanol to the graduation line of the volumetric flask. Transfer 80 mL of this solution to a 200 mL recovery flask, concentrate under reduced pressure in a water bath at 40 °C or

lower to ca. 5 mL to prepare a sample solution to be subjected to purification.

Purification. Transfer the sample solution to a 300 mL separating funnel A, add 50 mL of sodium chloride solution (5 w/v%), 4 mL of sodium hydroxide solution (1 mol/L) and 50 mL of hexane – ethyl acetate (1 : 1) to the separating funnel A, shake gently[2] and leave to stand. Transfer the the water layer (lower layer) to a 300 mL separating funnel B, add 50 mL of hexane – ethyl acetate (1 : 1), shake gently and leave to stand. Transfer the the water layer to a separating funnel C, add 2 mL of hydrochloric acid (6 mol/L) and 100 mL of hexane – ethyl acetate (1 : 1), shake vigorously for 5 minutes and leave to stand. Transfer the the water layer to a 300 mL separating funnel D, the hexane – ethyl acetate (1 : 1) layer (upper layer) to a 300 mL brown Erlenmeyer flask, respectively. Add 50 mL of hexane – ethyl acetate (1 : 1) to the separating funnel D, shake vigorously for 5 minutes and leave to stand. Transfer the hexane – ethyl acetate (1 : 1) layer to the Erlenmeyer flask. Dehydrate the hexane – ethyl acetate layer with a suitable amount of sodium sulfate (anhydrous) and filter through a filter paper (No. 5B) into a 500 mL recovery flask. Wash the Erlenmeyer flask above and the filter paper with a small amout of hexane – ethyl acetate (1 : 1) sequentially, filter the washings through this filter paper and combine the filtrates. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to ca. 1 mL and dry up by nitrogen gas flow.

Dissolve the residue with 1 mL of methanol to prepare a sample solution to be subjected to derivatization.

Derivatization. Add 0.5 mL of trimethylsilyldiazomethane solution to the sample solution, stopper the recovery flask that contains the sample solution air-tightly and leave to stand at room temperature for 30 minutes. Dry up the solution by nitrogen gas flow. Dissolve the residue with 5 mL of hexane to prepare a sample solution to be subjected to column treatment.

Derivatization of the standard stock solution. Transfer 1 mL of bentazone standard stock solution accurately to a 100 mL recovery flask, and dry up by nitrogen gas flow. Dissolve the residue by adding 1 mL of methanol and add 0.5 mL of trimethylsilyldiazomethane solution. Stopper the recovery flask above air-tightly and leave to stand at room temperature for 30 minutes. Dry up the solution by nitrogen gas flow. Dissolve the residue and dilute accurately with acetone to prepare several standard solutions that contain 0.02 – 1.2 μg of bentazone in 1 mL.

Column treatment. Wash a silica gel minicolumn (690 mg)[3] with 5 mL of hexane.

Load the sample solution on the minicolumn and let flow out by natural flow until the liquid level reaches the upper end of the column packing material. Wash the recovery flask that has contained the sample solution three times with 5 mL each of hexane, add the washings to the minicolumn in order of precedence. Let the washings flow out by natural flow in the similar way. Further, add 10 mL of hexane – diethyl ether (49 : 1) to the minicolumn and let flow out in the similar way.

Place a 50 mL recovery flask under the minicolumn, add 20 mL of hexane – diethyl ether (19 : 1) on the minicolumn to elute derivatized bentazone. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 2 mL of acetone accurately to prepare a sample solution to be subjected to gas chromatography.

Gas chromatography. Inject 2 μL each of the sample solution and respective standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Nitrogen-phosphorus detector

Column: Fused silica capillary column (5 % diphenyl/ 95 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)

Carrier gas: He (2.5 mL/min)

Make up gas: He (2 mL/min)

Hydrogen: 3 mL/min

Dry air: 60 mL/min

Sample injection : Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 70 °C (hold 1 min) → ramp 20 °C/min → 280 °C (hold 4 min)

Detector temperature: 280 °C

Calculation. Obtain respective peak heights or peak areas from the resulting chromatograms[4] to prepare a calibration curve and subsequently calculate the amount of bentazone present in the sample.

* 1. The quantification shall be performed under shielded from light.

«Summary of analysis method»

In this method, bentazone in feeds is extracted with hydrous methanol, purified by liquid-liquid extraction, methylation and a silica gel minicolumn and quantified by a gas chromatograph with a nitrogen-phosporus detector.

The flow sheet of the analysis method is shown in Figure 6.1.188-1.

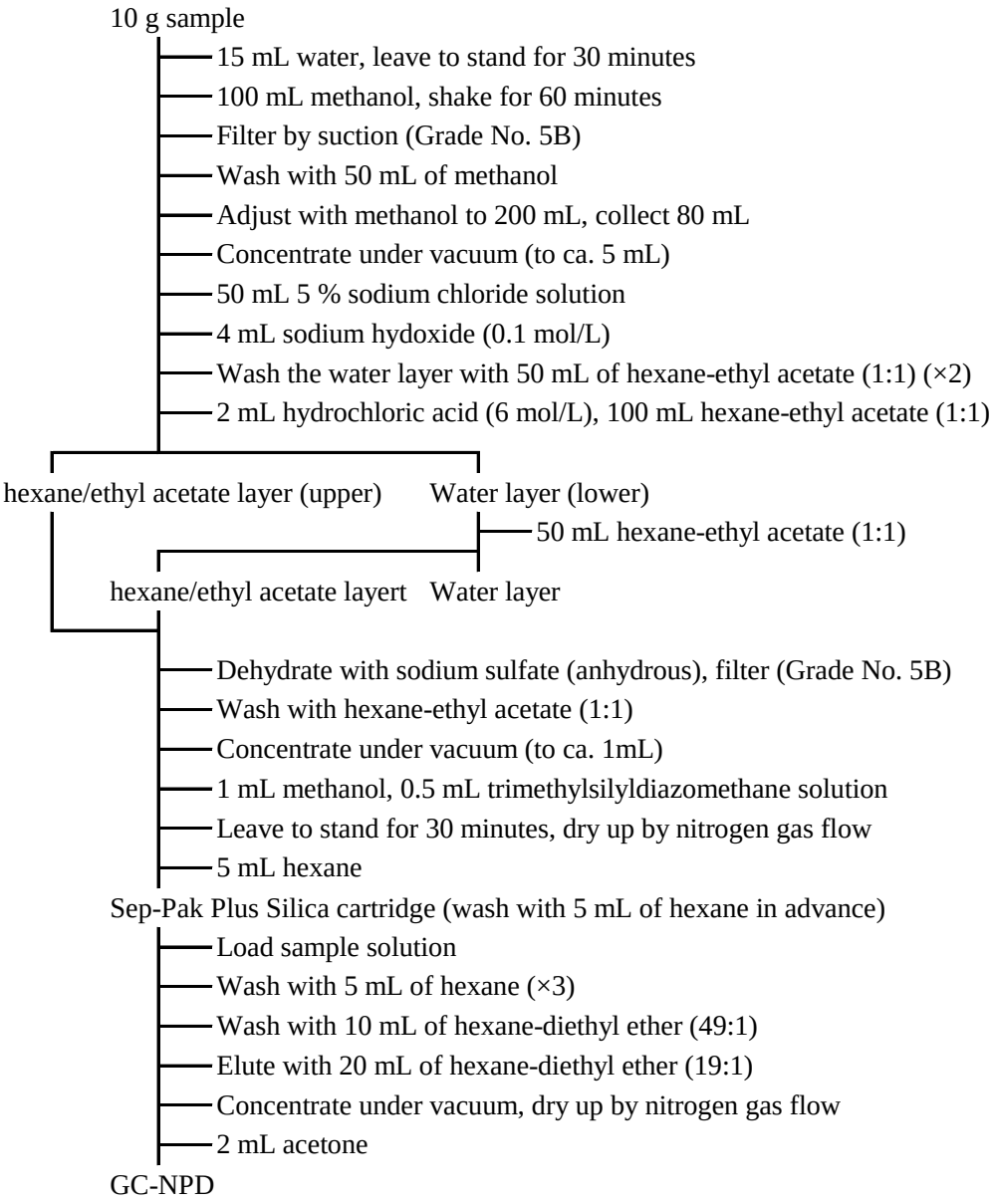


Figure 6.1.188-1. Flow sheet of the analysis method for bentazone

Reference: Miyuki Matsuzaki: Research Report of Animal Feed, 30, 26(2005)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike Recovery (%)	Repeatability RSD (% or less)
starting chick formula feed	50~500	3	89.4~91.8	7.8
sucking calf formula feed	50~500	3	91.6~95.4	7.5
ryegrass	50~500	3	85.3~88.1	7.7
timothy	50~500	3	83.5~86.4	3.7

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike Recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
finishinh period broiler formula feed	7	500	96.0	11.6	13.3	0.74
ryegrass	7	500	90.3	5.4	10.8	0.60

• Lower limit of quantification: 10 μg/kg (*SN* ratio)

• Lower limit ofdetection: 3 μg/kg (*SN* ratio)

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical and other manufacturers.

[2] Emulsion is likely to develop.

[3] Here, Sep-Pak Plus Silica Cartridge (Waters) was used.

[4] An example of chromatogram is shown in Figure 6.1.188-2.

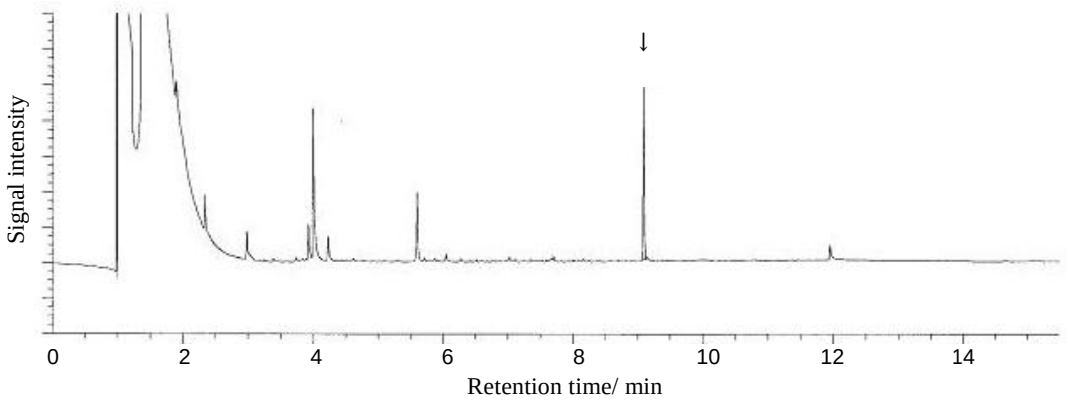
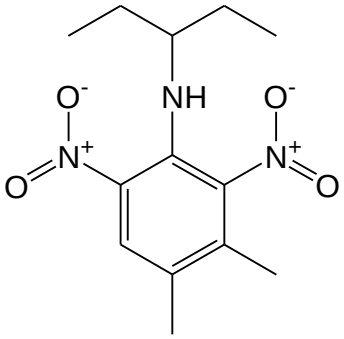


Figure 6.1.188-2. Chromatogram of a formula feed spiked with an amount equivalent to 0.5 mg/kg bentazone
(The arrow indicates the peak of methylated bentazone.)
Measurement conditions are as shown in the above example. The column used is J&W DB-5.

189 Pendimethalin



N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine

$C_{13}H_{19}N_3O_4$ MW: 281.3 CAS No.: 40487-42-1

[Summary of pendimethalin]

Pendimethalin is a dinitroaniline herbicide, developed by American Cyanamid (USA), which exhibits a potent effect against gramineous and broad-leaved annual weeds on dry fields.

Pendimethalin was registered as anagricultural chemical in 1981, in Japan. The trade name is "Go-Go-San".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

Oat, milo and pasture grass: 0.1 ppm / Barley, wheat, corn and rye: 0.2 ppm

Rice straw: 0.02 ppm

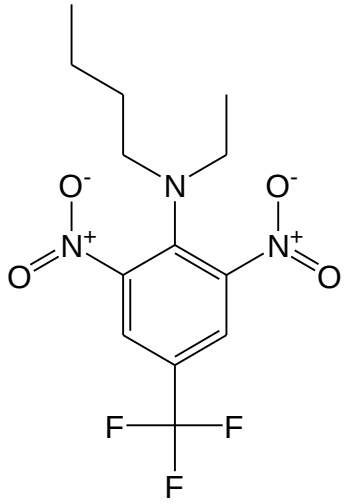
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

190 Benfluralin



N-butyl-*N*-ethyl-*α*,*α*,*α*-trifluoro-2,6-dinitro-*p*-toluidine

$C_{13}H_{16}F_3N_3O_4$ MW: 335.3 CAS No.: 1861-40-1

[Summary of benfluralin]

Benfluralin is a non-hormone, dinitroaniline herbicide for soil treatment, developed by Eli Lilly (USA) in1960.

Benfluralin was registered as an agricultural chemical to be used on lawn in 1968, in Japan. Registered name is bethrodine. The trade names are “Benefin” and “Benefix”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

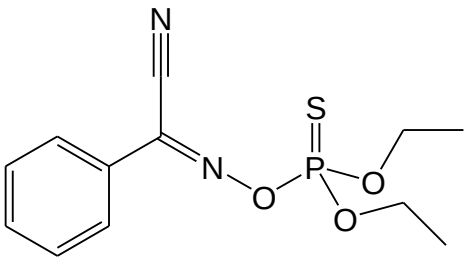
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

191 Phoxim



O,*O*-diethyl *α*-cyanobenzylideneaminooxyphosphonothioate

$C_{12}H_{15}N_2O_3PS$ MW: 298.3 CAS No.: 14816-18-3

[Summary of phoxim]

Phoxim is an organophosphorous insecticide. It is a yellow liquid, stable in water and acid, but unstable in alkali, and decomposed by ultraviolet rays gradually.

Phoxim has not been registered as an agricultural chemical in Japan. However, phoxim is used as a wood insecticide and an anti-termite.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.05 ppm

[Method listed in the Analytical Standards of Feeds]

1. Liquid chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 191.1]

A. Reagent Preparation

1) Phoxim standard solution. Weigh accurately 25 mg of phoxim [$C_{12}H_{15}N_2O_3PS$][1], transfer to a 50 mL volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the phoxim standard stock solution (1 mL of this solution contains 0.5 mg as phoxim).

Before use, dilute accurately a certain amount of the standard stock solution with methanol to prepare several phoxim standard solutions that contain 0.05 – 5 µg of phoxim in 1 mL.

2) Magnesium silicate. Wash synthetic magnesium silicate (particle size 149-250 µm (100-60 mesh))[2] with acetone, air-dry, and dry at 130 °C for 16 hours.

B. Quantification

Extraction. Weigh 10.0 g of an analysis sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 15 mL of water to moisten and leave to stand for 15 minutes. Further, add 80 mL of acetone (120 mL for grass hay) and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to ca. 15 mL, add 5 g of sodium chloride to prepare a sample solution to be subjected to column treatment I.

Column treatment I. Load the sample solution on the porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that has contained the sample solution three times with 10 mL each of hexane, add the washings to the column in order of precedence and elute phoxim by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 70 mL of the same solvent to the column to elute phoxim in the similar way.

Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow. Dissolve the residue by adding 10 mL of cyclohexane – acetone (4 : 1) accurately and filter the solution through a membrane filter (pore size: 0.5 µm) to prepare a sample solution to be subjected to gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph. Dispense an elution fraction into a 100 mL recovery flask, concentrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 5 mL of hexane – diethyl ether (9 : 1) to prepare a sample solution to be subjected to column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column[3] (20 mm in inner diameter, 300 mm in length, particle size 15 µm)

Guard column: Styrene-divinylbenzene copolymer column[3] (20 mm in inner diameter, 100 mm in length, particle size 15 µm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 80~100 mL[4]

Column treatment II. Suspend 5 g of magnesium silicate in hexane, pour into a column (15 mm inner diameter) and let flow out until the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Place a 200 mL recovery flask under the column and load the sample solution on the column. Wash the recovery flask that has contained the sample solution three times with 5 mL each of hexane – diethyl ether (9 : 1), add the washings to the column in order of precedence and elute phoxim by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Further, add 50 mL of the same solvent to the column and elute phoxim in the similar way. Concentrate the effluent under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 2 mL of methanol accurately, transfer this solution to a plastic centrifuge tube (1.5 mL), centrifuge at 5,000×*g* for 5 minutes to obtain a supernatant to be subjected to liquid chromatography.

Liquid chromatography. Inject 20 µL each of the sample solution and respective phoxim standard solutions into a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Ultraviolet spectrophotometer (Measurement wavelength: 284 nm)

Column: Octadecylsilylated silica gel column (4.6 mm in inner diameter, 250 mm in length, particle size 5 μm)*1[5]

Eluent: Methanol – water (7 : 3)

Flow rate: 1 mL/min

Column oven temperature: 40 °C

Calculation. Obtain the peak height or peak area from the resulting chromatograms[6] to prepare a calibration curve and subsequently calculate the amount of phoxim present in the sample.

* 1. Mightysil RP-18 GP (Kanto Chemical) or equivalents.

«Summary of analysis method»

The flow sheet of the analysis method is shown in Figure 6.1.191-1.

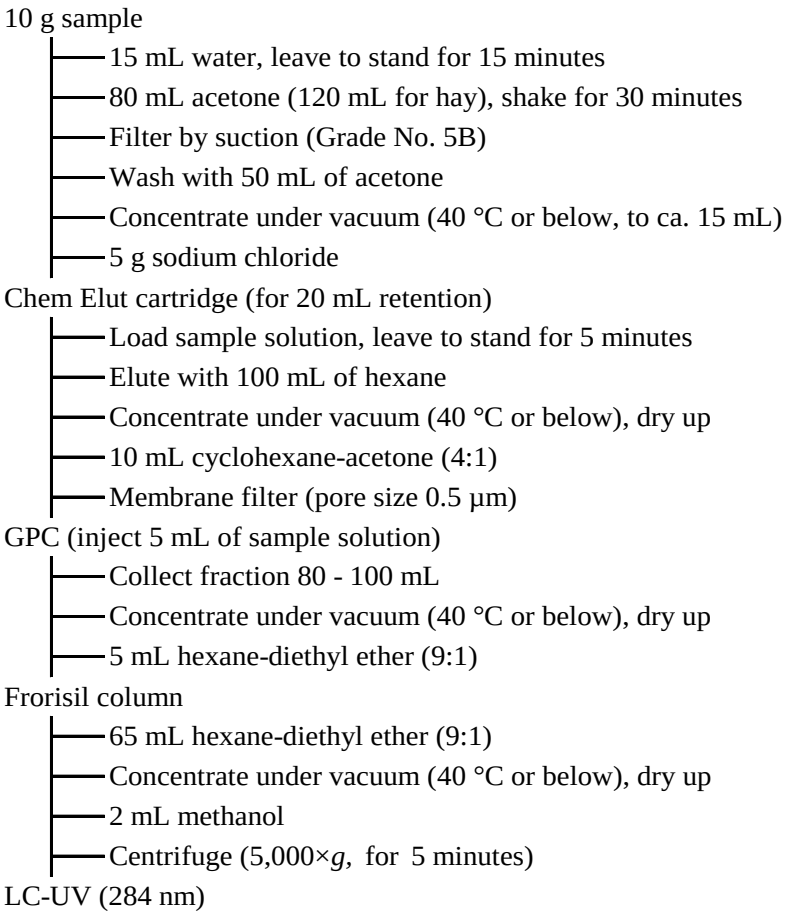


Figure 6.1.191-1. Flow sheet of the analysis method for phoxim

Reference: Ikumi Kobayashi, Yoshihiro Sekiguchi: Research Report of Animal Feed, 26, 41 (2001)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike Recovery (%)	Repeatability RSD (% or less)
chicken formula feed	100~500	3	88.2~88.8	5.4
pig formula feed	100~500	3	90.5~91.7	4.1
corn	100~500	3	90.4~90.6	4.4
ryegrass	100~500	3	89.7~91.6	7.3

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike Recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
finishing beef cattle formula feed	7	200	89.5	5.9	8.5	0.41

• Lower limit of quantification: 25 μg/kg in samples

«Notes and precautions»

[1] The standard is available from Wako Pure Chemical Industries.

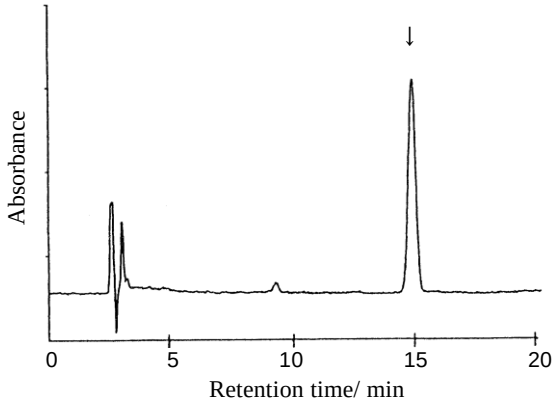
[2] Wash synthetic magnesium silicate with acetone several times, because of the possibility of elution of impurities that interfere with quantification of phoxim.

[3] A column packed with styrene-divinylbenzene copolymer hard gel with the use of eluent.

[4] Because elution fraction may vary among lots of column, depending on frequency of use, etc., it requires confirmation before use.

[5] Any columns packed with an end-capped packing material equivalent to this one may be used.

[6] An example of chromatogram is shown in Figure 6.1.191-2.



Measurement conditions

Detector: Ultraviolet spectrophotometer (Measurement wavelength: 284 nm)

Column: Mightysil RP-18 GP (4.6 mm in inner diameter, 250 mm in length, particle size 5 μm)

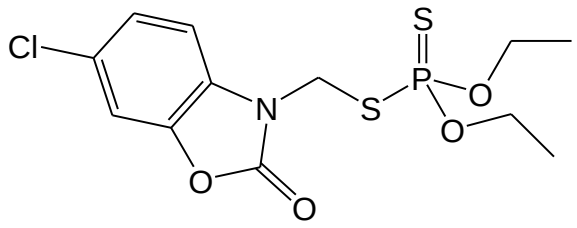
Eluent: Methanol – water (7 : 3)

Flow rate: 1 mL/min

Column oven temperature: 40 °C

Figure 6.1.191-2. Chromatogram of a formula feed spiked with an amount equivalent to 0.5 mg/kg phoxim
(The arrow indicates the peak of phoxim.)

192 Phosalone



S-6-chloro-2,3-dihydro-2-oxo-1,3-benzoxazol-3-ylmethyl *O*,*O*-diethyl phosphorodithioate

$C_{12}H_{15}ClNO_4PS_2$ MW: 367.8 CAS No.: 2310-17-0

[Summary of phosalone]

Phosalone is an organophosphorous insecticide, developed by Rhône-Poulenc (France). It is a white crystalline solid, soluble in many organic solvents, but practically insoluble in water.

Phosalone is a fast-acting contact insecticide against a wide range of insect pests on fruit trees and vegetables. It also exerts an insecticidal activity against spider mites.

Phosalone was registered as an agricultural chemical in 1966, in Japan. The trade name is "Rubitox".

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic Analysis of Organophosphorous Pesticides by Gas Chromatography (1) [Analytical Standards of Feeds, Chapter 6, Section 1 192.2]

Target analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds).

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

See Chapter 6, Section 2 [Multi-Componet Systematic Analysis Methods 2] (p.1027).

3. Systematic analysis methods for organophosphorus agricultural chemicals by gas

chromatography (2)

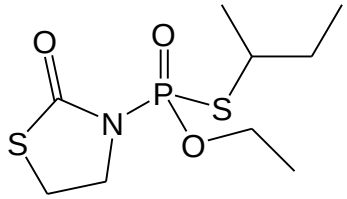
Target Analytes:

Group A: EPN, diazinon, parathion, fenitrothion, fenthion, phenthoate, phosalone and malathion (8 compounds).

Group B: Dichlorvos (1 compound)

Refer to Article 3, Section 2 in this chapter.

193 Fosthiazate



(*RS*)-[*S*-(*RS*)-*sec*-butyl *O*-ethyl 2-oxo-1,3-thiazolidin-3-ylphosphonothioate]

$C_9H_{18}NO_3PS_2$ MW: 283.3 CAS No.: 98886-44-3

[Summary of fosthiazate]

Fosthiazate is a phosphamide nematicide, developed by Ishihara Sangyo Kaisha.

Fosthiazate was registered as an agricultural chemical to be used on potatoes, vegetables, etc. in 1992, in Japan. The trade name is “Nematolin”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

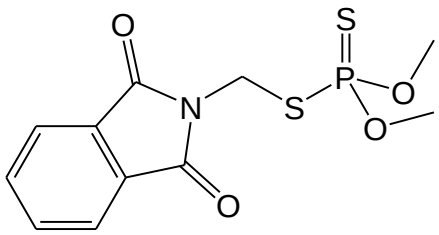
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

194 Phosmet (PMP)



O,*O*-dimethyl *S*-phthalimidomethyl phosphorodithioate

$C_{11}H_{12}NO_4PS_2$ MW: 317.3 CAS No.: 732-11-6

[Summary of phosmet (PMP)]

Phosmet (PMP), a white liquid, is an organophosphorous insecticide, developed by Stauffer Chemical (USA).

It is effective against not only insect pests on fruit trees such as apple, “mikan” (mandarine orange) etc. but also against rice stem borers, rice leaf beetles and spider mites.

Phosmet was registered as an agricultural chemical in 1963, in Japan. Registered name had been PMP. However, it was expired in 2002.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Oat, barley, wheat, corn, milo and rye: 0.05 ppm / Pasture grass: 40 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

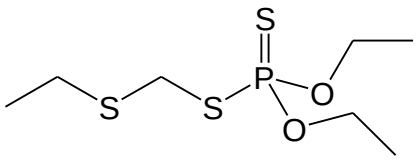
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

195 Phorate



O,*O*-diethyl *S*-ethylthiomethyl phosphorodithioate
$C_7H_{17}O_2PS_3$ MW: 260.4 CAS No.: 298-02-2

[Summary of phorate]

Phorate, a colorless liquid, is an organophosphorous insecticide being used on corn, cotton, etc.

Phorate has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Oat, barley, wheat, corn, milo and rye: 0.05 ppm / Pasture grass: 1 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

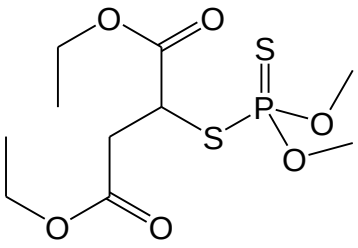
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

196 Malathion



S-1,2-bis(ethoxycarbonyl)ethyl *O*,*O*-dimethyl phosphorodithioate

$C_{10}H_{19}O_6PS_2$ MW: 330.4 CAS No.: 121-75-5

[Summary of malathion]

Malathion, a pale yellow liquid, is an organophosphorous insecticide.

It is a low-toxic, fast-acting, selective insecticide with fairly high systemic translocatability. It exhibits insecticidal activity against a broad spectrum of insect pests, especially against sucking insects such as planthoppers, leafhoppers, aphids and rice thrips. In the USA, malathion is being used as a post-harvest pesticide.

Malathion was registered as an agricultural chemical in 1953, in Japan. Registered name is malathon. The Trade name is "Malathon".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives] [Administrative Guidelines for Hazardous Substances in Feeds]

Oat, barley, corn, milo and rye: 2 ppm / Wheat: 8 ppm / Pasture grass: 135 ppm

Paddy rice: 2 ppm / Rice straw: 0.2 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

3. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (2)

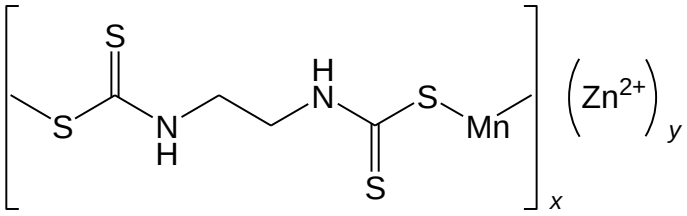
Target Analytes:

Group A: EPN, diazinon, parathion, fenitrothion, fenthion, phenthoate, phosalone and malathion (8 compounds).

Group B: Dichlorvos (1 compound)

Refer to Article 3, Section 2 in this chapter.

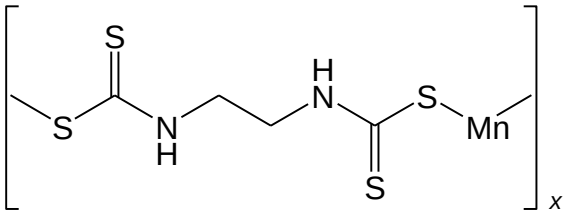
197 Mancozeb



manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt

$[C_4H_6N_2S_4Mn]_x(Zn)_y$ MW: 265.3 CAS No.: 8018-01-7

(Reference) Maneb



manganese ethylenebis(dithiocarbamate) (polymeric)

$C_4H_6N_2S_4Mn$ MW: 265.3 CAS No.: 12427-38-2

[Summary of Mancozeb]

Mancozeb is an ethylene bisdithiocarbamate fungicide developed by Rohm and Haas (USA). It is a grayish yellow powder that is insoluble in water or organic solvents and heat-, moisture- and acid-labile.

It has a fungicidal effect similar to that of maneb, but is widely used as a fungicide for home gardening, because its harmful effects on humans are milder than those of maneb.

Mancozeb was registered as an agricultural chemical in 1970, in Japan. Registered name is manzeb. The trade name is "Jimandaisen".

«Maximum Residue Limits in grains in the Food Sanitation Law»

(The sum of dithiocarbamate (zineb in carbon disulfide equivalent, ziram in carbon disulfide equivalent, thiram in carbon disulfide equivalent, nickelbis(dithiocarbamate) in carbon disulfide equivalent, ferbam in carbon disulfide equivalent, propineb in carbon disulfide equivalent, polycarbamate in carbon disulfide equivalent, mancozeb in carbon disulfide equivalent, maneb in carbon disulfide equivalent and metiram in carbon disulfide equivalent))

Corn: 0.1 ppm / Wheat, barley and rye: 1 ppm / Other grains: 0.1 ppm

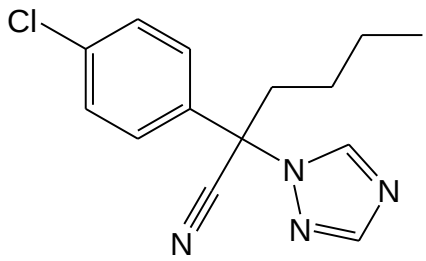
[Method listed in the Analytical Standards of Feeds]

1. Analysis method for zineb and mancozeb by liquid chromatography

Target analytes: Zineb and mancozeb (2 compounds)

Refer to Article 79.1 in this section.

198 Myclobutanil



(*RS*)-2-(4-chlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)hexanenitrile

$C_{15}H_{17}ClN_4$ MW: 288.8 CAS No.: 88671-89-0

[Summary of myclobutanil]

Myclobutanil is a conazole fungicide against powdery mildew on fruit trees and vegetables, developed by Rohm and Haas (USA).

Myclobutanil was registered as an agricultural chemical in 1990, in Japan. The trade names are “Rally”, etc.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Rye and corn: 0.03 ppm / Wheat: 0.3 ppm / Barley: 0.5 ppm / Other grains: 0.03 ppm

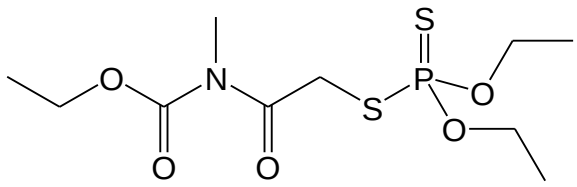
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method for cyanazine and myclobutanil by gas chromatograph-mass spectrometer

Target Analytes: Cyanazine and myclobutanil (2 compounds)

Refer to Article 14, Section 3 in this chapter.

199 Mecarbam



S-(*N*-ethoxycarbonyl-*N*-methylcarbamoylmethyl) *O*,*O*-diethyl phosphorodithioate

$C_{10}H_{20}NO_5PS_2$ MW: 329.37 CAS No.: 2595-54-2

[Summary of mecarbam]

Mecarbam, a brownish yellow liquid, is an organophosphorous insecticide and miticide that is used for control of planthoppers, spider mites, mealybugs and the like on rice, fruit trees and vegetables.

Mecarbam was registered as an agricultural chemical in 1961, in Japan. However, it was expired in 1987.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.05 ppm

[Method listed in the Analytical Standards of Feeds]

1. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

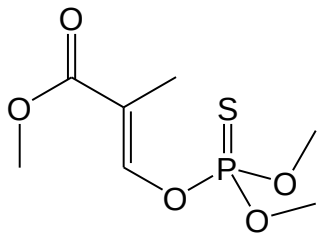
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

200 Methacrifos



(*E*)-*O*-2-methoxycarbonylprop-1-enyl *O*,*O*-dimethyl phosphorothioate

$C_7H_{13}O_5PS$ MW: 240.2 CAS No.: 62610-77-9

[Summary of methacrifos]

Methacrifos is an organophosphorous insecticide.

Methactifos has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley, rye, corn and other grains: 0.05 ppm

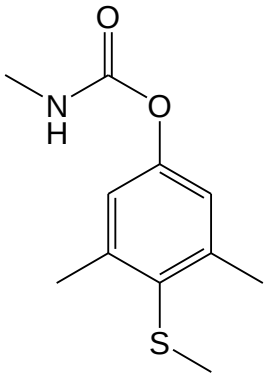
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

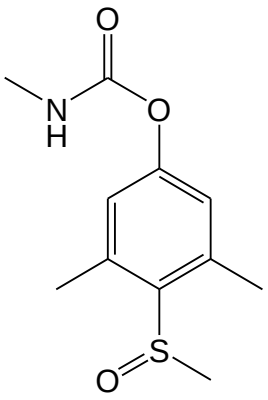
Refer to Article 1, Section 3 in this chapter.

201 Methiocarb
(including methiocarb sulfoxide and methiocarb sulfone)



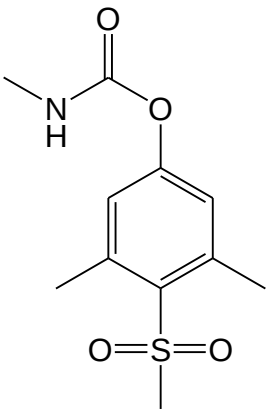
4-methylthio-3,5-xylyl methylcarbamate
$C_{11}H_{15}NO_2S$ MW: 225.31 CAS No.: 2032-65-7

Methiocarb sulfoxide



$C_{11}H_{15}NO_3S$ MW: 241.31
CAS No.: 2635-10-1

Methiocarb sulfone



$C_{11}H_{15}NO_4S$ MW: 257.31
CAS No.: 2179-25-1

[Summary of methiocarb]

Methiocarb is a carbamate insecticide used for control of slugs and snails on fruits and vegetables as well as of large insect pests such as lepidopteran, coleopteran, spiders and the like. The maximum residue level set in the Food Sanitation Law is the sum of methiocarb and its oxidative metabolites, methiocarb sulfoxide and methiocarb sulfone.

Methiocarb has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

(The sum of methiocarb, methiocarb sulfoxide in methiocarb equivalent and methiocarb sulfone in methiocarb equivalent)

Wheat, barley, rye, corn and other grains: 0.05 ppm

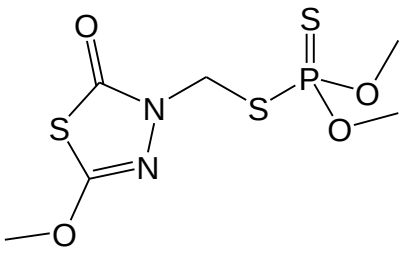
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (2)

Target Analytes: Ethiofencarb (including ethiofencarb sulfoxide and ethiofencarb sulfone), bendiocarb and methiocarb (including methiocarb sulfoxide and methiocarb sulfone) (3 compounds)

Refer to Article 4, Section 3 in this chapter.

202 Methidathion (DMTP)



S-2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl *O*,*O*-dimethyl phosphorodithioate

$C_6H_{11}N_2O_4PS_3$ MW: 302.3 CAS No.: 950-37-8

[Summary of methidathion (DMTP)]

Methidathion (DMTP) is a systemic organophosphorous insecticide effective against mealybugs, greenhouse whiteflies, thrips, etc., developed by Geigy (Switzerland). It is a clolorless crystalline solid and has a residual efficacy.

Methidathion was registered as an agricultural chemical in 1968, in Japan. Registered name is DMTP. The trade name is "Supracide".

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Barley, wheat and rye: 0.02 ppm / Corn: 0.1 ppm / Oat and milo: 0.2 ppm / Pasture grass: 12 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

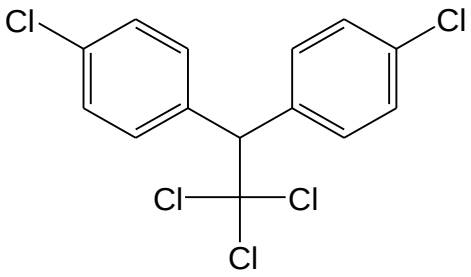
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

203 Methoxychlor (DMDT)



1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane
$C_{16}H_{15}Cl_3O_2$ MW: 345.7 CAS No.: 72-43-5

[Summary of methoxychlor (DMDT)]

Methoxychlor (DMDT) is an organochlorine insecticide that has an insecticidal activity and effect similar to that of DDT, but exhibits no bioconcentration potential. It is a colorless crystalline solid, insoluble in water and readily soluble in organic solvents.

Methoxychlor was registered as an agricultural chemical in 1950, in Japan. Registered name had been methoxychlor. However, it was expired in 1960.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Wheat, barley and rye: 2 ppm / Corn: 7 ppm / Other grains: 2 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

3. Systematic analysis methods for alachlor, allethrin, chlorpropham, dichloran and methoxychlor by gas chromatography

Target Analytes:

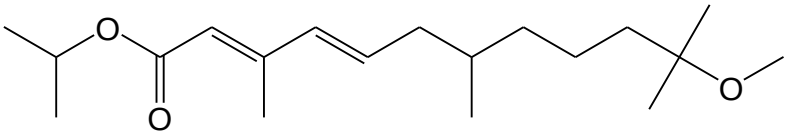
Group A: Alachlor, allethrin and dichloran (3 compounds)

Group B: Methoxychlor (1 compound)

Group C: Chlorpropham (1 compound)

Refer to Article 5, Section 2 in this chapter.

204 Methoprene



isopropyl (*E*,*E*)-(*RS*)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate

$C_{19}H_{34}O_3$ MW: 310.47 CAS No.: 40596-69-8

[Summary of methoprene]

Methoprene is an insect growth regulator developed by Sandoz (Switzerland). It is a brownish yellow liquid that exhibits an insecticidal effect by disrupting the hormonal balance and inhibiting the normal transformation of insects. Methoprene is water soluble with the solubility 1.4 mg/L, readily soluble in ordinary organic solvents, and UV-unstable.

Methoprene has not been registered as an agricultural chemical in Japan .

«Maximum Residue Limits in the Law Concerning Safety Assurance and Quality Improvement of Feeds» [Ordinance of the Standard of Feed and Feed Additives]

Oat, barley, wheat, corn, milo and rye: 5 ppm

[Method listed in the Analytical Standards of Feeds]

1. Liquid chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 204.1]

A. Reagent Preparation

Methoprene standard solution. Weigh accurately 20 mg of methoprene [$C_{19}H_{34}O_3$][1], transfer to a 100 mL volumetric flask and dissolve by adding acetonitrile. Further, add the same solvent up to the graduation line of the flask to prepare the methoprene standard stock solution (1 mL of this solution contains 0.2 mg as methoprene).

Before use, dilute accurately a certain amount of the standard stock solution with acetonitrile to prepare several methoprene standard solutions that contain 0.02 – 0.5 μg of methoprene in 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of an analysis sample, transfer it to a stoppered 200 mL Erlenmeyer flask,add 20 mL of acetonitrile – water (3 : 1) to moisten and leave to stand for 15 minutes. Further, add 100 mL of acetonitrile and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask above and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to almost dryness[2] and add 20 mL of saturated sodium chloride solution to prepare a sample solution to be subjected to column treatment I.

Column treatment I. Load the sample solution on the porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask

that has contained the sample solution three times with 20 mL each of hexane, add the washings to the column in order of precedence and elute methoprene by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 60 mL of hexane to the column and elute methoprene in the similar way. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and dry up by nitrogen gas flow.

Dissolve the residue by adding 10 mL of hexane accurately to prepare a sample solution to be subjected to column treatment II.

Column treatment II[3]. Load 2 mL of the sample solution accurately on the porous diatomite minicolumn (for 3 mL retention)*1 and remove the solvent to vacuum for 2 minutes[4]. Connect an octadecylsilylated silica minicolumn (360 mg) washed with 5 mL of acetonitrile in advance to the bottom of the porous diatomite minicolumn.

Place a 100 mL recovery flask under the minicolumn and elute methoprene by adding 20 mL of hexane-saturated acetonitrile. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and dry up by nitrogen gas flow.

Dissolve the residue with 2 mL of hexane – diethyl ether (19 : 1) to prepare a sample solution to be subjected to column treatment III.

Column treatment III. Wash a synthetic magnesium silicate minicolumn[5] (910 mg) with 5 mL of hexane.

Load the sample solution on the minicolumn. Wash the recovery flask that has contained the sample solution twice with 2 mL each of hexane – diethyl ether (19 : 1), add the washings to the minicolumn in order of precedence and let flow out by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 20 mL of hexane – diethyl ether (19 : 1) to the minicolumn and let it flow out in the similar way. Place a 50 mL recovery flask under the column, and add 10 mL of hexane – diethyl ether (17 : 3) to the minicolumn to elute methoprene. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 2 mL of acetonitrile accurately and filter the solution through a membrane filter (pore size: 0.5 µm or less) to prepare a sample solution to be subjected to liquid chromatography.

Liquid chromatography. Inject 20 µL each of the sample solution and respective standard solutions into a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Ultraviolet spectrophotometer (Measurement wavelength: 267 nm)

Column: Octadecylsilylated silica gel column (4.6 mm in inner diameter, 250 mm in length, particle size 5 µm)*2 [6]

Eluent: Acetonitrile – water (7 : 3)

Flow rate: 1 mL/min

Column oven temperature: 40 °C

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[7] to prepare a calibration curve and subsequently calculate the amount of methoprene in the sample.

* 1. Extrelut-3 (Merck) to which a reservoir with a suitable capacity is connected, or equivalents.
2. HAIsil C18 (Higgins Analytical) or equivalents.

«Summary of analysis method»

In this method, methoprene in samples is extracted with hydrous acetonitrile, purified with two kindsof porous diatomite columns, a C_{18} minicolumn and a Florisil minicolumn and quantified by a liquid chromatograph with an ultraviolet spectrophotometer.

The flow sheet of the analysis method is shown in Figure 6.1.204-1.

10 g sample
- 20 mL acetonitrile/water (3:1), leave to stand for 15 minutes
- 100 mL acetonitrile, shake for 30 minutes
- Filter by suction (Grade No. 5B)
- Wash with 50 mL of acetonitrile
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- 20 mL saturated saline

Chem Elut cartridge (for 20 mL retention)
- Load sample solution, leave to stand for 5 minutes
- Wash with 20 mL of hexane (×3)
- Elute with 60 mL of hexane
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- 10 mL hexane

EXtrelut-3 cartridge
- Load 2 mL of sample solution
- Expose to vacuum for 2 minutes

Connect Sep-Pak Plus C_{18} cartridge (wash with 5 mL of acetonitrile in advance) to the bottom of Extrelut-3 cartridge
- Elute with 20 mL of hexane-saturated acetonitrile
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- 2 mL hexane-diethyl ether (19:1)

Sep-Pak Plus Florisil cartridge (wash with 5 mL of hexane in advance)
- Wash with 2 mL of hexane-diethyl ether (19:1) (×2)
- Wash with 20 mL of hexane-diethyl ether (19:1)
- Elute with 10 mL of hexane-diethyl ether (17:3)
- Concentrate under vacuum (40 °C or below), dry up by nitrogen gas flow
- 2 mL acetonitrile
- Membrane filter (pore size 0.5 μm or less)

LC-UV (267 nm)

Figure 6.1.204-1. Flow sheet of the analysis method for methoprene

Reference: Akito Ikezawa, Yuji Shirai: Research Report of Animal Feed, 23, 62(1998)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike Recovery (%)	Repeatability RSD (% or less)
chicken formula feed	50~500	3	91.7~99.0	8.5
finishing pig formula feed	50~500	3	85.7~86.0	14.9
alfalfa	50~500	3	92.0~95.0	4.9

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike Recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
growing chick formula feed	7	100	85.6	6.8	8.3	0.38

• Lower limit of quantification: 50 μg/kg in samples

«Notes and precautions»

[1] The standards are available from Hayashi Pure Chemical, GL Sciences, and other manufacturers.

[2] Because of the risk of sudden boiling, concentrate carefully.

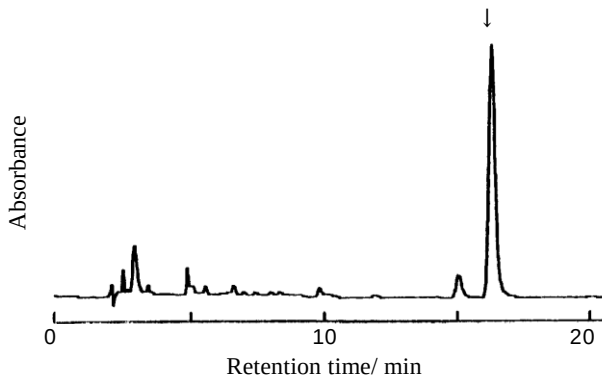
[3] Conducted to degrease fat in sample.

[4] Expose to vacuum for 2 minutes with the use of an vacuum manifold and the like.

[5] Use an unopened Florisil cartridge, because, once opened, Florisil cartridges are at risk for adsorption and fluctuation of elution patterns, even if stored in a sealed desiccator.

[6] Any columns packed with an end-capped packing material equivalent to this one may be used.

[7] An example of chromatogram is shown in Figure 6.1.204-2.



Measurement conditions

Detector: Ultraviolet spectrophotometer (Measurement wavelength: 267 nm)

Column: Higgins Analytical HAIsilC18 (4.6 mm in inner diameter, 250 mm in length, particle size 5 μm)

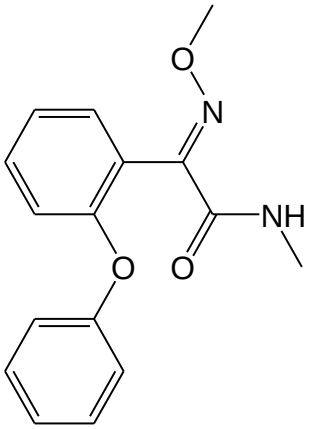
Eluent: Acetonitrile – water (7 : 3)

Flow rate: 1 mL/min

Column oven temperature: 40 °C

Figure 6.1.204-2. Chromatogram of a formula feed spiked with an amount equivalent to 0.5 mg/kg methoprene
(The arrow indicates the peak of methoprene.)

205 (*E*)-Metominostrobin



(*E*)-2-(methoxyimino)-*N*-methyl-2-(2-phenoxyphenyl)acetamide

$C_{16}H_{16}N_2O_3$ MW: 284.3 CAS No.: 133408-50-1

[Summary of (*E*)-metominostrobin]

(*E*)-Metominostrobin is a strobilurin fungicide developed by Shionogi.

Metominostrobin (as (*E*)-metominostrobin) was registered as an agricultural chemical to be used on rice in 1998, in Japan. The trade name is "Oribright".

«Maximum Residue Limits in grains in the Food Sanitation Law»

(The sum of metominostorbin *E*-isomer and *Z*-isomer)

0.01 ppm (Uniform limit)

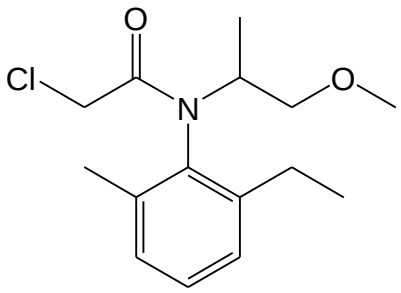
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

206 Metolachlor



2-chloro-*N*-(6-ethyl-*o*-tolyl)-*N*-[(1*RS*)-2-methoxy-1-methylethyl]acetamide

$C_{15}H_{22}ClNO_2$ MW: 283.8 CAS No.: 51218-45-2

[Summary of metolachlor]

Metolachlor is a non-hormone anilide herbicide for soil treatment, developed by Ciba-Geigy (Switzerland).

Metolachlor was registered as an agricultural chemical in 1987, in Japan. The trade name is "Dual".

«Maximum Residue Limits in grains in the Food Sanitation Law»

(Including metolachlor and S- metolachlor)

Wheat, barley, rye and corn: 0.1 ppm / Other grains: 0.3 ppm

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

2. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography

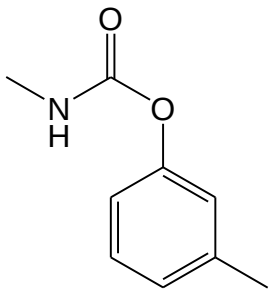
Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

Refer to Article 1, Section 2 in this chapter.

207　Metolcarb (MTMC)



m-tolyl methylcarbamate

$C_9H_{11}NO_2$　MW: 165.189　CAS No.: 1129-41-5

[Summary of metolcarb (MTMC)]

Metolcarb (MTMC) is a carbamate insecticide developed by Nihon Nohyaku. It is a white crystalline solid that is effective against green rice leafhopper, planthopper, etc.

Metolcarb was registered as an agricultural chemical in 1967, in Japan. Registered name had been MTMC. However, it was expired in 1997.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)

Target Analytes: XMC, aldicarb (including aldicarb sulfoxide and aldicarb sulfone), isoprocarb, carbaryl, carbofuran, xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb (12 compounds)

Refer to Article 3, Section 3 in this chapter.

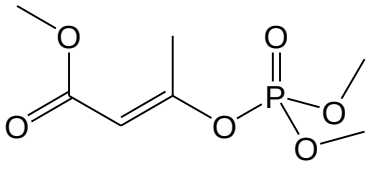
2. Simultaneous analysis method of carbamate agricultural chemicals by gas chromatography

Target Analytes: XMC, isoprocarb, carbaryl, xylylcarb, fenobucarb, propoxur and metolcarb (7 compounds)

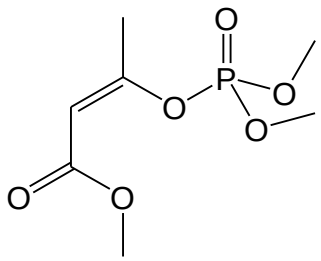
Refer to Article 5, Section 3 in this chapter.

208 Mevinphos

(*E*)-Mevinphos



(*Z*)-Mevinphos



(*E*)-2-methoxycarbonyl-1-methylvinyl dimethyl phosphate

(*Z*)-2-methoxycarbonyl-1-methylvinyl dimethyl phosphate

$C_7H_{13}O_6P$ MW: 224.1 CAS No.: 7786-34-7 (*EZ*), 26718-65-0 (*E*), 298-01-1 (*E*), 339-45-4 (*Z*)

[Summary of mevinphos]

Mevinphos is an organophosphorous insecticide.

Mevinphos has not been registered as an agricultural chemical in Japan.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

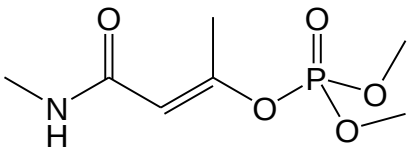
[Method listed in the Analytical Standards of Feeds]

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer

Target analytes: agricultural chemicals (138 compounds)

Refer to Article 1, Section 3 in this chapter.

209 Monocrotophos



dimethyl (*E*)-1-methyl-2-(methylcarbamoyl)vinyl phosphate

$C_7H_{14}NO_5P$ MW: 223.164 CAS No.: 6923-22-4

[Summary of monocrotophos]

Monocrotophos, a white crystalline solid, is an organophosphorous insecticide, developed by Shell (USA). It is a systemic, translocating insecticide, which, when applied to the soil, is absorbed through the roote of plants and exerts its insecticidal effect on both sucking and chewing insecticide pests.

Monocrotophos was registered as an agricultural chemical in 1980, in Japan. However, it was expired in 2004.

«Maximum Residue Limits in grains in the Food Sanitation Law»

0.01 ppm (Uniform limit)

[Methods listed in the Analytical Standards of Feeds]

1. Simultaneous Analysis of Pesticides by Gas Chromatograph Mass Spectrometer [Analytical Standards of Feeds, Chapter 6, Section 1 209.1]

Target analytes: Agricultural chemical s (138 compounds)

See Chapter 6, Section 3 [Multi-Component Simultaneous Analysis Methods 1] (p.1064).

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1)

Target Analytes:

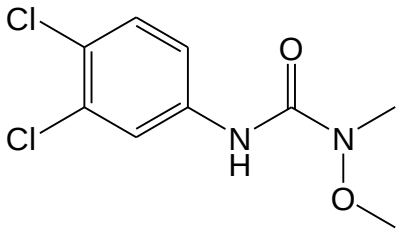
Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

Refer to Article 2, Section 2 in this chapter.

210 Linuron



3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea

$C_9H_{10}Cl_2N_2O_2$ MW: 249.09 CAS No.: 330-55-2

[Summary of linuron]

Linuron is a non-hormone phenylurea herbicide, which exerts its herbicidal effect through pre- and post-emergent soil application.

Linuron was registered as an agricultural chemical in 1964, in Japan. The trade names are “Afalon” and “Lorox”.

«Maximum Residue Limits in grains in the Food Sanitation Law»

Barley and rye: 0.1 ppm / Wheat and corn: 0.2 ppm / Other grains: 0.1 ppm

[Method listed in the Analytical Standards of Feeds]

1. Liquid chromatography [Analytical Standards of Feeds, Chapter 6, Section 1, Article 210.1]

A. Reagent preparation

Linuron standard solution. Weigh accurately 20 mg of linuron[$C_9H_{10}Cl_2N_2O_2$][1], transfer to a 100 mL volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the linuron standard stock solution (1 mL of this solution contains 0.2 mg as linuron).

Before use, dilute accurately a certain amount of the standard stock solution with methanol to prepare several linuron standard solutions that contain 0.05 – 5 μg of linuron in 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of an analysis sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 15 mL of water to moisten and leave to stand for 30 minutes. Further, add it 100 mL of acetone and extract by shaking for 60 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way.

Concentrate the filtrate under reduced pressure in a water bath at 40 °C or lower to ca. 15 mL to prepare a sample solution to be subjected to column treatment I.

Column treatment I Load the sample solution on the porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that has contained the sample solution three times with 10 mL each of hexane, add the washings to the

column in order of precedence and elute linuron by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 70 mL of the same solvent to the column and elute linuron in the similar way. Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 10 mL of cyclohexane – acetone (4 : 1) accurately, filter the solution through a membrane filter (pore size: 0.5 µm or less) to prepare a sample solution to be subjected to gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph. Dispense each elution fraction into a 100 mL recovery flask, concentrate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 10 mL of hexane – diethyl ether (19 : 1) accurately to prepare a sample solution to be subjected to column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column[2] (20 mm in inner diameter, 300 mm in length, particle size 15 µm)

Guard column: Styrene-divinylbenzene copolymer column[2] (20 mm in inner diameter, 100 mm in length, particle size 15 µm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 85~105 mL[3]

Column treatment II. Wash a synthetic magnesium silicate minicolumn (910 ng) with 5 mL of hexane.

Load the sample solution on the minicolumn and let flow out until the liquid level reaches the upper end of the column packing material. Wash the recovery flask that has contained the sample solution three times with 2 mL each of hexane – diethyl ether (19 : 1), add the washings to the minicolumn in order of precedence and let them flow out in the similar way.

Place a 50 mL recovery flask under the minicolumn and elute linuron by adding 25 mL of hexane – diethyl ether (7 : 3). Concentrate the eluate under reduced pressure in a water bath at 40 °C or lower to almost dryness and further dry up by nitrogen gas flow.

Dissolve the residue by adding 1 mL of methanol accurately, transfer this solution to a plastic centrifuge tube (1.5 mL), centrifuge at 5,000×*g* for 5 minutes to obtain a supernatant to be subjected to liquid chromatography.

Liquid chromatography. Inject 20 µL each of the sample solution and respective linuron standard solutions into a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Ultraviolet spectrophotometer (Measurement wavelength: 254 nm)

Column: Octadecylsilylated silica gel column (4.6 mm in inner diameter, 250 mm in length, particle size: 5 µm)*1 [4]

Eluent: Water – acetonitrile – methanol (6 : 4 : 1)

Flow rate: 1 mL/min

Column oven temperature: 40 °C

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[5] to prepare a calibration curve and subsequently calculate the amount of linuron in the sample.

* 1. Mightysil RP-18 GP (Kanto Chemical) or equivalents.

«Summary of analysis method»

In this method, linuron in feeds is extracted with hydrous acetone, purified with a porous diatomite column, a GPC and a Florisil minicolumn and quantified by a liquid chromatograph with an ultraviolet spectrophotometer.

The flow sheet of the analysis method is shown in Figure 6.1.210-1.

10 g sample
- 15 mL water, leave to stand for 30 minutes
- 100 mL acetone, shake for 60 minutes
- Filter by suction (Grade No. 5B)
- Wash with 50 mL of acetone
- Concentrate under vacuum (to 15 mL)

Chem Elut cartridge (for 20 mL retention)
- Load sample solution, leave to stand for 5 minutes
- Elute wirh 100 mL of hexane
- Concentrate under vacuum, dry up by nitrogen gas flow
- 10 mL cyclohexane-acetone (8:2)

GPC (Load 5 ml of sample solution)
- Collect fraction 85 - 105 mL
- Concentrate under vacuum, dry up by nitrogen gas flow
- 10 mL hexane-diethyl ether (95:5)

Sep-Pak Plus Florisil cartridge (wash with 5 mL of hexane in advance)
- Load sample solution
- Elute with 25 mL of hexane-diethyl ether (7:3)
- Concentrate under vacuum, dry up by nitrogen gas flow
- 1 mL methanol
- Centrifuge (5,000×*g*, for 5 minutes)

LC-UV (254 nm)

Figure 6.1.210-1. Flow sheet of the analysis method for linuron

Reference: Masato Funatsu: Research Report of Animal Feed, 27, 38(2002)

«Method validation»

• Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Replicate	Spike Recovery (%)	Repeatability RSD (% or less)
Adult chicken formula feed	100~500	3	79.3~87.9	1.5
cattle formula feed	100~500	3	79.2~82.0	7.4
corn	100~500	3	80.1~85.7	2.5
timothy	100~500	3	90.5~93.4	2.8

• Collaborative study

Sample type	No. of labs	Spike concentration (μg/kg)	Spike Recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
growing chick formula feed	7	100	85.6	6.8	8.3	0.38
milo	7	100	89.7	4.4	7.8	0.35

• Lower limit of quantification: 10 μg/kg in samples

«Notes and precautions»

[1] Available from Wako Pure Chemical Industries and other manufacturers.

[2] A column packed with styrene-divinylbenzene copolymer hard gel with the eluent. The column and the guard column listed in Appendix 2 of the Analytical Standards of Feeds are Shodex CLNpak EV-2000 AC and Shodex CLNpak EV-G AC manufactured by Showa Denko K.K. respectively.

[3] Because elution fraction may vary among lots of column, depending on frequency of use, etc., it requires confirmation in advance in each laboratory.

[4] Any columns packed with an end-capped packing material equivalent to this one may be used.

[5] Examples of chromatogram are shown in Figure 6.1.210-2.

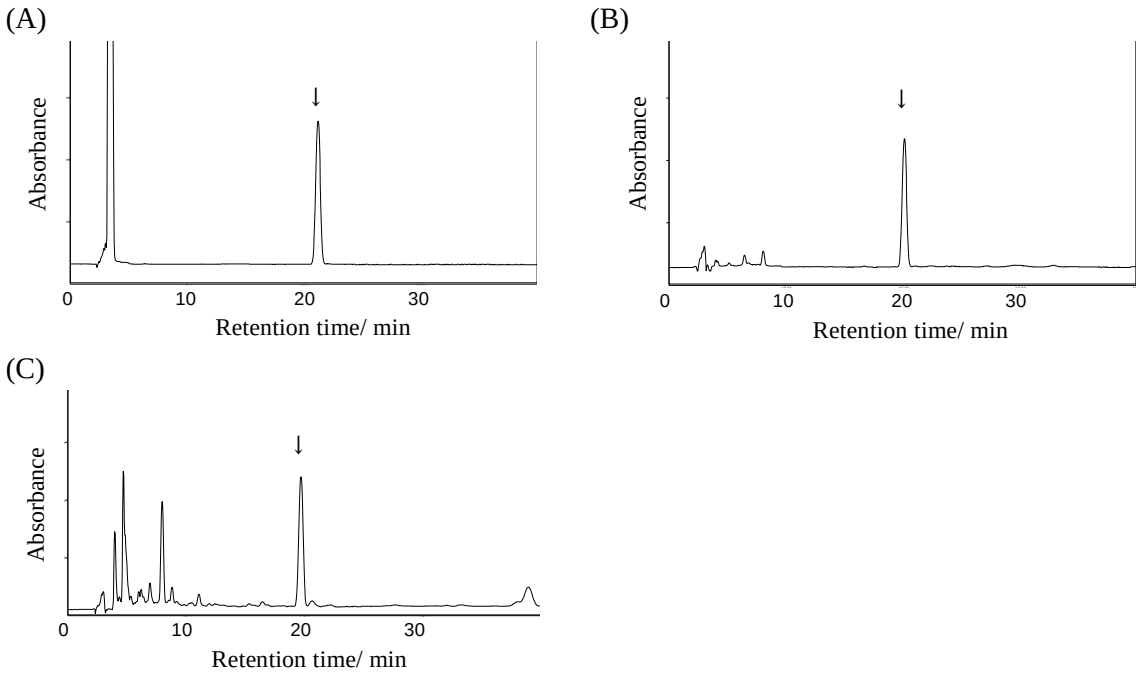


Figure 6.1.210-2. Chromatogram of linuron
(The arrow indicates the peak of linuron.)

(A) Standard solution (25 ng)

(B) Chicken formula feed (Spike 500 μg/kg)

(C) Grass hay (Spike 500 μg/kg)

211 Hydrogen phosphide

PH_3

phosphine
H_3P MW: 34.0 CAS No.: 7803-51-2

[Summary of hydrogen phosphide]

Phosphorus compound with hydrogen and its alkyl or allyl substitution products are collectively referred to as phosphine, among which the best-known substance is a gas, hydrogen phosphide (PH_3).

Hydrogen phosphide is a colorless gas with a characteristic bad odor like rotten fish or acetylene.

Hydrogen phosphide is highly toxic to humans, and exposure to 2,000 ppm causes acute symptoms such as severe hypotension, dyspnea and pulmonary edema, and eventually results in death. The concentration of 100 ppm is said to lead to poisoning death within 2 – 3 hours.

Hydrogen phosphide is now being used as a fumigant for straw of wheat and foliage of agropyron mixed in imported hay (bales) produced in USA as well as stored grains.

Hydrogen phosphide was registered as an agricultural chemical in 1994, in Japan. The trade name is "Phosphine".

«Maximum Residue Limits in grains in the Food Sanitation Law»

(The sum of hydrogen phosphide, aluminum phosphide in hydrogen phosphide equivalent, magnesium phosphide in hydrogen phosphide equivalent and zinc phosphide in hydrogen phosphide equivalent)

Wheat, barley, rye, corn and other grains: 0.1 ppm

[Method listed in the Analytical Standards of Feeds]

1. Absorption spectrophotometry [Analytical Standards of Feeds, Chapter 6, Section 1, Article 211.1]

A. Reagent preparation[1]

1) Phosphorus standard stock solution. Transfer 0.439 g of potassium dihydrogenphosphate [KH_2PO_4] (dried in a desiccator for more than 24 hours) to a 1,000 mL volumetric flask and dissolve with water. Further, add water up to the graduation line of the flask to prepare the phosphorus standard stock solution (1 mL of this solution contains 0.1 mg as phosphorus [P]).

2) Bromine saturated solution[*1]. Dissolve bromine in chilled water until saturation is reached[2].

B. Quantification[3]

Separation and absorption. Weigh 200 g of analysis sample[4] and transfer to a 5 L round-bottom flask (separable cover, three-necked). Connect air-tightly this round-bottom flask to an apparatus for separation, absorption and oxidation of hydrogen phosphide[5] (A hydrogen phosphide separation apparatus to which one empty gas absorption tube and two gas absorption tubes containing 100 mL each of bromine saturated solution are connected air-tightly. The three gas absorption tubes are cooled in an ice water bath.). Pour 2 L of water into the round-bottom flask and feed nitrogen gas into the flask at a flow rate of 200 mL/min for 30 minutes. Further, feed nitrogen gas into the flask on heating[6] by a

mantle heater in the same manner for two hours to separate and absorb hydrogen phosphide.

Then, transfer the reaction liquid in the gas absorption tubes with a small amount of water to a 500 mL beaker and concentrate on heating on a hot plate[7] to ca. 10 mL to prepare a sample solution.

Concurrently, the same procedure is performed without the analysis sample to prepare a blank test solution.

Measurement. Transfer the sample solution with a small amount of water to a 25 mL volumetric flask, add 2.5 mL of sulfuric acid (3+7) solution, 3 mL of ammonium molybdate solution (2.5 w/v%) and 1.5 mL of hydrazinium sulfate solution (0.15 w/v%) to the volumetric flask and further add water up to the graduation line of the flask. Heat this liquid in a boiling bath for 10 minutes for color development and allow to cool[8]. Measure the absorbance at wavelength of 820 nm using water a control liquid[9].

Measure the absorbance of the blank test solution in the similar way and correct the result[10].

Concurrently, the absorbance of each certain amount of phosphorus standard solution under the same measurement conditions as those for the sample solution.

Calculation. Prepare a calibration curve[11] from the resulting absorbance values and calculate the amount of hydrogen phosphide [PH_3] in the sample using the following formula.

$$\text{The amount of hydrogen phosphide in the sample (mg/kg)} = \frac{A}{200} \times 1.0976$$

A : The weight of phosphorus obtained fron the calibration curve (μg)

* 1. Bromine water (2 - 3 w/v%) (Wako Pure Chemical Industries) or equivalents.

«Summary of analysis method»

In this method, residual hydrogen phosphide in feeds fumigated with aluminum phosphide is oxidized in the presence of saturated bromine water to phosphoric acid, reacted with ammonium molybdate in acidic solution, reduced with hydrazine sulfate, heated in a boiling bath for ca. 10 minutes for color development and quantified by a spectrophotometer, by taking the advantage of the property of this chromogenic substance that it takes its maximum value at the wavelength of ca. 820 nm (Figure 6.1.211-1).

Reference: R. B. Bruce, A. J. Robbins, T. O. Tuft: J. Agric. Food Chem., 10, 18 (1962)
Hiroshi Akiyama, et al.: Plant Protection Station Research Report, 14, 38 (1977)
Kiyoshi Sugano: Research Report of Animal Feed, 6, 78 (1980)

$$AlP \xrightarrow{} PH_3\uparrow$$

aluminum phosphide → hydrogen phosphide

$$PH_3 \xrightarrow[\text{oxidation}]{\text{saturated bromine water}} H_3PO_4$$

$$H_3PO_4 \xrightarrow[\text{in acidic solution}]{(NH_4)_6Mo_7O_{24}\ (\text{ammonium molybdate})} (NH_4)_3[PMo_{12}O_{40}]$$

ammonium phosphomolybdate

$$\xrightarrow[\text{reduction}]{(NH_2)_2\cdot H_2SO_4\ (\text{hydrazine sulfate})} Mo_2O_3\cdot 3MoO_3\cdot 6H_2O$$

molybdenum blue (chromogenic substance)

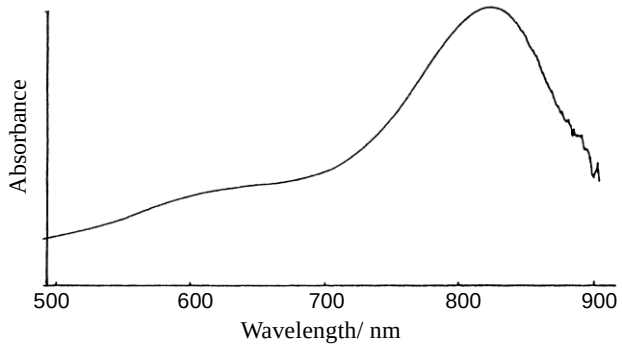


Figure 6.1.211-1. Absorption curve of color developed by hydrogen phosphide

«Notes and precautions»

[1] Water to be used is distilled ion-exchanged water.

[2] Because of high toxicity of bromine, this procedure shall be carried out carefully in a well-ventilated environment, such as in a draft chamber. The concentration of the saturated bromine water is 3.42 g/100 mL (25 °C) and that of commercially available bromine water is 2 – 3 g/100 mL. Therefore, when new commercial bromine water is used, this procedure may be skipped, provided that its bottle is tightly stoppered and stored in a refrigerator.

[3] Before use, wash lab ware once with hydrochloric acid (1:1) and twice with distilled water.

[4] Weigh hay etc. cut into ca. 2 cm pieces using a manual straw cutter, and corns etc. unground.

[5] The apparatus for separation, absorption and oxidation of hydrogen phosphide as shown in Figure 6.1.211-2 is placed in a draft chamber.

[6] Heat to ca. 90 °C.

[7] Concentrate the reaction liquid in a draft chamber, because bromine gas is generated.

[8] This liquid may be cooled in a water bath.

[9] The chromogenic substance is relatively stable. Its absorbance measured again after having been stored for ca. 1 week in the refrigerator remained

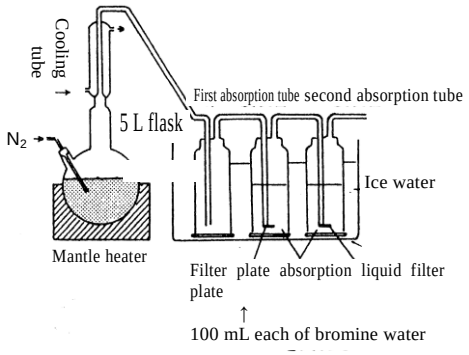


Figure 6.1.211-2. Hydrogen phosphide separation and absorption apparatus

almost the same level.

[10] The absorbance values measured for nonfumigated feeds (hay A and B) and a reagent alone (saturated bromine water and coloring reagent) are as follows.

(A) Absorbance of nonfumigant feeds

Hay A		Hay B	
0.035	0.036 in average	0.050	0.045 in average
0.036		0.040	

(B)Absorbance of reagent alone

0.036	0.042 in average
0.048	

From these results, it is thought that the amount of phosphorus measured has derived almost entirely from the saturated bromine water and the coloring reagent and that the content of phosphorus in the nonfumigated feeds is negligible small.

Therefore, when nonfumigated feeds are difficult to obtain, it is reasonable to measure the absorbance of a blank test solution and to correct the result obtained.

[11] An example of calibration curve is shown in Table 6.1.211-1 and Figure 6.1.211-3.

Table 6.1.211-1. Absorbance of phosphorus standard solutions

Standard solution(phosphorus content)	First	Second	Third
Standard blank test solution	0.020	0.015	0.018
Standard solution1(1 µg)	0.053	0.053	0.053
Standard solution2(2 µg)	0.094	0.091	0.093
Standard solution3(4 µg)	0.160	0.154	0.157
Standard solution4(8 µg)	0.299	0.294	0.297

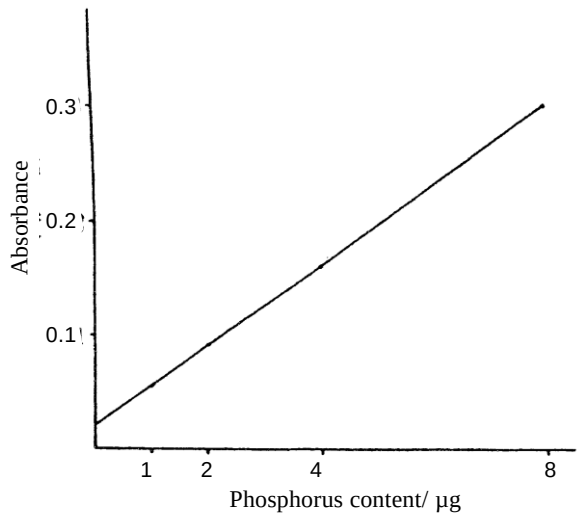


Figure 6.1.211-3. Calibration curve of phosphorus

Section 2. Systematic Analysis Methods for Agricultural Chemicals

1. Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 2, Article 1]

Target Analytes:

Group A: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, aldrin, *α*-endosulfan[1], endrin, oxychlordane, *cis*-chlordane, *trans*-chlordane, dieldrin, nitrofen, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene, heptachlor, heptachlor epoxide and methoxychlor (24 compounds)

Group B: Alachlor, *β*-endosulfan[1], endosulfan sulfate[1], chlorobenzilate, dichloran, butachlor, pretilachlor and metolachlor (8 compounds)

A. Reagent Preparation

Mixed standard solution (group A). Weigh accurately 20 mg each of *α*-BHC [$C_6H_6Cl_6$][2], *β*-BHC [$C_6H_6Cl_6$][2], *γ*-BHC [$C_6H_6Cl_6$][2], *δ*-BHC [$C_6H_6Cl_6$][2], *o,p'*-DDD [$C_{14}H_{10}Cl_4$][2], *p,p'*-DDD [$C_{14}H_{10}Cl_4$][2], *o,p'*-DDE [$C_{14}H_8Cl_4$][2], *p,p'*-DDE [$C_{14}H_8Cl_4$][2], *o,p'*-DDT [$C_{14}H_9Cl_5$][2], *p,p'*-DDT [$C_{14}H_9Cl_5$][2], aldrin [$C_{12}H_8Cl_6$][2], *α*-endosulfan [$C_9H_6Cl_6O_3S$][2], endrin [$C_{12}H_8Cl_6O$][2], oxychlordane [$C_{10}H_4Cl_8O$][2], *cis*-chlordane [$C_{10}H_6Cl_8$][2], *trans*-chlordane [$C_{10}H_6Cl_8$][2], dieldrin [$C_{12}H_8Cl_6O$][2], nitrophen [$C_{12}H_7Cl_2NO_3$][2], *cis*-nonachlor [$C_{10}H_5Cl_9$][2], *trans*-nonachlor [$C_{10}H_5Cl_9$][2], hexachlorobenzene [C_6Cl_6][2], heptachlor [$C_{10}H_5Cl_7$][2], heptachlor epoxide [$C_{10}H_5Cl_7O$][2] and methoxychlor [$C_{16}H_{15}Cl_3O_2$][2]. Transfer each of them to a 100 mL volumetric flask and dissolve by adding 20 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks, and use these solutions as the standard stock solutions (Each 1 mL of these solutions contains 0.2 mg of the respective group A agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare mixed standard solutions containing 0.01 – 0.2μg each of the group A agricultural chemicals per 1 mL.

Mixed standard solution (group B). Weigh accurately 20 mg each of alachlor [$C_{14}H_{20}ClNO_2$][2], *β*-endosulfan [$C_9H_6Cl_6O_3S$][2], endosulfan sulfate [$C_9H_6Cl_6O_4S$][2], chlorobenzilate [$C_{16}H_{14}Cl_2O_3$][2], dichloran [$C_6H_4Cl_2N_2O_2$][2], butachlor [$C_{17}H_{26}ClNO_2$][2], pretilachlor [$C_{17}H_{26}ClNO_2$][2] and metolachlor [$C_{15}H_{22}ClNO_2$][2]. Transfer each of them to a 100 ml volumetric flask and dissolve with 20 ml of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks, and use these solutions as the standard stock solutions (Each 1 ml of these solutions contains 0.2 mg of the respective group B agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare mixed standard solutions containing 0.01 – 0.2μg each of the group B agricultural chemicals per 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 20 mL of acetonitrile – water mixture (3 : 1) to moisten, and leave to stand for 10 minutes. Then, add it 100 mL of

acetonitrile and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness[3], add 20 mL of saturated sodium chloride solution and use the mixture as a sample solution for column treatment I.

Column treatment I. Load the sample solution on the porous diatomite column (for 20 mL retention)[4] and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that had contained the sample solution three times with 20 mL each of hexane, add the washings to the column in order of precedence and elute by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 60 mL of hexane to the column and elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas. Dissolve the residue by adding 10 mL of cyclohexane – acetone mixture (4 : 1) accurately, filter the solution through a membrane filter (pore size: 0.5 µm or less)[5], and use the filtrate as a sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph[6]. Dispense each elution fraction into a 100 mL recovery flask, concentrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 2 mL of hexane and use this solution as a sample solution for Column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column[7] (20 mm in inner diameter, 300 mm in length, particle size 15 µm)

Guard column: Styrene-divinylbenzene copolymer column[7] (20 mm in inner diameter, 100 mm in length, particle size 15 µm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 70-120 mL

Column treatment II. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane.

Place a 50 mL recovery flask A under the minicolumn and load the sample solution on the minicolumn. Wash the recovery flask which had contained the sample solution twice with 2 mL each of hexane, add the washings to the column in order of precedence and elute until the liquid level reaches the upper end of the column packing material. Add 15 mL of hexane – diethyl ether (9 : 1) to the minicolumn to elute the group A agricultural chemicals (sample solution A).

Then, replace the recovery flask A by a 50 mL recovery flask B, add 15 mL of hexane – acetone (19 : 1) to the minicolumn to elute the group B agricultural chemicals (sample solution B).

Concentrate each of the eluates under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the respective residues by adding exactly 2 mL each of 2,2,4-trimethylpentane – acetone (4 : 1) and use these solutions as sample solutions A and B for gas chromatography.

Gas chromatography. Inject 1 μL each of the sample solution A, the respective mixed standard solutions (group A) the sample solution B and the respective mixed standard solutions (group B) into the gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Electron capture detector

Column: Fused silica capillary column (14 % cyanopropylphenyl / 86 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)[8]

Carrier gas: He (1.5 mL/min)

Make up gas: N_2 (60 mL/min)

Sample injection[9]: Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 60 °C (hold 1 min) → ramp 20 °C/min → 180 °C → ramp 2 °C/min → 260 °C → ramp 5 °C/min → 275 °C (hold 1 min)

Detector temperature: 280 °C

Calculation. Obtain the peak height from the resulting chromatograms[10] to prepare a calibration curve and subsequently calculate the amount of the respective pesticides present in the sample.

«Summary of analysis method»

This method is a systematic analysis method for 32 organochlorine and acid-amide agricultural chemicals of 16 types.

In this method, each agricultural chemicals in feeds is extracted with acetonitrile/water, purified by a porous diatomite column and GPC, further purified and fractionated with a Florisil minicolumn and quantified by a gas chromatograph equipped with an electron capture detector.

The flow sheet of the analysis method is shown in Figure 6.2.1-1.

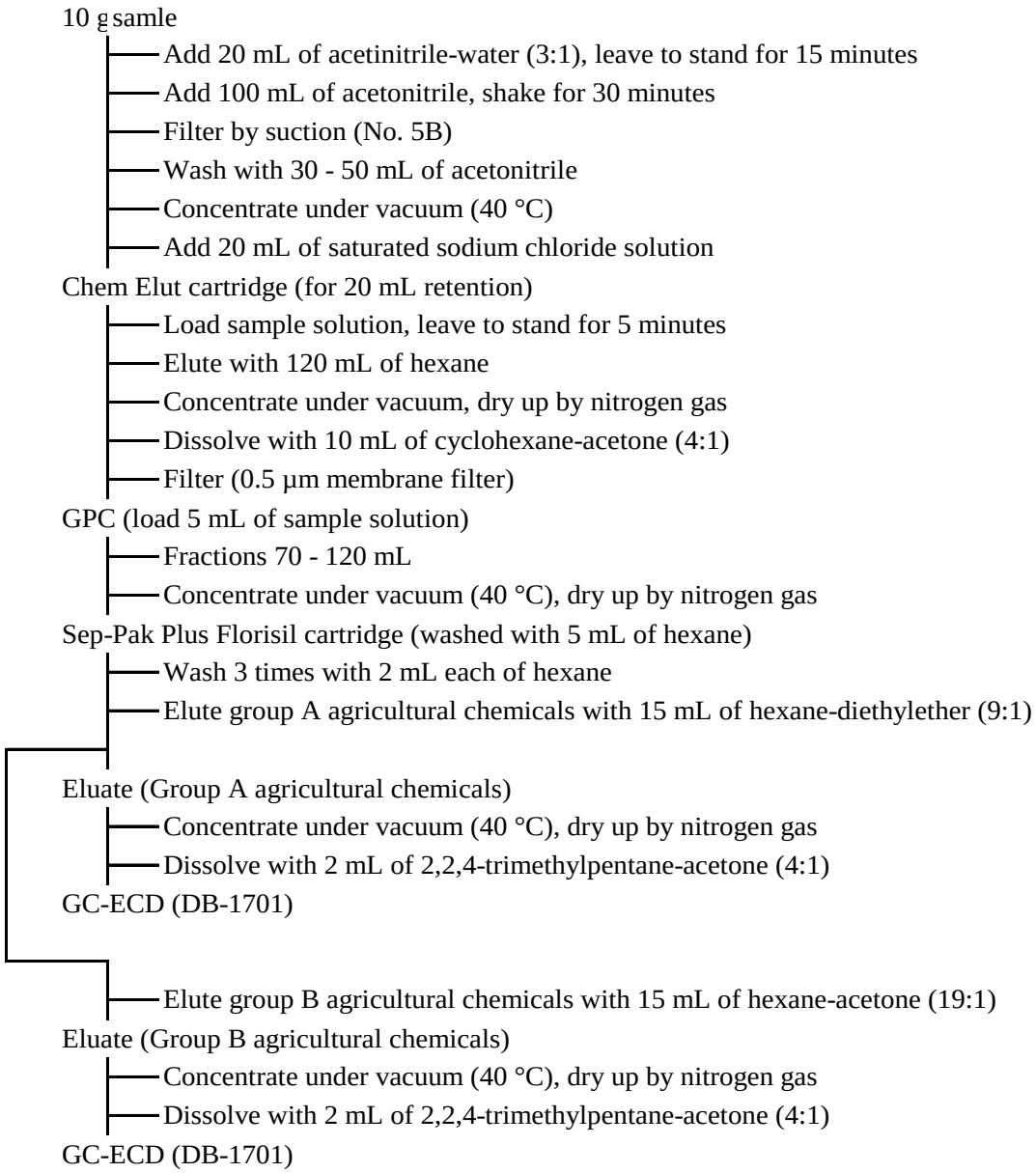


Figure 6.2.1-1. Flow sheet of the systematic analysis method for organochlorine and acid-amide agricultural chemicals

Reference: Yuji Shirai, Reiko Kazama: Research Report of Animal Feed, 22, 48 (1997).
Tomoharu Nozaki, Yoshiyuki Sunaga, Koji Aoyama, Toshiharu Yagi: Research Report of Animal Feed, 30, 17 (2005).

«Method validation»

• Spike recovery and repeatability

(Group A)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
α-BHC	chicken formula feed	10~100	3	82.7~97.7	5.7
	swine formula feed	10~100	3	78.3~87.7	7.5
	alfalfa	10~100	3	77.0~92.0	6.0
β-BHC	chicken formula feed	10~100	3	87.7~96.3	7.5
	swine formula feed	10~100	3	87.7~100.0	6.1
	alfalfa	10~100	3	85.0~96.0	3.5
γ-BHC	chicken formula feed	10~100	3	85.3~102.3	5.5
	swine formula feed	10~100	3	79.0~94.7	8.3
	alfalfa	10~100	3	85.7~94.7	6.5
δ-BHC	chicken formula feed	10~100	3	83.3~100.3	2.5
	swine formula feed	10~100	3	79.0~96.0	12.6
	alfalfa	10~100	3	80.0~91.3	3.3
o,*p*'-DDD	chicken formula feed	10~100	3	90.0~94.0	3.1
	swine formula feed	10~100	3	85.0~97.7	6.2
	alfalfa	10~100	3	85.3~105.7	8.2
p,*p*'-DDD	chicken formula feed	10~100	3	91.0~94.7	1.3
	swine formula feed	10~100	3	87.3~98.7	7.4
	alfalfa	10~100	3	82.7~105.0	4.0
o,*p*'-DDE	chicken formula feed	10~100	3	91.3~102.3	6.4
	swine formula feed	10~100	3	86.0~97.3	6.3
	alfalfa	10~100	3	87.3~101.3	1.7
p,*p*'-DDE	chicken formula feed	10~100	3	92.3~100.3	3.0
	swine formula feed	10~100	3	86.7~96.7	7.6
	alfalfa	10~100	3	93.3~104.3	2.0
o,*p*'-DDT	chicken firmula feed	10~100	3	93.7~99.0	4.0
	swine formula feed	10~100	3	92.0~96.0	9.5
	alfalfa	10~100	3	97.7~104.3	3.1
p,*p*'-DDT	chicken formula feed	10~100	3	86.7~105.3	6.2
	swine formula feed	10~100	3	90.0~94.0	6.3
	alfalfa	10~100	3	90.7~105.0	2.5
aldrin	chicken formula feed	10~100	3	86.7~108.3	3.6
	swine formula feed	10~100	3	84.7~92.3	6.0
	alfalfa	10~100	3	85.3~97.3	4.6
α-endosulfan	chicken formula feed	10~100	3	87.7~97.0	9.6
	swine formula feed	10~100	3	81.0~93.7	5.6
	alfalfa	10~100	3	93.7~96.3	8.0

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (%or less)
endrin	chicken formula feed	10~100	3	91.3~106.7	8.5
	swine formula feed	10~100	3	91.7~96.7	7.5
	alfalfa	10~100	3	92.7~97.0	2.2
oxychlordane	chicken formula feed	10~100	3	90.7~93.0	4.4
	swine formula feed	10~100	3	87.3~92.7	9.3
	alfalfa	10~100	3	84.0~97.3	3.6
cis -chlordane	chicken formula feed	10~100	3	96.0~103.3	5.1
	swine formula feed	10~100	3	89.7~95.0	6.4
	alfalfa	10~100	3	94.7~103.7	8.3
trans -chlordane	chicken formula feed	10~100	3	94.0~105.3	3.0
	swine formula feed	10~100	3	91.0~92.3	7.7
	alfalfa	10~100	3	95.3~108.7	4.4
dieldrin	chicken formula feed	10~100	3	88.0~92.7	5.0
	swine formula feed	10~100	3	86.0~93.0	4.2
	alfalfa	10~100	3	84.3~92.7	3.9
nitrophen	finishing beef cattle formula feed	10~100	3	95.9~100.2	10.7
	dairy cattle formula feed	10~100	3	110.3~113.0	13.8
	rye	10~100	3	97.3~104.0	10.7
	alfalfa	10~100	3	98.6~102.1	3.8
cis -nonachlor	chicken formula feed	10~100	3	90.0~96.7	2.2
	swine formula feed	10~100	3	87.3~94.0	6.6
	alfalfa	10~100	3	89.7~97.3	1.6
trans -nonachlor	chicken formula feed	10~100	3	91.0~98.7	4.1
	swine formula feed	10~100	3	88.7~94.7	4.4
	alfalfa	10~100	3	95.3~108.3	4.5
hexachlorobenzene	chicken formula feed	10~100	3	80.7~88.0	7.3
	swine formula feed	10~100	3	80.3~84.0	9.1
	alfalfa	10~100	3	78.7~81.7	5.1
heptachlor	chicken formula feed	10~100	3	90.7~106.0	3.4
	swine formula feed	10~100	3	86.0~101.7	3.9
	alfalfa	10~100	3	92.7~105.3	8.2
heptachlor epoxide	chicken formula feed	10~100	3	86.7~94.3	2.7
	swine formula feed	10~100	3	84.7~94.3	6.0
	alfalfa	10~100	3	85.7~95.3	2.4
methoxychlor	chicken formula feed	50~500	3	87.3~105.7	8.0
	swine formula feed	50~500	3	92.3~101.3	6.0
	alfalfa	50~500	3	98.7~104.7	2.3

(Group B)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
alachlor	chicken formula feed	50~500	3	94.3~105.7	5.1
	swine formula feed	50~500	3	94.3~99.0	5.3
	alfalfa	50~500	3	97.7~103.0	3.4
β-endosulfan	chicken formula feed	10~100	3	91.3~100.7	5.0
	swine formula feed	10~100	3	85.7~98.7	10.2
	alfalfa	10~100	3	92.3~98.7	3.5
endosulfan sulfate	chicken formula feed	10~100	3	89.7~91.0	6.7
	swine formula feed	10~100	3	84.0~92.3	9.3
	alfalfa	10~100	3	80.7~107.3	5.0
chlorobenzilate	chicken formula feed	50~500	3	78.7~87.7	4.6
	swine formula feed	50~500	3	77.0~86.7	6.0
	alfalfa	50~500	3	78.3~93.7	6.0
dichloran	chicken formula feed	10~100	3	94.7~98.7	7.4
	swine formula feed	10~100	3	94.7~97.3	6.8
	alfalfa	10~100	3	96.3~100.0	2.1
butachlor	chicken formula feed	50~500	3	92.3~101.0	6.9
	swine formula feed	50~500	3	89.7~96.7	8.7
	alfalfa	50~500	3	88.7~102.0	2.8
pretilachlor	chicken formula feed	50~500	3	93.3~102.3	7.9
	swine formula feed	50~500	3	89.3~98.3	4.5
	alfalfa	50~500	3	93.3~100.0	3.1
metolachlor	chicken formula feed	50~500	3	94.7~105.7	6.3
	swine formula feed	50~500	3	90.0~100.7	5.6
	alfalfa	50~500	3	88.0~104.0	3.0

• Collaborative study

(Group A)

Component	Sample type	No. of labs	Spike concent-ration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
α-BHC	chicken formula feed	4	20	84.2	10.0	7.8	0.36
β-BHC	chicken formula feed	4	20	99.0	11.2	11.5	0.52
γ-BHC	chicken formula feed	4	20	89.3	9.0	8.9	0.41
δ-BHC	chicken formula feed	4	20	92.7	9.0	9.0	0.41
o,*p*'-DDD	chicken formula feed	4	20	94.6	8.2	8.4	0.38
p,*p*'-DDD	chicken formula feed	4	20	91.1	8.1	8.7	0.40
o,*p*'-DDE	chicken formula feed	4	20	94.8	8.0	17.1	0.78
p,*p*'-DDE	chicken formula feed	4	20	92.4	8.9	9.3	0.42
o,*p*'-DDT	chicken formula feed	4	20	93.3	8.0	8.5	0.39
p,*p*'-DDT	chicken formula feed	4	20	93.5	7.6	8.0	0.36
aldrin	chicken formula feed	4	20	90.9	7.9	13.2	0.60
α-endosulfan	chicken formula feed	4	20	95.8	7.6	7.9	0.36
endrin	chicken formula feed	4	20	102.0	9.0	17.0	0.77
oxychlordane	chicken formula feed	4	20	88.1	9.0	9.5	0.43
cis-chlordane	chicken formula feed	4	20	98.8	7.7	7.0	0.32
trans-chlordane	chicken formula feed	4	20	101.2	7.3	7.6	0.35
dieldrin	chicken formula feed	4	20	86.0	11.8	15.3	0.70
nitrofen	dairy cattle formula feed	6	100	85.5	2.4	14.4	0.65
	rye		100	94.4	6.3	8.3	0.38
cis-nonachlor	chicken formula feed	4	20	92.3	8.2	6.6	0.30
trans-nonachlor	chicken formula feed	4	20	100.0	7.1	5.9	0.27
hexachlorobenzene	chicken formula feed	4	20	78.0	13.6	12.3	0.56
heptachlor	chicken formula feed	4	20	90.3	8.5	11.6	0.53
heptachlor epoxide	chicken formula feed	4	20	90.6	8.3	6.3	0.29
methoxychlor	chicken formula feed	4	100	97.3	7.2	7.8	0.35

(Group B)

Component	Sample type	No. of labs	Spike concent-ration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Intra-laboratory reproducibility RSD_R (%)	HorRat
alachlor	chicken formula feed	4	100	95.5	6.0	9.1	0.41
β-endosulfan	chicken formula feed	4	20	98.5	4.3	13.0	0.59
endosulfan sulfate	chicken formula feed	4	20	100.0	6.0	16.6	0.75
chlorobenzilate	chicken formula feed	4	100	89.1	8.2	25.6	1.16
dichloran	chicken formula feed	4	20	93.1	12.6	14.0	0.64
butachlor	chicken formula feed	4	100	94.7	5.3	11.8	0.54
pretilachlor	chicken formula feed	4	100	99.7	8.0	13.6	0.62
metolachlor	chicken formula feed	4	100	98.0	5.0	13.1	0.59

• Lower limit of quantification: 10 μg/kg in sample for mrthoxychlor, alachlor, chlorobenzilate, butachlor, pretilachlor and metolachlor; 2 μg/kg in sample for the other pesticides (spike recovery and relative standard deviation)

«Notes and precautions»

[1] The relevant 3 components of endosulfan (*α*- and *β*-isomers as well as endosulfan sulfate) are divided into group A or group B agricultural chemicals.

[2] The standard reagents are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers.

[3] To prevent bumping, warm up filtrates and eluates on a water bath by holding a recovery flask on the level where its bottom touches the hot water.

[4] On the porous diatomite column, organochlorine and acid-amide agricultural chemicals in aqueous solutions are transferred to hexane.

[5] For Example, Millex®-FH 0.45 µm (hydrophobic PTFE, 25 mm) from Millipore.

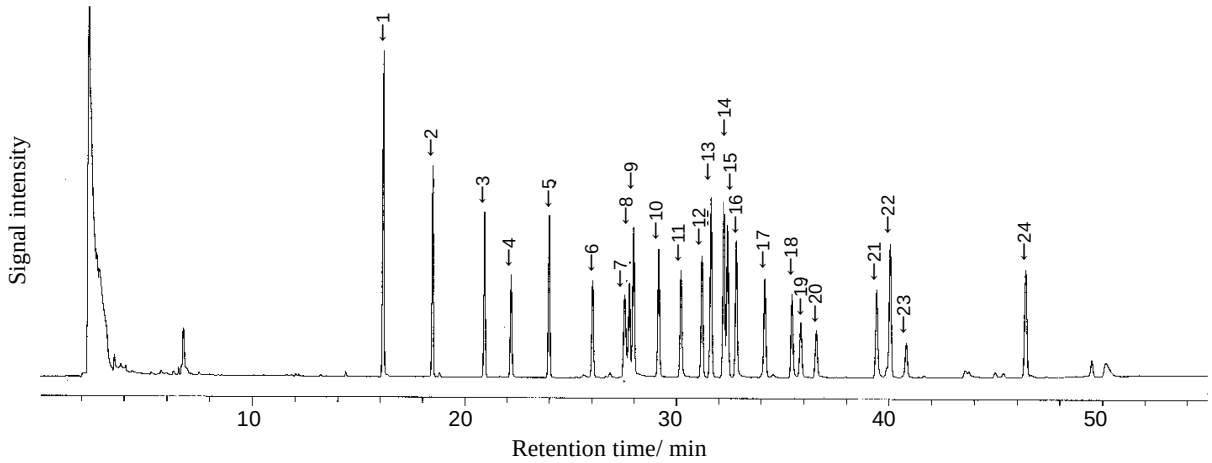
[6] Gel permeation chromatography (GPC) is a liquid chromatographic method which separates molecules in solution by their molecular size with the use of column packing material for GPC and take fractions of analyte substances using a fraction collector. Because eluted fractions may vary among lots of the column or depending on frequency of use, prior confirmation in each laboratory is required.

[7] Column packed with styrene-divinylbenzene copolymer hard gel using elute. The column and guard column provided in Appended Table 2 of Analytical Standards of Feeds are Shodex CLNpak EV-2000 AC and Shodex CLNpak EV-G AC from Showa Denko respectively.

[8] For example, DB-1701 (Agilent Technologies).

[9] Use an inactivated (silanized) inlet insert, such as Tapered Liner with glass wool [Part number: 5062-3587] from Agilent Technologies, silanized SPL insert, [Part number: 225-09145] from Shimadzu Corporation or equivalents.

[10] Examples of gas chromatogram are shown in Figure 6.2.1-2, 6.2.1-3 and 6.2.1-4.



Figutre 6.2.1-2. Chromatogram of group A agricultural chemicals (A-1: excluding nitrophen)

For gas chromatographic conditions, see the measurement conditions described above.

Names of spiked compounds

1	hexachlorobenzene	9	oxychlordane	17	dieldrin
2	*α*-BHC	10	heptachlor epoxide	18	*o,p'*-DDD
3	*γ*-BHC	11	*o,p'*-DDE	19	endrin
4	heptachlor	12	*α*-endosulfan	20	*o,p'*-DDT
5	aldrin	13	*trans*-chlordane	21	*p,p'*-DDD
6	*β*-BHC	14	*cis*-chlordane	22	*cis*-nonachlor
7	dicofol*	15	*trans*-nonachlor	23	*p,p'*-DDT
8	*δ*-BHC	16	*p,p'*-DDE	24	methoxychlor

* Because dicofol may be unmeasurable by some models, it is unlisted in Analytical Standards of Feeds.

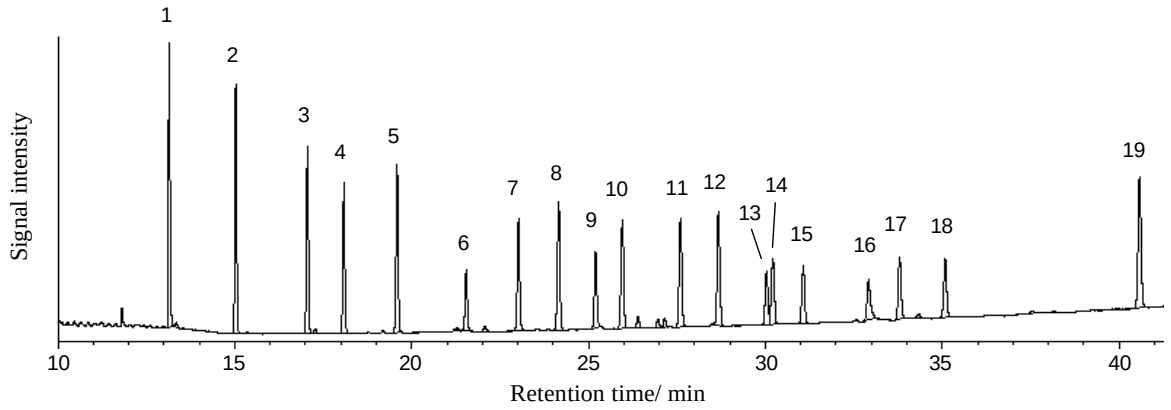


Figure 6.2.1-3. Chromatogram of group A agricultural chemicals (A-2: excluding chlordane and nonachlor)

For gas chromatographic conditions, see the measurement conditions described above.

Names of spiked compounds

1	hexachlordane	8	heptachlor epoxide	15	*o,p'*-DDT
2	*α*-BHC	9	*o,p'*-DDE	16	nitrofen
3	*γ*-BHC	10	*α*-endosulfan	17	*p,p'*-DDD
4	heptachlor	11	*p,p'*-DDE	18	*p,p'*-DDT
5	aldrin	12	dieldrin	19	methoxychlor
6	*β*-BHC	13	*o,p'*-DDD		
7	*δ*-BHC	14	endrin		

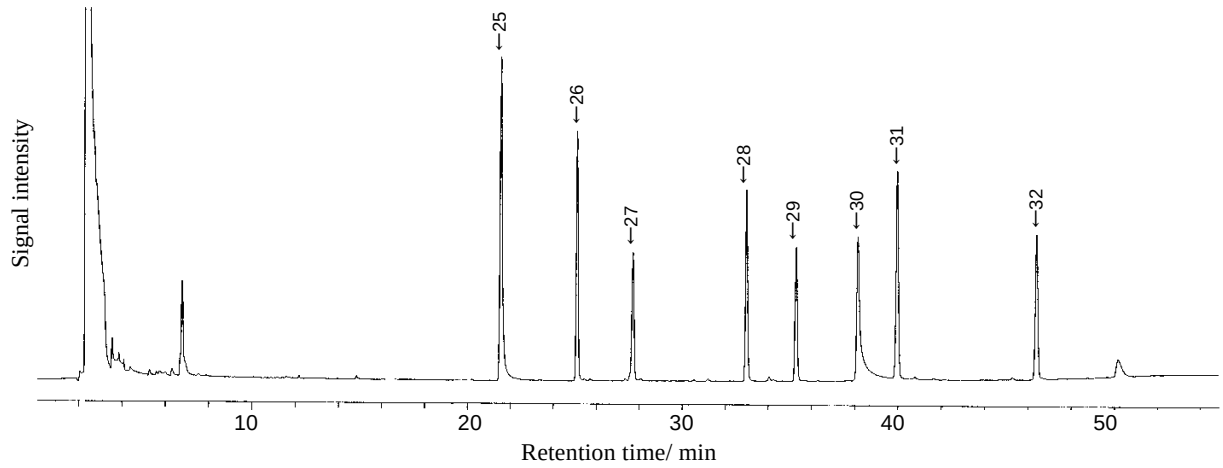


Figure 6.2.1-4. Chromatogram of group B agricultural chemicals

For gas chromatogram conditions, see the measurement conditions described above.

Names of spiked compounds

25	dichloran	29	pretilachlor	32	endosulfan sulfate
26	alachlor	30	chlorobenzilate		
27	metolachlor	31	*β*-endosulfan		

2. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (1) [Analytical Standards of Feeds, Chapter 6, Section 2, Article 2]

Target Analytes:

Group A: EPN, iprobenfos, ethion, edifenphos, etrimfos, carbophenothion, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), diazinon, terbufos, tolclofos-methyl, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, prothiofos, phosalone, phosmet, phorate, malathion and methidathion (21 compounds).

Group B: Ethoprophos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, parathion, parathion-methyl and mecarbam (7 compounds)

Group C: Acephate, isofenphos, isofenphos oxon, dichlorvos, dimethoate, fensulfothion and monocrotophos (7 compounds).

A. Reagent Preparation

Mixed standard solution (group A). Weigh accurately 20 mg each of EPN [$C_{14}H_{14}NO_4PS$][1], iprobenfos [$C_{13}H_{21}O_3PS$][1], ethion [$C_9H_{22}O_4P_2S_4$][1], edifenphos [$C_{14}H_{15}O_2PS_2$][1], etrimfos [$C_{10}H_{17}N_2O_4PS$][1], carbophenothion [$C_{11}H_{16}ClO_2PS_3$][1], chlorfenvinphos (*E*-isomer) [$C_{12}H_{14}Cl_3O_4P$][1], chlorfenvinphos (*Z*-isomer) [$C_{12}H_{14}Cl_3O_4P$][1], diazinon [$C_{12}H_{21}N_2O_3PS$][1], terbufos [$C_9H_{21}O_2PS_3$][1], tolclofos-methyl [$C_9H_{11}Cl_2O_3PS$][1], pirimiphos-methyl [$C_{11}H_{20}N_3O_3PS$][1], fenitrothion [$C_9H_{12}NO_5PS$][1], fenthion [$C_{10}H_{15}O_3PS_2$][1], phenthoate [$C_{12}H_{17}O_4PS_2$][1], prothiofos [$C_{11}H_{15}Cl_2O_2PS_2$][1], phosalone [$C_{12}H_{15}ClNO_4PS_2$][1], phosmet [$C_{11}H_{12}NO_4PS_2$][1], phorate [$C_7H_{17}O_2PS_3$][1], malathion [$C_{10}H_{19}O_6PS_2$][1] and methidathion [$C_6H_{11}N_2O_4PS_3$][1]. Transfer each of them to a 100 mL brown volumetric flask and dissolve by adding 20 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks, and use these solutions as the standard stock solutions of the group A agricultural chemicals (Each 1 mL of these solutions contains 0.2 mg of the respective agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare mixed standard solutions containing 0.1 – 2μg each of the group A agricultural chemicals per 1 mL.

Mixed standard solutions (group B and C). Weigh accurately 20 mg each of acephate [$C_4H_{10}NO_3PS$][1], isofenphos [$C_{15}H_{24}NO_4PS$][1], isofenphos oxon [$C_{15}H_{24}NO_5P$][1], ethoprophos [$C_8H_{19}O_2PS_2$][1], quinalphos [$C_{12}H_{15}N_2O_3PS$][1], chlorpyrifos [$C_9H_{11}Cl_3NO_3PS$][1], chlorpyrifos-methyl [$C_7H_7Cl_3NO_3PS$][1], dichlorvos [$C_4H_7Cl_2O_4P$][1], dimethoate [$C_5H_{12}NO_3PS_2$][1], parathion [$C_{10}H_{14}NO_5PS$][1], parathion-methyl [$C_8H_{10}NO_5PS$][1], fensulfothion [$C_{11}H_{17}O_4PS_2$][1], mecarbam [$C_{10}H_{20}NO_5PS_2$][1]and monocrotophos [$C_7H_{14}NO_5P$][1]. Transfer each of them to a 100 mL brown volumetric flask and dissolve by adding 20 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks, and use these solutions as the standard stock solutions of the group B and C agricultural chemicals (Each 1 mL of these solutions contains 0.2 mg of the respective agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare mixed standard solutions

containing 0.1 – 2μg each of the group B and C agricultural chemicals per 1 mL.

B. Quantification

Extraction. Weigh 5 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 5 mL of water, and leave to stand for 30 minutes. Then, add 50 mL of acetonitrile and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness[2] and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 10 mL of cyclohexane – acetone mixture (7 : 3) accurately, transfer the solution to a 10 mL centrifuge tube and centrifuge at 1,500×*g* for 5 minutes. Filter the supernatant through a membrane filter (pore size: 0.5 μm or less)[3], and use the filtrates as sample solutions for gel permeation chromatography (A+B) or (C) to quantify group A and B or group C agricultural chemicals respectively.

Gel permeation chromatography (A+B). Inject 5.0 mL of the sample solution into the gel permeation chromatograph[4]. Dispense each elution fraction for quantifying group A and B agricultural chemicals into a 200 mL recovery flask, add a drop of acetone – diethylene glycol (100 : 1), concentrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution for column treatment (A+B).

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 300 mm in length, particle size 15 μm)

Eluent: Cyclohexane – acetone (7 : 3)

Flow rate: 5 mL/min

Fraction collection: 50~90 mL

Column treatment (A+B). Load the sample solution on the synthetic magnesium silicate minicolumn (500 mg)[*1], discard the first 1 mL of the effluent and use the subsequent 1 – 2 mL each of the effluent as the respective sample solution for gas chromatography (A) and (B+C) respectively

Gel permeation chromatography (C). Inject 5.0 mL of the sample solution into the gel permeation chromatograph[4]. Dispense each elution fraction for quantifying group C agricultural chemicals into a 200 mL recovery flask, concentrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of acetone and use this solution as a sample solution for column treatment (C).

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 300 mm in length, particle size 15 μm)

Eluent: Cyclohexane – acetone (7 : 3)

Flow rate: 5 mL/min

Fraction collection: 45~90 mL

Column treatment (C). Wash an activated carbon minicolumn (400 mg)[*2] with 5 mL of acetone.

Place a 100 mL recovery flask under the minicolumn, load the sample solution on the minicolumn and elute group C agricultural chemicals.

Wash the recovery flask which had contained the sample solution three times with 5 mL each of acetone, add the washings to the minicolumn in order of precedence and elute in the similar way. Further, add 10 mL of acetone to the minicolumn to elute in the similar way. Concentrate the effluent on a water bath at 40 °C or lower to almost dryness under reduced pressure and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution for gas chromatography (B+C).

Gas chromatography (Group A). Inject 1 µL each of the sample solution and mixed standard solutions (group A) to obtain chromatograms.

Example of measurement conditions

Detector: Flame photometric detector (Filter for phosphorus detection)

Column: Fused silica capillary column (5 % diphenyl / 95 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)[5]

Carrier gas: He (1.5 mL/min)

Make up gas: N_2 (30 mL/min)

Hydrogen : 75 mL/min

Dry air : 100 mL/min

Sample injection[6]: Splitless mode (60 s)

Injection port temperature: 240 °C

Column oven temperature: Initial temperature 60 °C (hold 1 min) → ramp 20 °C/min → 170 °C → ramp 2 °C/min → 260 °C

Detector temperature: 270 °C

Gas chromatography (Group B+C). Inject 1 µL each of the sample solution and mixed standard solutions (group B and C) to obtain chromatograms.

Example of measurement conditions

Detector: Flame photometric detector (Filter for phosphorus detection)

Column: Fused silica capillary column (50 % trifluoropropyl methyl / 50 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)[7]

Carrier gas: He (1.5 mL/min)

Make up gas: N_2 (30 mL/min)

Hydrogen : 75 mL/min

Dry air : 100 mL/min

Sample injection[6]: Splitless mode (60 s)

Injection port temperature: 210 °C

Column oven temperature: Initial temperature 60 °C (hold 1 min) → ramp 20 °C/min → 150 °C → ramp 2 °C/min → 210 °C → ramp 5 °C/min → 235 °C (hold 7.5 min)

Detector temperature: 240 °C

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[8] to prepare a calibration curve and subsequently calculate the amount of the respective pesticides in the sample*3.

* 1. AccuBOND^{II} Florisil (reservoir volume 3 mL, Agilent Technologies)[9] or equivalents.

2. Sep-Pak Plus AC-1 Cartridge (Waters)[10] connected to a reservoir of an suitable volume, or equivalents.

3. In this method, naled [$C_4H_7Br_2Cl_2O_4P$] in the sample, if any, could be converted to dichlorvos and included in the amount of dichlorvos in the sample.

«Summary of analysis method»

This method is a systematic analysis method for 35 organophosphorus agricultural chemicals of 33 types.

For analysis of group A and B agricultural chemicals, each agricultural chemical in feeds is extracted with acetonitrile/water, purified by GPC and Florisil minicolumn and quantified by a gas chromatograph equipped with a flame photometric detector (filter for phosphorus detection) with the use of two types of capillary columns.

For analysis of group C agricultural chemicals, each agricultural chemical in feeds is extracted with acetonitrile/water, purified by GPC and activated carbon minicolumn and quantified by a gas chromatograph equipped with a flame photometric detector (filter for phosphorus detection).

The flow sheet of the analysis method is shown in Figure 6.2.2-1

Reference: Yuji Shirai, Shigetaka Suzuki, Hiroshi Hibino, Toshiaki Hayakawa: Research Report of Animal Feed, 21, 40 (1996).

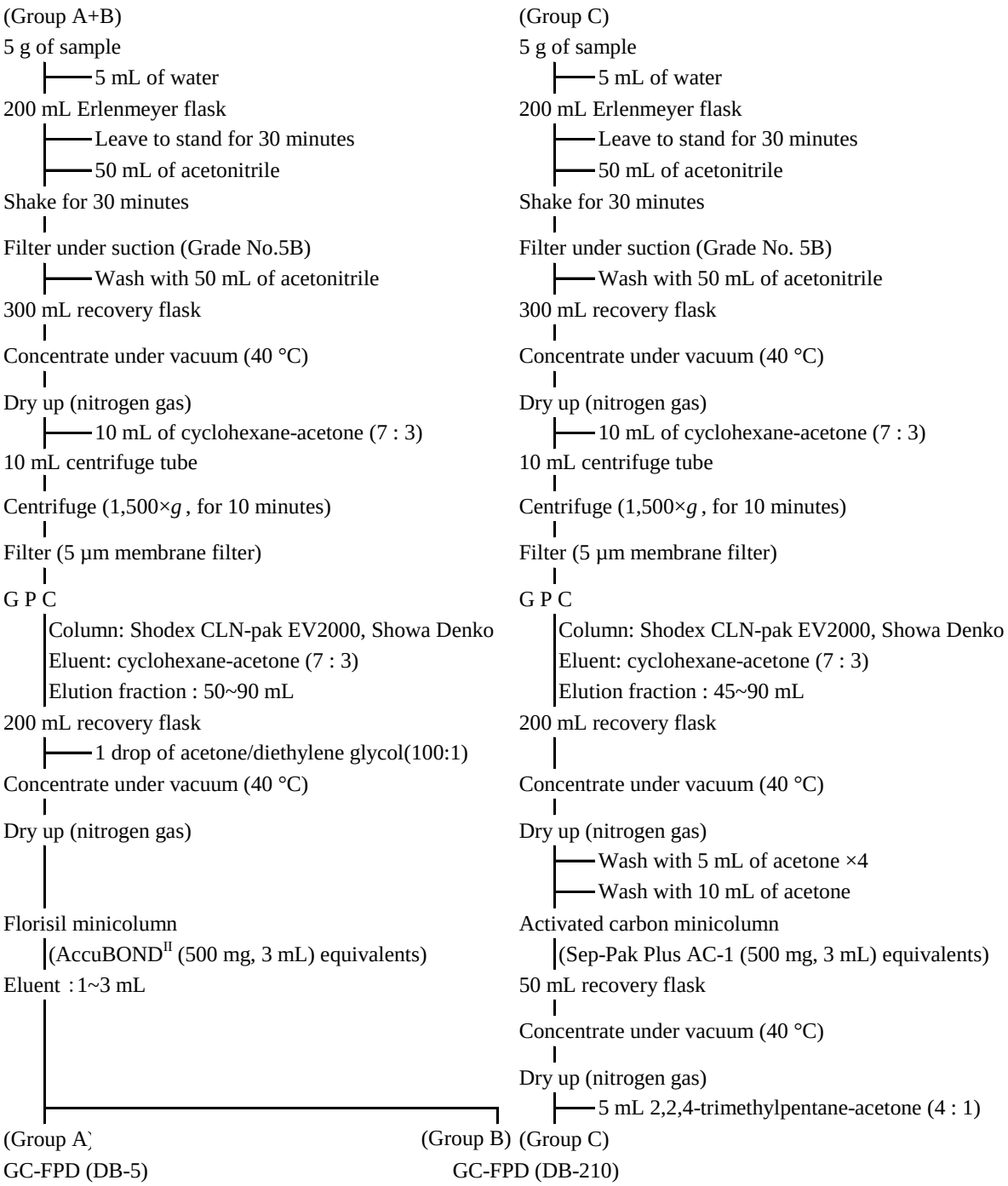


Figure 6.2.2-1. Flow sheet of the systematic analysis method for organophosphorus agricultural chemicals (35 compounds of 33 types)

«Method validation»

• Spike recovery and repeatability

(Group A)

Component	Sample type	Spike concentra-tion (µg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
EPN	adult hen formula feed	200~2,000	3	98.3~105.0	9.1
	suckling pig formula feed	200~2,000	3	99.7~104.7	7.2
	alfalfa	200~2,000	3	85.3~97.7	7.4

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
iprobenfos	adult hen formula feed	200~2,000	3	87.7~99.3	10.2
	suckling pig formula feed	200~2,000	3	93.0~101.3	11.2
	alfalfa	200~2,000	3	86.0~94.0	7.3
edifenphos	adult hen formula feed	200~2,000	3	101.3~113.7	3.1
	suckling pig formula feed	200~2,000	3	100.3~114.0	6.1
	alfalfa	200~2,000	3	94.7~101.0	6.9
ethion	adult hen formula feed	200~2,000	3	84.0~97.0	6.2
	suckling pig formula feed	200~2,000	3	90.3~96.7	8.4
	alfalfa	200~2,000	3	73.0~90.7	9.9
etrimfos	adult hens formula feed	200~2,000	3	95.3~101.7	5.0
	suckling pig formula feed	200~2,000	3	99.7~105.0	4.4
	alfalfa	200~2,000	3	88.3~96.3	7.3
carbophenothion	adult hen formula feed	200~2,000	3	98.7~106.0	5.0
	suckling pig formula feed	200~2,000	3	97.3~104.7	9.8
	alfalfa	200~2,000	3	93.3~97.0	10.0
chlorfenvinphos	adult hen formula feed	200~2,000	3	89.3~97.3	10.4
(*E* -isomer)	suckling pig formula feed	200~2,000	3	91.0~95.7	9.4
	alfalfa	200~2,000	3	79.0~93.7	9.1
chlorfenvinphos	adult hen formula feed	200~2,000	3	92.3~98.0	4.9
(*Z* -isomer)	suckling pig formula feed	200~2,000	3	95.3~103.3	5.3
	alfalfa	200~2,000	3	81.3~96.0	6.1
diazinon	adult hen formula feed	200~2,000	3	77.3~82.7	6.2
	suckling pig formula feed	200~2,000	3	81.7~83.7	4.6
	alfalfa	200~2,000	3	74.3~83.7	13.3
terbufos	adult hen formula feed	200~2,000	3	77.3~87.3	10.0
	suckling pig formula feed	200~2,000	3	80.0~87.0	10.0
	alfalfa	200~2,000	3	72.7~80.0	14.4
tolclofos-methyl	adult hen formula feed	200~2,000	3	94.3~104.3	10.6
	suckling pig formula feed	200~2,000	3	97.3~102.7	6.8
	alfalfa	200~2,000	3	89.3~99.0	8.1
pirimiphos-methyl	adult hen formula feed	200~2,000	3	98.0~101.0	6.9
	suckling pig formula feed	200~2,000	3	94.0~107.0	8.9
	alfalfa	200~2,000	3	86.3~93.7	5.4
fenitrothion	adult hen formula feed	200~2,000	3	97.0~109.7	6.4
	suckling pig formula feed	200~2,000	3	99.0~110.3	6.9
	alfalfa	200~2,000	3	97.0~98.0	5.8
fenthion	adult hen formula feed	200~2,000	3	91.0~94.7	11.5
	suckling pig formula feed	200~2,000	3	84.0~93.0	8.2
	alfalfa	200~2,000	3	84.0~89.3	7.3

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
phenthoate	adult hen formula feed	200~2,000	3	95.0~102.7	4.9
	suckling pig formula feed	200~2,000	3	97.0~102.7	4.7
	alfalfa	200~2,000	3	85.0~98.0	5.4
prothiofos	adult hen formula feed	200~2,000	3	96.0~104.0	3.8
	suckling pig formula feed	200~2,000	3	98.7~102.7	6.2
	alfalfa	200~2,000	3	86.0~97.3	5.2
phosalone	adult hen formula feed	200~2,000	3	94.7~112.3	6.6
	suckling pig formula feed	200~2,000	3	99.7~110.3	3.2
	alfalfa	200~2,000	3	91.3~97.3	5.9
phosmet	adult hen formula feed	200~2,000	3	97.7~112.3	2.6
	suckling pig formula feed	200~2,000	3	100.7~106.7	8.7
	alfalfa	200~2,000	3	90.7~98.0	13.7
phorate	adult hen formula feed	200~2,000	3	87.7~88.3	8.3
	suckling pig formula feed	200~2,000	3	86.3~88.7	8.5
	alfalfa	200~2,000	3	85.0~88.7	7.2
malathion	adult hen formula feed	200~2,000	3	95.3~110.3	6.7
	suckling pig formula feed	200~2,000	3	99.3~110.3	6.0
	alfalfa	200~2,000	3	91.3~99.7	7.1
methidathion	adult hen formula feed	200~2,000	3	103.3~107.3	8.6
	suckling pig formula feed	200~2,000	3	101.7~104.0	7.7
	alfalfa	200~2,000	3	92.7~104.3	3.3

(Group B)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
ethoprophos	adult hen formula feed	200~2,000	3	104.0~112.0	11.1
	suckling pig formula feed	200~2,000	3	101.7~106.0	7.4
	alfalfa	200~2,000	3	95.7~98.3	7.8
quinalphos	adult hen formula feed	200~2,000	3	104.0~111.7	12.2
	suckling pig formula feed	200~2,000	3	100.7~110.3	7.4
	alfalfa	200~2,000	3	98.0~102.0	8.1
chlorpyrifos	adult hen formula feed	200~2,000	3	102.7~108.0	11.2
	suckling pig formula feed	200~2,000	3	99.7~104.7	7.4
	alfalfa	200~2,000	3	93.3~103.0	8.9
chlorpyrifos-methyl	adult hen formula feed	200~2,000	3	106.3~107.7	11.2
	suckling pig formula feed	200~2,000	3	99.7~111.7	9.6
	alfalfa	200~2,000	3	93.0~100.3	9.4
parathion	adult hen formula feed	200~2,000	3	104.0~106.0	11.8
	suckling pig formula feed	200~2,000	3	96.7~104.0	9.6
	alfalfa	200~2,000	3	95.7~99.7	8.5

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
parathion-methyl	adult hen formula feed	200~2,000	3	103.7~107.7	7.6
	suckling pig formula feed	200~2,000	3	96.3~105.3	10.7
	alfalfa	200~2,000	3	91.0~94.7	14.4
mecarbam	adult hen formula feed	200~2,000	3	106.7~112.7	12.1
	suckling pig formula feed	200~2,000	3	100.7~107.7	7.4
	alfalfa	200~2,000	3	93.7~100.0	14.5

(Group C)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike concentration (%)	Repeatability RSD (% or less)
acephate	adult hen formula feed	200~2,000	3	97.0~112.3	10.7
	suckling pig formula feed	200~2,000	3	75.3~108.7	68.3
	alfalfa	200~2,000	3	90.0~103.0	8.6
isofenphos	adult hen formula feed	200~2,000	3	101.7~108.0	2.0
	suckling pig formula feed	200~2,000	3	98.3~111.7	7.8
	alfalfa	200~2,000	3	89.3~92.0	5.0
isofenphos oxon	adult hen formula feed	200~2,000	3	108.0~110.7	5.8
	suckling pig formula feed	200~2,000	3	107.7~110.0	7.5
	alfalfa	200~2,000	3	95.0~101.7	8.2
dichlorvos	adult hen formula feed	200~2,000	3	100.7~107.3	10.9
	suckling pig formula feed	200~2,000	3	104.0~111.7	3.5
	alfalfa	200~2,000	3	86.7~92.3	4.7
dimethoate	adult hen formula feed	200~2,000	3	105.7~110.7	12.5
	suckling pig formula feed	200~2,000	3	98.0~110.7	5.4
	alfalfa	200~2,000	3	90.0~98.3	10.9
fensulfothion	adult hen formula feed	200~2,000	3	110.3~113.3	3.7
	suckling pig formula feed	200~2,000	3	105.0~113.0	11.2
	alfalfa	200~2,000	3	94.0~101.3	8.8
monocrotophos	adult hen formula feed	200~2,000	3	102.7~109.0	12.9
	suckling pig formula feed	200~2,000	3	102.0~111.7	5.3
	alfalfa	200~2,000	3	86.0~95.3	10.9

• Collaborative study

(Group A)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
EPN	suckling pig formula feed	3	1,000	100.7	8.2	14.1	0.88
iprobenfos	suckling pig formula feed	3	1,000	107.7	6.7	23.9	1.51
edifenphos	suckling pig formula feed	3	1,000	117.5	9.1	24.4	1.56
ethion	suckling pig formula feed	3	1,000	100.1	6.8	19.4	1.21
etrimfos	suckling pig formula feed	3	1,000	100.8	7.5	7.3	0.46
carbophenothion	suckling pig formula feed	3	1,000	104.4	7.5	14.7	0.92
chlorfenvinphos (*E*-isomer)	suckling pig formula feed	3	1,000	100.1	7.7	13.4	0.84
chlorfenvinphos(*Z*-isomer)	suckling pig formula feed	3	1,000	102.2	7.1	8.3	0.52

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
diazinon	suckling pig formula feed	3	1,000	94.0	7.4	23.5	1.45
terbufos	suckling pig formula feed	3	1,000	92.7	9.3	17.0	1.05
tolclofos-methyl	suckling pig formula feed	3	1,000	100.5	6.4	10.2	0.64
pirimiphos-methyl	suckling pig formula feed	3	1,000	98.5	5.4	7.1	0.44
fenitrothion	suckling pig formula feed	3	1,000	100.0	5.4	7.1	0.44
fenthion	suckling pig formula feed	3	1,000	82.9	8.4	9.7	0.59
phenthoate	suckling pig formula feed	3	1,000	98.1	6.7	7.8	0.48
prothiofos	suckling pig formula feed	3	1,000	102.4	6.9	14.3	0.90
phosalone	suckling pig formula feed	3	1,000	106.1	7.9	23.9	1.51
phosmet	suckling pig formula feed	3	1,000	99.5	9.4	14.6	0.91
phorate	suckling pig formula feed	3	1,000	84.7	10.2	11.6	0.71
malathion	suckling pig formula feed	3	1,000	102.8	6.6	7.2	0.45
methidathion	suckling pig formula feed	3	1,000	105.4	6.9	14.1	0.89

(Group B)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
ethoprophos	suckling pig formula feed	3	1,000	102.3	5.9	5.6	0.35
quinalphos	suckling pig formula feed	3	1,000	103.8	4.7	9.5	0.60
chlorpyrifos	suckling pig formula feed	3	1,000	105.9	5.4	4.3	0.27
chlorpyrifos-methyl	suckling pig formula feed	3	1,000	106.8	5.2	5.0	0.31
parathion	suckling pig formula feed	3	1,000	104.3	5.5	5.7	0.36
parathion-methyl	suckling pig formula feed	3	1,000	101.7	6.0	8.6	0.54
mecarbam	suckling pig formula feed	3	1,000	95.6	6.5	24.9	1.54

(Group C)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
acephate	suckling pig formula feed	3	1,000	129.3	5.8	22.4	1.46
isofenphos	suckling pig formula feed	3	1,000	98.5	7.0	10.7	0.67
isofenphos oxon	suckling pig formula feed	3	1,000	112.5	3.2	4.7	0.30
dichlorvos	suckling pig formula feed	3	1,000	87.1	6.8	20.5	1.26
dimethoate	suckling pig formula feed	3	1,000	105.7	2.5	8.6	0.54
fensulfothion	suckling pig formula feed	3	1,000	96.1	4.8	31.3	1.94
monocrotophos	suckling pig formula feed	3	1,000	114.3	4.4	5.5	0.35

• Lower limit of quantification: 20 μg/kg in sample for all pesticides.

«Notes and precautions»

[1] The standard reagents are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers.

[2] To prevent bumping, warm up filtrates and eluates on a water bath by holding a recovery flask on the level where its bottom touches the hot water.

[3] For example, Millex®-FH 0.45 μm (hydrophobic PTFE, 25 mm) from Millipore.

[4] For gel permeation chromatograph (GPC), see also [6] and [7] of **«Notes and precautions»** in Article 1 "Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography" in this section.

[5] For example, DB-5 (Agilent Technologies).

[6] Use an inactivated (silanized) inlet insert, such as Tapered Liner with glass wool [Part number: 5062-

3587] from Agilent Technologies, silanized SPL insert [Part number: 225-09145] from Shimadzu Corporation or equivalents.

[7] For example, Rtx-200 (Restek).

[8] Examples of chromatogram are shown in Figure 6.2.2-2 and 6.2.2-3.

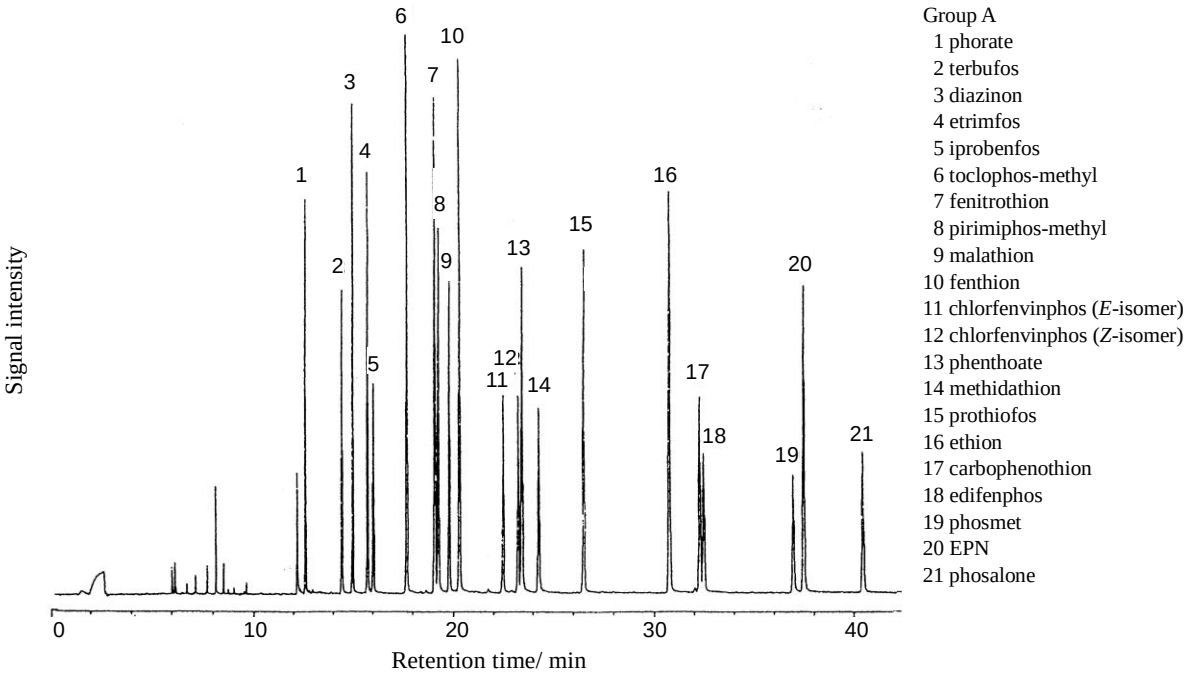


Figure 6.2.2-2. Chromatogram of group A agricultural chemicals mixed standard solutions (each 500 ng)

For measurement conditions, see the example. The column is DB-5 from J&W scientific.

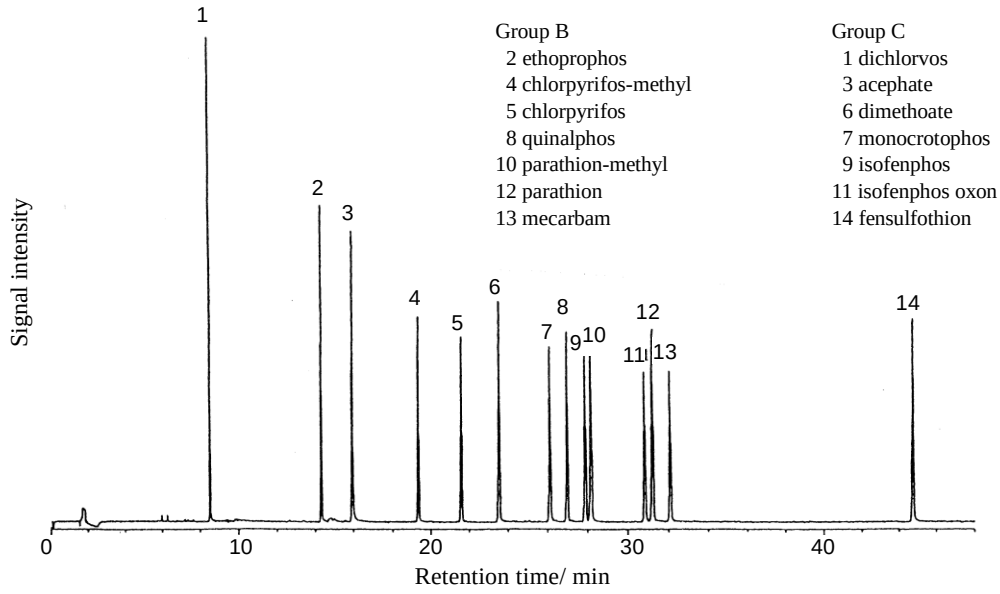


Figure 6.2.2-3. Chromatogram of sample solution spiked with group B+C agricultural chemicals mixed standard solution equivalent to 500 µg/kg each

For measurement conditions, see the example. The column is DB-210 from J&W scientific.

[9] The distribution of the said minicolumn was terminated and now, SampliQ Florisil PR (Agilent Technologies) is available as its substitute. Before use, confirm fractions.

[10] Sep-Pak Plus AC-2 (Waters) is now available.

3. Systematic analysis methods for organophosphorus agricultural chemicals by gas chromatography (2) [Analytical Standards of Feeds, Chapter 6, Section 2, Article 3]

Target Analytes:

Group A: EPN, diazinon, parathion, fenitrothion, fenthion, phenthoate, phosalone and malathion (8 compounds).

Group B: Dichlorvos (1 compound)

Scope of application: Feeds

A. Reagent preparation

1) Mixed standard solution. Weigh accurately 20 mg each of EPN [$C_{14}H_{14}NO_4PS$][1], dichlorvos [$C_4H_7Cl_2O_4P$][1], diazinon [$C_{12}H_{21}N_2O_3PS$][1], parathion [$C_{10}H_{14}NO_5PS$][1], fenitrothion [$C_9H_{12}NO_5PS$][1], fenthion [$C_{10}H_{15}O_3PS_2$][1], phenthoate [$C_{12}H_{17}O_4PS_2$][1], phosalone [$C_{12}H_{15}ClNO_4PS_2$][1] and malathion [$C_{10}H_{19}O_6PS_2$][1]. Transfer each of them to a 100 mL volumetric flask and dissolve by adding 20 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks, and use these solutions as the standard stock solutions of each agricultural chemical (Each 1 mL of these solutions contains 0.2 mg of the respective agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare mixed standard solutions containing 0.1 – 2μg each of agricultural chemicals per 1 mL.

2) Magnesium silicate. Dry synthetic magnesium silicate (particle size 149-250 μm (100-60 mesh)) at 130 °C for 16 hours[2].

3) L-Cysteine hydrochloride solution[3]. Dissolve 4.2 g of L-cysteine hydrochloride monohydrate in water to be 250 mL.

4) Activated carbon mixture. Mix 10 g of activated carbon[*1] [4] 10 g and 90 g of cellulose[*2].

B. Quantification

Extraction. Weigh 10.0 – 20.0 g of the sample[5], transfer it to a 300 mL separating funnel, add 30 mL of L-cysteine hydrochloride solution to moisten, and leave to stand for 30 minutes. Further, add it 70 mL of acetone and extract by shaking for 30 minutes. Place a 200 mL recovery flask under a Büchner funnel[*2] and filter the extract through a filter paper (No. 5B) by suction. Then, wash the separating funnel and the residue with 50 mL of acetone – water (7 : 3) sequentially, and filter the washings by suction in the similar way. Add acetone – water (7 : 3) up to the graduation line of the volumetric flask, and use this solution as a sample solution for purification.

Purification. Transfer 100 mL of sample solution[6] to a 500 mL separating funnel already containing 250 mL of sodium chloride solution (5 w/v%) and 50 mL of dichloromethane, shake for 5 minutes and leave to stand. Transfer the dichloromethane layer (lower layer) to an Erlenmeyer flask. Add 50 mL of dichloromethane to the remaining liquid, swirl gently and leave to stand. Combine the dichloromethane layer to the Erlenmeyer flask. Dehydrate the dichloromethane layer with a suitable amount of sodium sulfate (anhydrous) and filter into a 300 mL recovery flask with filter paper (No. 2S). Wash the Erlenmeyer flask above and the filter paper with a small amount of dichloromethane, filter the washings through the filter paper and combine the filtrate with the filtrate above. Concentrate the combined filtrate

on a water bath at 40 °C or lower to almost dryness under reduced pressure[3] and dry up by the flow of nitrogen gas.

Transfer the residue with 30 mL of hexane to a 200 mL separating funnel, add 30 mL of acetonitrile – water (100 : 1), shake for 5 minutes and leave to stand. Transfer the acetonitrile layer (lower layer) to a 300 mL recovery flask[7]. Add 30 mL of acetonitrile – water (100 : 1) and operate in the similar way. Concentrate the acetonitrile layer on a water bath at 40 °C or lower to almost dryness under reduced pressure and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of hexane – acetone (19 : 1) and use this solution as a sample solution to be subjected to column treatment (A) for group A agricultural chemicals quantification and to column treatment (B) for dichlorvos quantification respectively.

Column treatment (A). Suspend 2 g of sodium sulfate (anhydrous), 5 g of magnesium silicate and 2 g of sodium sulfate (anhydrous) respectively in hexane – acetone (19 : 1), pour into a column (15 mm inner diameter) sequentially and elute so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Place a 300 mL recovery flask under the column and load the sample solution on the column. Wash the recovery flask that had contained the sample solution five times with 5 mL each of hexane – acetone (19 : 1), add the washings to the column in order of precedence and elute by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material to elute group A agricultural chemicals. Further, add 70 mL of hexane – acetone (19 : 1) to the column to elute in the similar way. Add 2 – 3 drops of acetone – diethylene glycol (99 : 1)[8] to the eluate, concentrate it on a water bath at 40 °C or lower to almost dryness under reduced pressure and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution A for gas chromatography.

Column treatment (B). Suspend 2 g of sodium sulfate (anhydrous), 5 g of activated carbon mixture and 2 g of sodium sulfate (anhydrous) respectively in acetone, pour into a column (15 mm inner diameter) sequentially and elute so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Place a 300 mL recovery flask under the column and load the sample solution on the column. Wash the recovery flask that had contained the sample solution five times with 5 mL each of acetone, add the washings to the column in order of precedence and elute dichlorvos by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material to elute dichlorvos. Further, add 20 mL of acetone to the column to elute in the similar way. Add 2 – 3 drops of acetone – diethylene glycol (99 : 1)[9] to the eluate, concentrate it on a water bath at 40 °C or lower to almost dryness under reduced pressure and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution B for gas chromatography.

Gas chromatography. Inject 1 μL each of the sample solution A and B as well as respective mixed standard solutions into the gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame photometric detector (Filter for phosphorus detection)[10]

Column: Fused silica capillary column (5 % diphenyl / 95 % dimethyl-polysiloxane coating, 0.32 mm in inner diameter, 30 m in length, film thickness 0.25 μm)[11]

Carrier gas: He (1.5 mL/min)

Make up gas: N_2 (30 mL/min)

Hydrogen : 75 mL/min

Dry air : 100 mL/min

Sample injection: Splitless mode (60 s)[12]

Injection port temperature: 230 °C

Column oven temperature: Initial temperature 60 °C (hold 1 min) → ramp 10 °C/min → 170 °C (hold 21 min) → ramp 20 °C/min → 240 °C (hold 12 min)

Detector temperature: 250 °C

Calculation. Obtain the peak area from the resulting chromatograms[13] to prepare a calibration curve and subsequently calculate the amount of the respective agricultural chemicals present in the sample*3.

* 1. Charcoal, activated for chromatography (Wako Pure Chemical Industries)[9] or equivalents.

2. Cellulose microcrystalline for thin-layer chromatography (Merck) or equivalents.

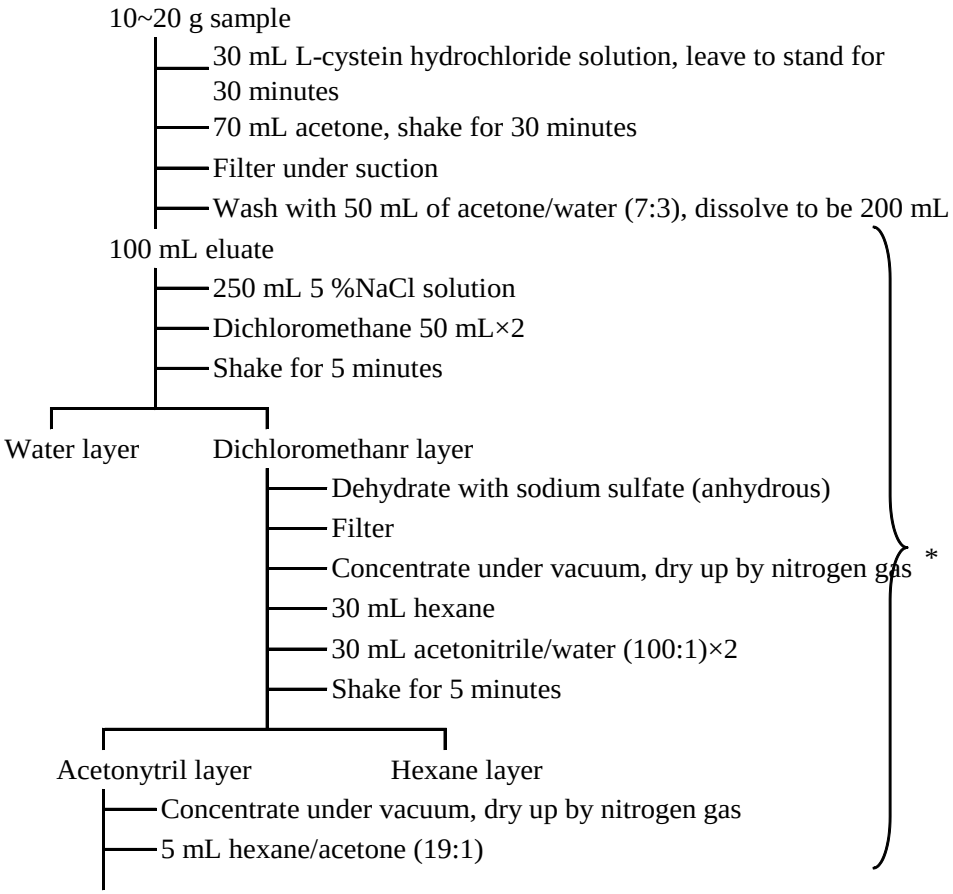
3. In this method, naled [$C_4H_7Br_2Cl_2O_4P$] in the sample, if any, could be converted to dichlorvos and included in the amount of dichlorvos in the sample.

«Summary of analysis method»

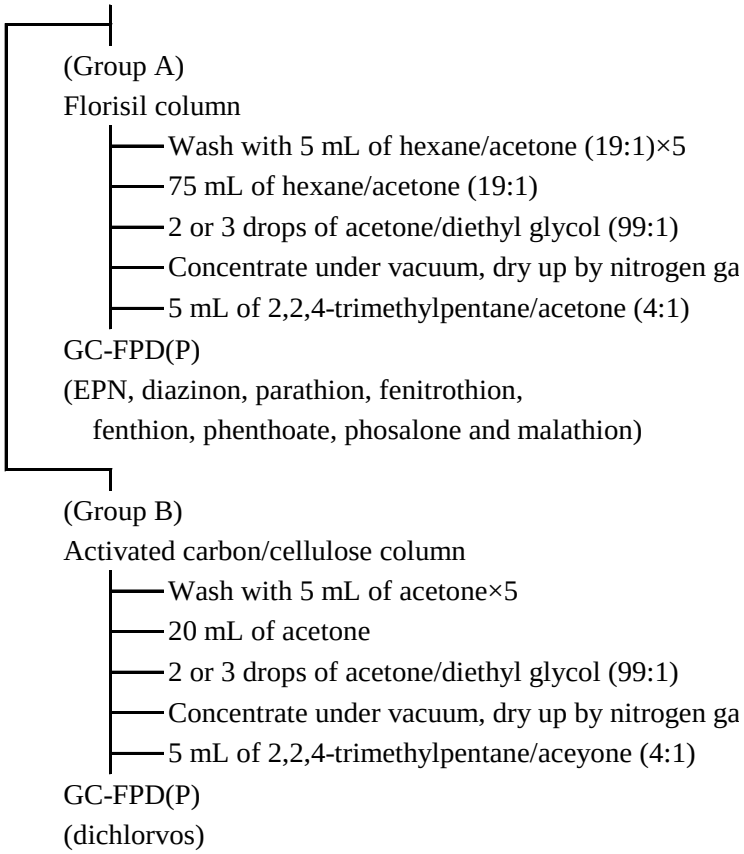
This method is a systematic analysis method for 9 organophosphorus pesticides.

Each agricultural chemical in feeds is extracted with L-cysteine-containing acetone and purified by liquid-liquid partition to obtain sample solutions. With the use of these sample solutions purified by each groups using synthetic magnesium silicate column or activated carbon/cellulose column respectively, each agricultural chemical is quantified by a gas chromatograph equipped with a flame photometric detector.

The flow sheet of the analysis method is shown in Figure 6.2.3-1.



To be continued



* Perform the above operation for Group A and B respectively.

Figure 6.2.3-1. Flow sheet of the systematic analysis method for organophosphorus agricultural chemicals

Reference: Fumio Kojima, Yukie Ishida: Research Report of Animal Feed, 17, 26 (1992).
Yuji Shirai, Hiroshi Ogata, Fumio Kojima, Yukie Ishida: Research Report of Animal Feed, 18, 1 (1993)

«Method validation»

• Spike recovery and repeatability

(Group A)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
EPN	adult hen fprmula feed	100~500	3	99.0~106.5	8.3
	hay cube	100~500	3	95.6~96.8	11.3
diazinone	adult hen formula feed	100~500	3	88.5~91.1	5.4
	hay cube	100~500	3	86.7~90.2	10.4
parathion	adult hen formula feed	100~500	3	94.3~97.6	3.2
	hay cube	100~500	3	92.5~93.5	10.5
fenitrothion	adult hen formula feed	100~500	3	103.9~108.2	10.6
	hay cube	100~500	3	94.7~95.3	5.3
fenthion	adult hen formula feed	100~500	3	94.6~96.6	4.9
	hay cube	100~500	3	83.8~88.4	14.3
phenthoate	adult hen formula feed	100~500	3	93.5~98.8	9.1
	hay cube	100~500	3	92.0~94.7	5.9
phosalone	adult hen formula feed	50~1,000	3	96.2~101.7	13.2
	finishing pig formula feed	50~1,000	3	100.2~102.0	3.9
	hay cube	50~1,000	3	92.6~100.4	10.1
malathion	adult hen formula feed	100~500	3	101.7~114.9	14.7
	hay cube	100~500	3	94.8~96.1	4.2

(Group B)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
dichlorvos	adult hen formula feed	100~500	3	94.8~98.8	13.6
	hay cube	100~500	3	89.4~90.4	8.3

• Collaborative study

(Group A)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
EPN	suckling pig formula feed	7	500	96.2	4.9	8.3	0.47
diazinon	suckling pig formula feed	7	500	90.8	7.4	8.3	0.46
parathion	suckling pig formula feed	7	500	94.4	3.3	4.6	0.26
fenitrothion	suckling pig formula feed	7	500	98.4	5.5	9.6	0.54
fenthion	suckling pig formula feed	7	500	94.7	5.6	5.7	0.32
phenthoate	suckling pig formula feed	7	500	95.7	4.9	6.0	0.34
phosalone	suckling pig formula feed	7	250	99.4	4.1	9.0	0.45
malathion	suckling pig formula feed	7	500	101.9	4.3	8.4	0.48

(Group B)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
dichlorvos	suckling pig formula feed	7	500	100.7	6.4	12.3	0.69

«Notes and precautions»

[1] The standard reagents are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi

Pure Chemical, GL Sciences and other manufacturers.

[2] Prepare immediately before use, allow to cool in the desiccators and then use.

[3] Use to prevent oxidation of fenthion.

For feeds with a lot of interfering substances such as oil, it is advisable to perform congealing by the use of ammonium chloride and phosphoric acid solution after extraction and purification.

[4] Because of the possible differences in activity level of activated carbon, confirmation of the recovery with the use of the standard solution is required before use.

[5] For samples having a high pigment content, such as grass hay, use 10 g of samples.

[6] For a simultaneous quantification of group A and B agricultural chemicals, purify 100 mL each of the extracts concurrently.

[7] For removal of low polarity substances.

[8] Use as a keeper to prevent diazinon loss.

[9] Use as a keeper to prevent dichlorvos loss.

[10] NPD may also be used.

[11] For example, DB-5 (Agilent Technologies).

[12] Injection method may either be splitless or cool-on.

[13] Example of chromatogram is shown in Figure 6.2.3-2.

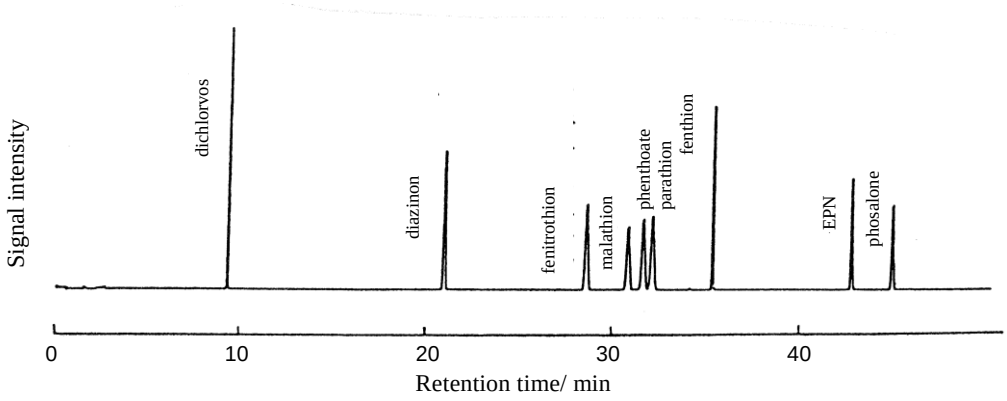


Figure 6.2.3-2. Chromatogram of sample solution spiked with organophosphorus agricultural chemicals equivalent to 2 ng each

Measurement Conditions

Detector: FPD (Filter for phosphorus detection)

Column: DB-5 (0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)

Carrier gas: He (1.5 mL/min, initial flow rate)

Make up gas: N_2 (30 mL/min)

Hydrogen : 75 mL/min

Dry air : 100 mL/min

Sample injection: Splitless mode

Injection port temperature: 230 °C

Column oven temperature: Initial temperature 60 °C (hold 1 min) → ramp 10 °C/min → 170 °C (hold 21 min) → ramp 20°C/min → 240 °C (hold 8 min)

Detector temperature: 250 °C

4. Systematic analysis methods for pyrethroid agricultural chemicals by gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 2, Article 4]

Target Analytes:

Group A: Tefluthrin, bifenthrin and permethrin (3 compounds)

Group B: Cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate and fluvalinate (8 compounds)

Group C: Allethrin and tetramethrin (2 compounds)

A. Reagent preparation

1) Mixed standard solution (group A). Weigh accurately 20 mg each of tefluthrin [$C_{17}H_{14}ClF_7O_2$][1], bifenthrin [$C_{23}H_{22}ClF_3O_2$][1] and permethrin [$C_{21}H_{20}Cl_2O_3$][1]. Transfer each of them to a 100 mL brown volumetric flask and dissolve by adding 20 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks, and use these solutions as the standard stock solutions (Each 1 mL of these solutions contains 0.2 mg of the respective group A agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare mixed standard solutions containing 0.1 – 2μg each of the group A agricultural chemicals (0.01-0.2 for tefluthrin) per 1 mL.

2) Mixed standard solution (group B). Weigh accurately 20 mg each of cyhalothrin [$C_{22}H_{18}Cl_2FNO_3$][1], cyfluthrin [$C_{22}H_{18}Cl_2FNO_3$][1], cypermethrin [$C_{22}H_{19}Cl_2NO_3$][1], deltamethrin [$C_{22}H_{19}Br_2NO_3$][1], fenvalerate [$C_{25}H_{22}ClNO_3$][1], fenpropathrin [$C_{22}H_{23}NO_3$][1], flucythrinate [$C_{26}H_{23}F_2NO_4$][1] and fluvalinate [$C_{26}H_{22}ClF_3N_2O_3$][1]. Transfer each of them to a 100 mL brown volumetric flask and dissolve by adding 20 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks, and use these solutions as the standard stock solutions (Each 1 mL of these solutions contains 0.2 mg of the respective group B agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare mixed standard solutions containing 0.1 – 2μg each of the group B agricultural chemicals per 1 mL.

3) Mixed standard solution (group C). Weigh accurately 20 mg each of allethrin [$C_{19}H_{26}O_3$][1] and tetramethrin [$C_{19}H_{25}NO_4$][1]. Transfer each of them to a 100 mL brown volumetric flask and dissolve by adding 20 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks, and use these solutions as the standard stock solutions (Each 1 mL of these solutions contains 0.2 mg of the respective group C agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare mixed standard solutions containing 0.1 – 2μg each of the group C agricultural chemicals per 1 mL.

B. Quantification

Extraction. Weigh 10 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 20 mL of acetonitrile – water (3 : 1), and leave to stand for 15 minutes. Then, add it 100 mL of acetonitrile and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the

extract through filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness, add 20 mL of saturated sodium chloride solution and use the mixture as a sample solution for column treatment I.

Column treatment I. Load the sample solution on the porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that had contained the sample solution three times with 20 mL each of hexane, add the washings to the column in order of precedence and elute by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 60 mL of hexane to the column and elute agricultural chemicals in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 10 mL of cyclohexane – acetone (4 : 1) accurately, filter the solution through a membrane filter (pore size: 0.5 µm or less)[3], and use the filtrate as a sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph[4]. Dispense each elution fraction into a 100 mL recovery flask, concentrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of hexane accurately and use this solution as a sample solution for column treatment (A) and (B+C) respectievly.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 300 mm in length, particle size 15 µm)

Guard column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 100 mm in length, particle size 15 µm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 60-85 mL

Column treatment (A). Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane.

Load 2 mL of the sample solution accurately to the minicolumn and elute by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 15 mL of hexane to the minicolumn and elute in the similar way. Place a 50 mL recovery flask A under the minicolumn and add 15 mL of hexane – diethylether (19 : 1) to the minicolumn to elute the group A agricultural chemicals. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 2 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution A for gas chromatography.

Column treatment (B+C). Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane.

Load 2 mL of the sample solution accurately to the minicolumn and elute by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 15 mL of hexane to the minicolumn and elute in the similar way. Place a 50 mL recovery flask B under the minicolumn and add 15 mL of hexane – diethylether (4 : 1) to the minicolumn to elute the group B agricultural chemicals. Then, replace the minicolumn by a 50 mL recovery flask C, add 15 mL of hexane – acetone (19 : 1) to the minicolumn to elute the group C agricultural chemicals. Concentrate each of the eluates under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the respective residues by adding accurately 2 mL each of 2,2,4-trimethylpentane – acetone (4 : 1) and use these solutions as sample solutions B and C for gas chromatography.

Gas chromatography. Inject 1 µL each of the sample solutions A, B and C as well as the respective mixed standard solutions (group A, B and C) into the gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Electron capture detector

Column: Fused silica capillary column (50 % trifluoropropyl methyl/ 50 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)[5]

Carrier gas: He (1.5 mL/min)

Make up gas: N_2 (60 mL/min)

Sample injection[6]: Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 1 min) → ramp 20 °C/min → 200 °C → ramp 2 °C/min → 290 °C (hold 10 min)

Detector temperature: 300 °C

Calculation. Obtain the peak height from the resulting chromatograms[7] to prepare a calibration curve and subsequently calculate the amount of the respective pesticides in the sample*1.

* 1. In this method, tralomethrin in the sample, if any, could be converted to deltamethrin and included in the amount of deltamethrin in the sample.

«Summary of analysis method»

This method is a systematic analysis method for 13 pyrethroid pesticides.

In this method, each agricultural chemical in feeds is extracted with acetonitrile/water, purified by a porous diatomite column, a gel permeation chromatograph (GPC) and Florisil minicolumn and quantified by a gas chromatograph equipped with an electron capture detector.

The flow sheet of the analysis method is shown in Figure 6.2.4-1.

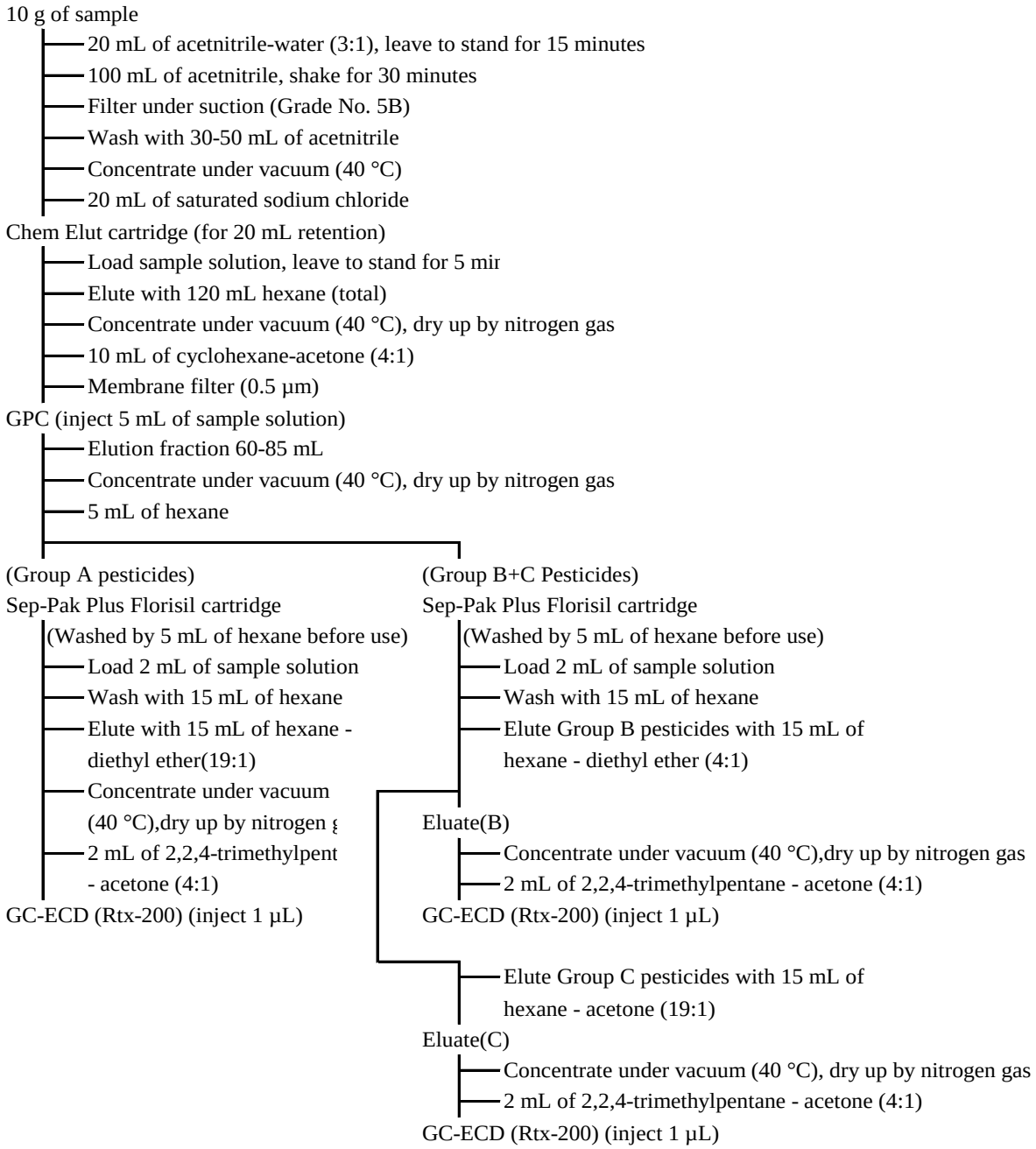


Figure 6.2.4-1. Flow sheet of the systematic analysis method for pyrethroid agricultural chemicals (13 compounds of 13 types)

Reference: Yuji Shirai, Yoshihiro Sekiguchi, Masato Funatsu: Research Report of Animal Feed, 23, 73 (1998).

«Method validation»

• Spike recovery and repeatability

(Group A)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
tefluthrin	chicken formula feed	10~100	3	92.0~103.0	5.6
	swine formula feed	10~100	3	96.0~103.0	8.1
	timothy hay	10~100	3	90.7~108.0	5.5
bifenthrin	chicken formula feed	100~1,000	3	82.0~98.0	5.7
	swine formula feed	100~1,000	3	94.0~102.3	9.8
	timothy hay	100~1,000	3	90.0~95.3	2.3
permethrin	chicken formula feed	100~1,000	3	86.7~95.0	6.4
	swine formula feed	100~1,000	3	98.3~103.7	3.1
	timothy hay	100~1,000	3	89.0~101.0	2.6

(Group B)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
cyhalothrin	chicken formula feed	100~1,000	3	92.3~102.3	9.1
	swine formula feed	100~1,000	3	101.3~113.7	6.9
	timithy hay	100~1,000	3	98.0~102.0	4.4
cyfluthrin	chicken formula feed	100~1,000	3	100.3~113.7	5.7
	swine formula feed	100~1,000	3	98.0~111.7	4.4
	timothy hay	100~1,000	3	92.7~99.3	6.1
cypermethrin	chicken formula feed	100~1,000	3	94.0~107.3	7.4
	swine formula feed	100~1,000	3	98.0~114.0	6.4
	timothy hay	100~1,000	3	96.3~102.3	5.6
deltamethrin	chicken formula feed	100~1,000	3	107.0~115.3	12.6
	swine formula feed	100~1,000	3	108.0~114.0	7.6
	timothy hay	100~1,000	3	96.3~108.3	2.8
fenvalerate	chicken formula feed	100~1,000	3	100.7~106.3	6.2
	swine formula feed	100~1,000	3	101.3~111.7	9.0
	timithy hay	100~1,000	3	92.7~105.7	5.2
fenpropathrin	chicken formula feed	100~1,000	3	97.3~105.0	7.6
	swine gormula feed	100~1,000	3	96.3~109.3	3.6
	timothy hay	100~1,000	3	99.3~104.7	5.2
flucythrinate	chicken formula feed	100~1,000	3	97.3~112.3	6.3
	swine formula feed	100~1,000	3	97.7~115.7	7.0
	timothy hay	100~1,000	3	100.3~103.3	4.0
fluvalinate	chicken formula feed	100~1,000	3	108.3~117.3	6.3
	swine formula feed	100~1,000	3	105.3~118.7	7.2
	timothy hay	100~1,000	3	100.3~109.0	3.2

(Group C)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
allethrin	chicken formula feed	100~1,000	3	93.0~105.3	2.6
	swine formula feed	100~1,000	3	101.0~110.3	5.5
	timothy hay	100~1,000	3	91.7~103.7	2.0
tetramethrin	chicken formula feed	100~1,000	3	97.3~106.0	5.7
	swine formula feed	100~1,000	3	91.3~102.7	2.1
	timothy hay	100~1,000	3	96.0~100.7	4.8

• Collaborative study

(Group A)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
tefluthrin	chicken formula feed	5	25	86.4	5.8	15.8	0.72
bifenthrin	chicken formula feed	5	250	93.0	3.3	21.1	1.06
permethrin	chicken formula feed	5	250	93.1	5.1	24.7	1.24

(Group B)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
cyhalothrin	chicken formula feed	5	250	93.7	3.8	9.9	0.50
cyfluthrin	chicken formula feed	5	250	93.8	4.1	17.9	0.90
cyoermethrin	chicken formula feed	5	250	93.8	3.4	12.9	0.65
deltamethrin	chicken formula feed	5	250	92.8	4.6	15.8	0.79
fenvalerate	chicken formula feed	5	250	94.6	3.8	18.5	0.93
fenpropathrin	chicken formula feed	5	250	92.3	3.2	15.0	0.75
flucythrinate	chicken formula feed	5	250	95.3	3.9	15.9	0.80
fluvalinate	chicken formula feed	5	250	96.7	5.7	19.1	0.96

(Group C)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
allethrin	chicken formula feed	5	250	99.8	9.7	13.3	0.68
tetramethrin	chicken formula feed	5	250	102.5	5.8	18.5	0.94

• Lower limit of quantification: 5 μg/kg in sample for tefluthrin; 50 μg/kg each in sample for the other pesticides

• Lower limit of detection: 2 μg/kg in sample for tefluthrin; 20 μg/kg each in sample for the other pesticides

«Notes and precautions»

[1] The standard reagents are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers.

[2] To prevent bumping, warm up filtrates and eluates on a water bath by holding a recovery flask on the level where its bottom touches the hot water.

[3] For Example, Millex®-FH 0.45 μm (hydrophobic PTFE, 25 mm) from Millipore.

[4] For gel permeation chromatograph (GPC), see also [6] and [7] of **«Notes and precautions»** in Article 1 “Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas

chromatography" of this Section.

[5] For example, Rtx-200 (Restek).

[6] Use an inactivated (silanized) inlet insert.

[7] Examples of chromatogram are shown in Figure 6.2.4-2 – 4.

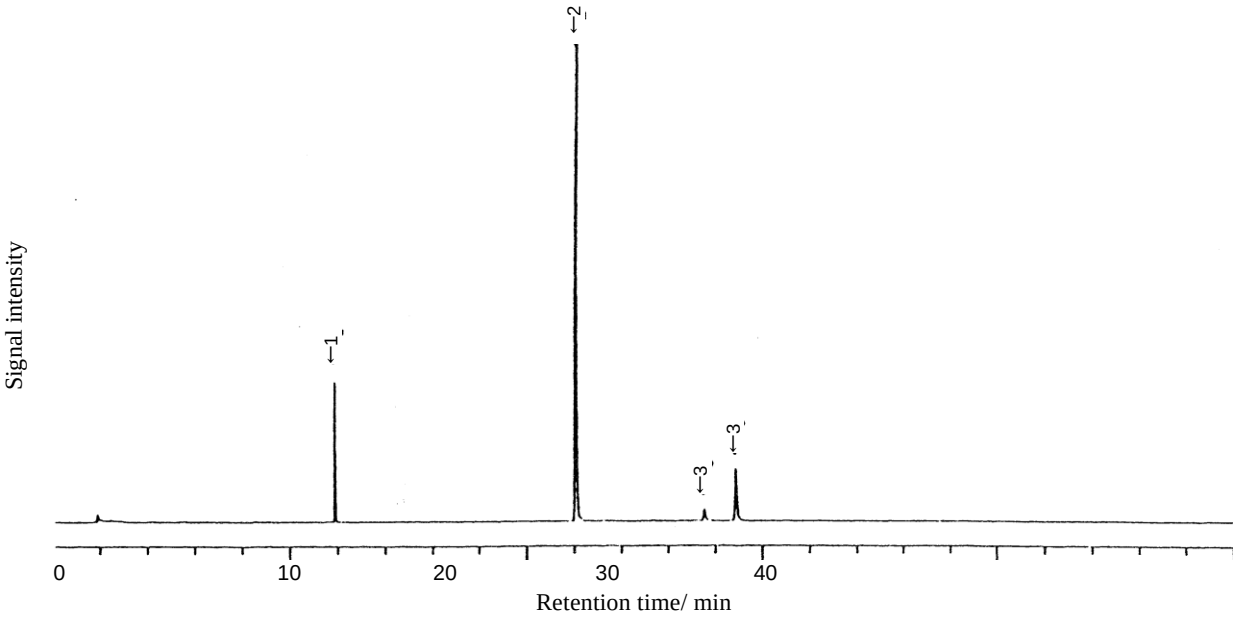


Figure 6.2.4-2. Chromatogram of pyrethroid agricultural chemicals (group A)

For gas chromatographic conditions, see the measurement conditions described above.

1 tefluthrin 2 bifenthrin 3 permethrin

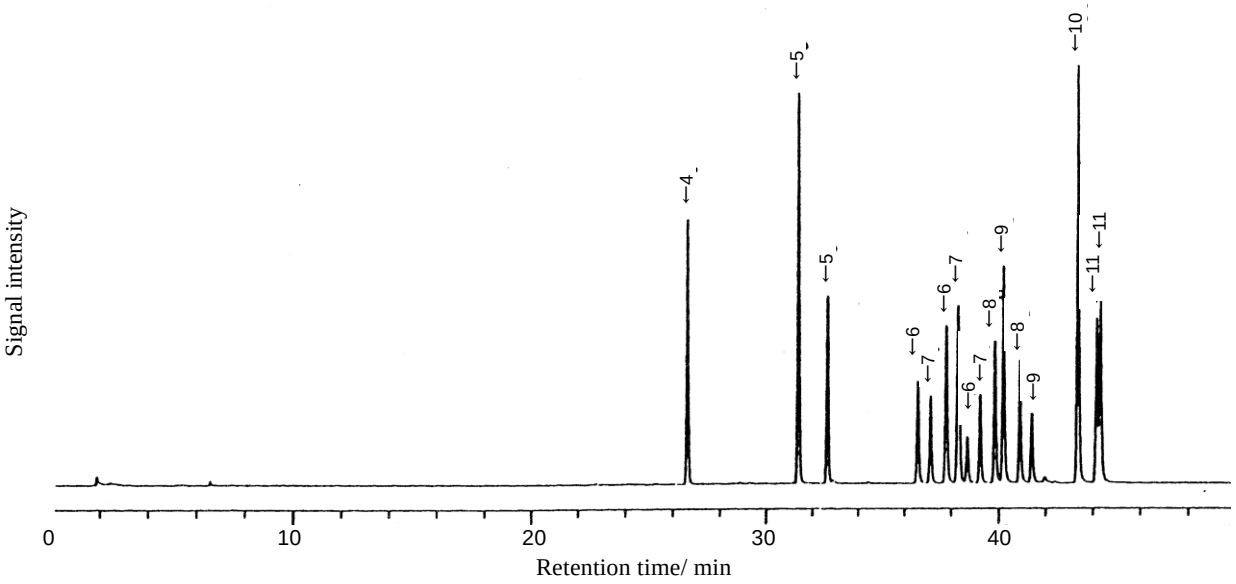


Figure 6.2.4-3. Chromatogram of pyrethroid agricultural chemicals (group B)

For gas chromatographic conditions, see the measurement conditions described above.

4 fenpropathrin 7 cyfluthrin 10 deltamethrin

5 cyhalothrin 8 flucythrinate 11 fluvalinate

6 cypermethrin 9 fenvalerate

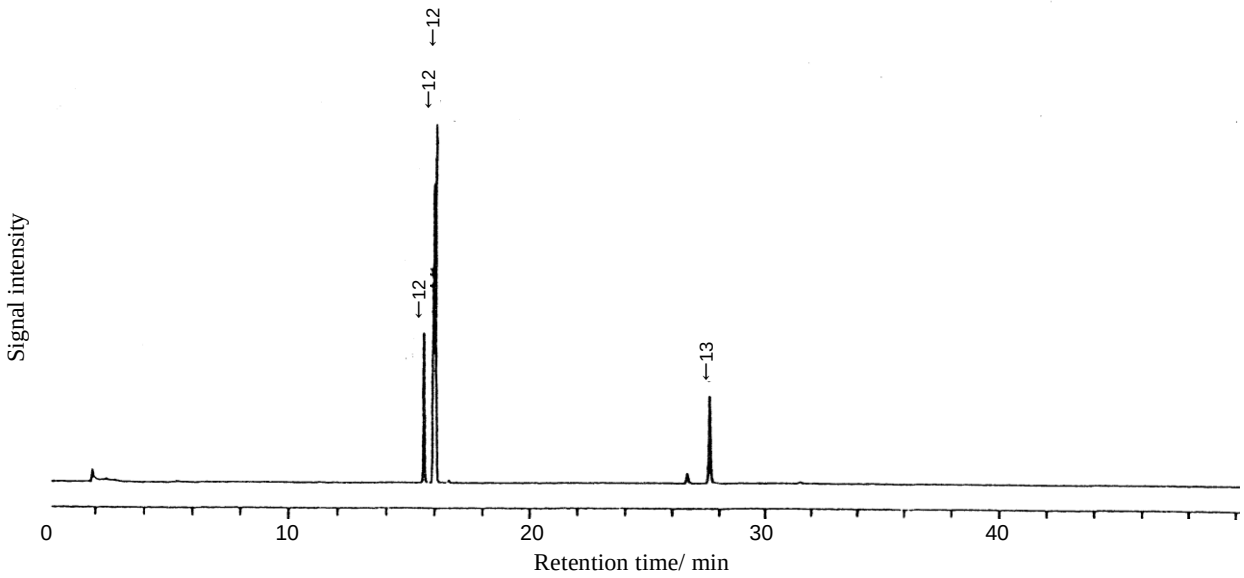


Figure 6.2.4-4. Chromatogram of pyrethroid agricultural chemicals (group C)

For gas chromatographic conditions, see the measurement conditions described above.

12 allethrin 13 tetramethrin

5. Systematic analysis methods for alachlor, allethrin, chlorpropham, dichloran and methoxychlor by gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 2, Article 5]

Target Analytes:

Group A: Alachlor, allethrin and dichloran (3 compounds)

Group B: Methoxychlor (1 compound)

Group C: Chlorpropham (1 compound)

A. Reagent Preparation

1) Mixed standard solution (group A and B). Weigh accurately 20 mg each of alachlor [$C_{14}H_{20}ClNO_2$][1], allethrin [$C_{19}H_{26}O_3$][1], dichloran [$C_6H_4Cl_2N_2O_2$][1] and methoxychlor [$C_{16}H_{15}Cl_3O_2$][1]. Transfer each of them to a 100 mL brown volumetric flask and dissolve by adding 20 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks, and use these solutions as the standard stock solutions (Each 1 mL of these solutions contains 0.2 mg of the respective agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare mixed standard solutions containing 0.025 – 0.75μg each of the group A and B agricultural chemicals per 1 mL.

2) Chlorpropham standard solution. Weigh accurately 20 mg of chlorpropham [$C_{10}H_{12}ClNO_2$][1], transfer to a 100 mL brown volumetric flask and dissolve by adding 20 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the flask and use this solution as the chlorpropham standard stock solution (Each 1 mL of this solution contains 0.2 mg of chlorpropham.).

Before use, dilute accurately a certain amount of the standard stock solution with 2,2,4-trimethylpentane – acetone (4 : 1) to prepare chlorpropham standard solutions containing 0.1 – 3μg of chlorpropham per 1 mL.

3) Magnesium silicate. Dry synthetic magnesium silicate (particle size: 149-250 μm (100-60 mesh)) at 130 °C for 16 hours[2].

B. Quantification

Extraction. Weigh 10.0 – 20.0 g of the sample[3], transfer it to a 300 mL separating funnel, add 30 mL of water to moisten and leave to stand for 30 minutes. Further, add it 70 mL of acetone and extract by shaking for 30 minutes. Place a 200 mL volumetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the separating funnel and the residue with 50 mL of acetone – water (7 : 3) sequentially, and filter the washings by suction in the similar way. Add acetone – water (7 : 3) up to the graduation line of the volumetric flask, and use this solution as a sample solution for purification.

Purification. Transfer 50 mL of sample solution to a 300 mL separating funnel A already containing 100 mL of sodium chloride solution (5 w/v%) and 100 mL of hexane, shake vigorously for 5 minutes and leave to stand. Transfer the water layer (lower layer) to a 300 mL separating funnel B and the hexane layer (upper layer) to an Erlenmeyer flask respectively. Add 50 mL of hexane to the separating funnel, swirl gently and leave to stand. Combine the hexane layer to the Erlenmeyer flask above. Dehydrate the hexane layer with a suitable amount of sodium sulfate (anhydrous) and filter into a 300 mL recovery

flask with filter paper (No. 2S). Wash the Erlenmeyer flask above and the filter paper with a small amount of hexane sequentially, filter the washings through the filter and combine the filtrate with the filtrate. Concentrate the combined filtrate on a water bath at 40 °C or lower to almost dryness under reduced pressure and dry up by the flow of nitrogen gas.

Transfer the residue with 30 mL of hexane to a 200 mL separating funnel, add 30 mL of acetonitrile – water (100 : 1), shake intensely for 5 minutes and leave to stand. Add 30 mL of acetonitrile – water (100 : 1) to the remaining liquid, operate twice in the similar way, and combine the acetonitrile layer to the recovery flask. Concentrate the acetonitrile layer on a water bath at 40 °C or lower to almost dryness under reduced pressure and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of hexane – diethyl ether (19 : 1) and use this solution as a sample solution to be subjected to column treatment.

Column treatment. Suspend 5 g of magnesium silicate and 2 g of sodium sulfate (anhydrous) in hexane respectively, pour into a column (15 mm inner diameter) sequentially and elute so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Place a 300 mL recovery flask under the column and load the sample solution on the column. Wash the recovery flask that had contained the sample solution three times with 5 mL each of hexane – diethyl ether (19 : 1), add the washings to the column in order of precedence and elute methoxychlor by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Further, add 80 mL of hexane – diethyl ether (19 : 1) to the column to elute[4] methoxychlor in the similar way.

Concentrate the effluent on a water bath at 40 °C or lower to almost dryness under reduced pressure and dry up by the flow of nitrogen gas. Dissolve the residue by adding 2 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution B to be subjected to gas chromatography (B).

Then, replace the recovery flask by a 200 mL recovery flask, add 50 mL of hexane – acetone (19 : 1) to the minicolumn to elute[4] the group A agricultural chemicals and chlorpropham.

Concentrate the eluate on a water bath at 40 °C or lower to almost dryness under reduced pressure and dry up by the flow of nitrogen gas. Dissolve the residue by adding 2 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution AC to be subjected to gas chromatography (A) and (C).

Gas chromatography (A). Inject 2 µL each of the sample solution AC and the respective mixed standard solutions (group A and B) into the gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Electron capture detector

Column: Fused silica capillary column (14 % cyanopropylphenyl / 86 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)[5]

Carrier gas: He (1.5 mL/min, initial flow rate)

Make up gas: N_2 (60 mL/min)

Sample injection[6]: Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 2 min) → ramp 30 °C/min → 140 °C → ramp 3 °C/min → 220 °C

Detector temperature: 300 °C

Gas chromatography (B). Inject 2 µL each of the sample solution B and the respective mixed standard solutions (group A and B) into the gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Electron capture detector

Column: Fused silica capillary column (14 % cyanopropylphenyl / 86 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)[5]

Carrier gas: He (1.5 mL/min)

Make up gas: N_2 (60 mL/min)

Sample injection[6]: Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 2 min) → ramp 30 °C/min → 140 °C → ramp 3 °C/min → 220 °C (hold 15 min) → ramp 2 °C/min → 250°C

Detector temperature: 300 °C

Gas chromatography (C). Inject 2 µL each of the sample solution AC and the chlorpropham standard solution into the gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame thermionic detector

Column: Fused silica capillary column (100 % dimethylpolysiloxane coating, 0.32 mm in inner diameter, 30 m in length, film thickness 0.25 µm)[7]

Carrier gas: He(1.5 mL/min)

Make up gas: N_2 (30 mL/min)

Hydrogen : 4 mL/min

Dry air : 100 mL/min

Sample injection[6]: Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 2 min) → ramp 30 °C/min → 140 °C → ramp 3 °C/min → 180 °C

Detector temperature: 280 °C

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[8] to prepare a calibration curve and subsequently calculate the amount of the respective pesticides present in the sample.

«Summary of analysis method»

This method is a systematic analysis method for alachlor, allethrin, chlorpropham, dichloran and methoxychlor.

Each agricultural chemical in feeds is extracted with acetone/water, purified by liquid-liquid extraction and synthetic magnesium silicate column and quantified by gas chromatographs equipped with an electron capture detector or a flame thermionic detector respectively.

The flow sheet of the analysis method is shown in Figure 6.2.5-1.

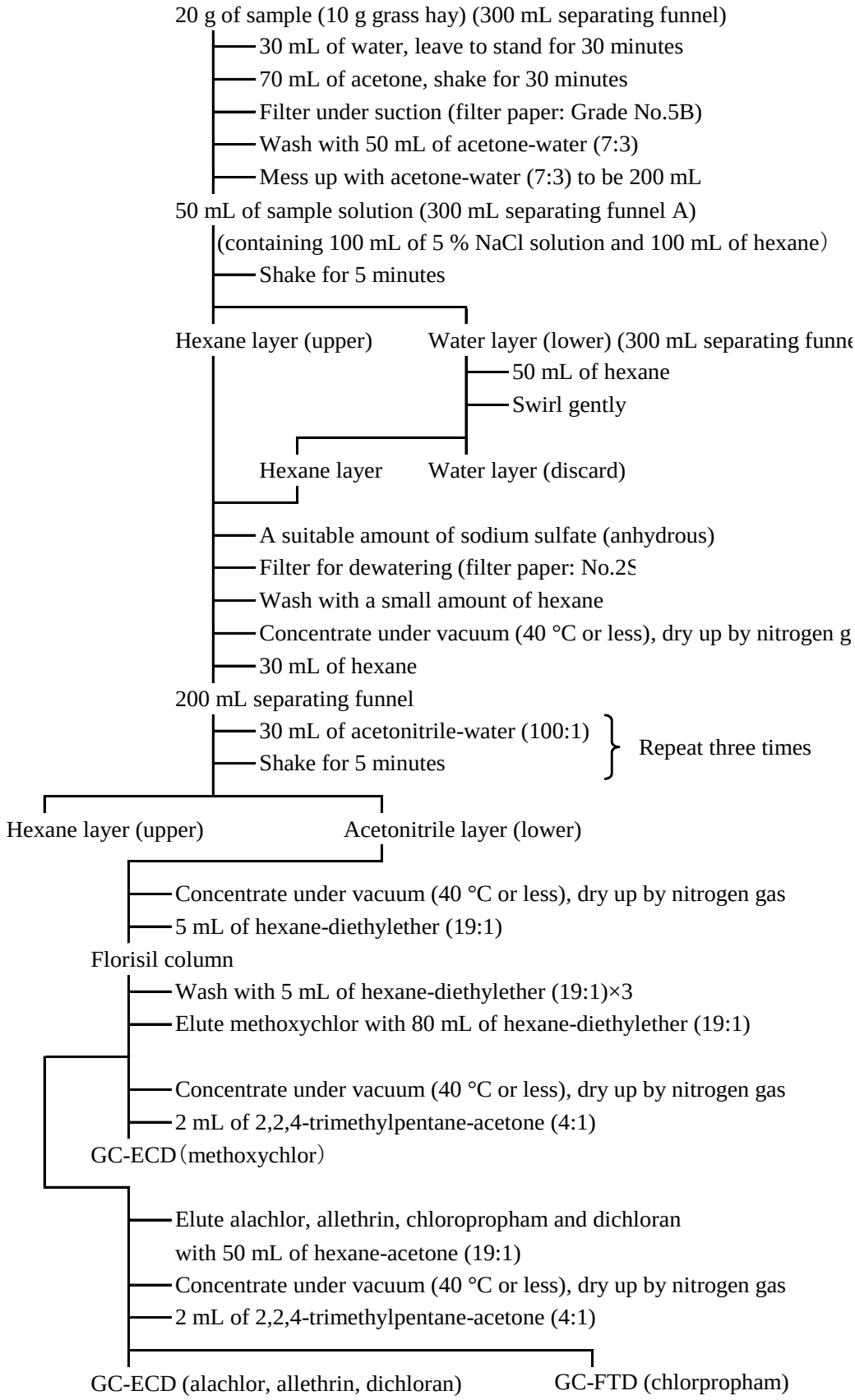


Figure 6.2.5-1. Flow sheet of the systematic analysis method for alachlor, allethrin, chlorpropham, dichloran and methoxychlor

Reference: Norio Saito: Research Report of Animal Feed, 18, 26 (1993).

«Method validation»

• Spike recovery and repeatability

(Group A)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
alachlor	corn	10~100	3	103.3~109.2	9.7
	milo	10~100	3	102.2~109.2	10.3
	adult hen formula feed	10~100	3	103.2~107.0	14.1
allethrin	corn	20~200	3	102.5~110.0	6.0
	milo	20~200	3	104.0~110.0	3.6
	adult hen formula feed	20~200	3	99.3~108.7	8.1
dichloran	corn	20~200	3	95.3~98.7	11.2
	milo	20~200	3	96.8~104.0	12.4
	adult hen formula feed	20~200	3	94.8~102.3	10.0

(Group B)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
methoxychlor	corn	20~200	3	99.0~111.7	10.5
	milo	20~200	3	113.0~114.0	7.6
	adult hen formula feed	20~200	3	103.9~117.7	13.1

(Group C)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
chlorpropham	corn	40~400	3	88.3~97.7	12.8
	milo	40~400	3	93.5~109.4	15.6
	adult hen formula feed	40~400	3	88.8~103.9	9.0

• Collaborative study

(Group A)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
alachlor	adult hen formula feed	5	100	101	3.5	10.9	0.50
allethrin	adult hen formula feed	5	100	93	3.1	12.2	0.56
dichloran	adult hen formula feed	5	100	102	4.2	12.0	0.55

(Group B)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
methoxychlor	adult hen formula feed	5	100	106	4.6	10.7	0.49

(Group C)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
chlorpropham	adult hen formula feed	5	200	94	7.0	13.1	0.64

• Lower limit of quantification: 20 μg/kg in sample for chlorpropham; 10 μg/kg in sample for the other pesticides

«Notes and precautions»

[1] The standard reagents are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers. The gas chromatographic conditions for chlorpropham are different from those for the other agricultural chemicals, therefore, it is required to prepare a standard solution of chlorpropham alone instead of adding it to the mixed standard solution.

[2] Prepare just before use, allow to cool in a desiccators and use thereafter.

[3] Usually, use 10 g of grass hay and 20 g of other samples for analysis.

[4] The flow rate at the column outlet shall be about 1~2 mL/min.

[5] For example, DB-1701 (Agilent Technologies).

[6] Splitless mode is preferred.

[7] For example, DB-1 (Agilent Technologies).

[8] Examples of chromatogram are shown in Figure 6.2.5-2 - 4.

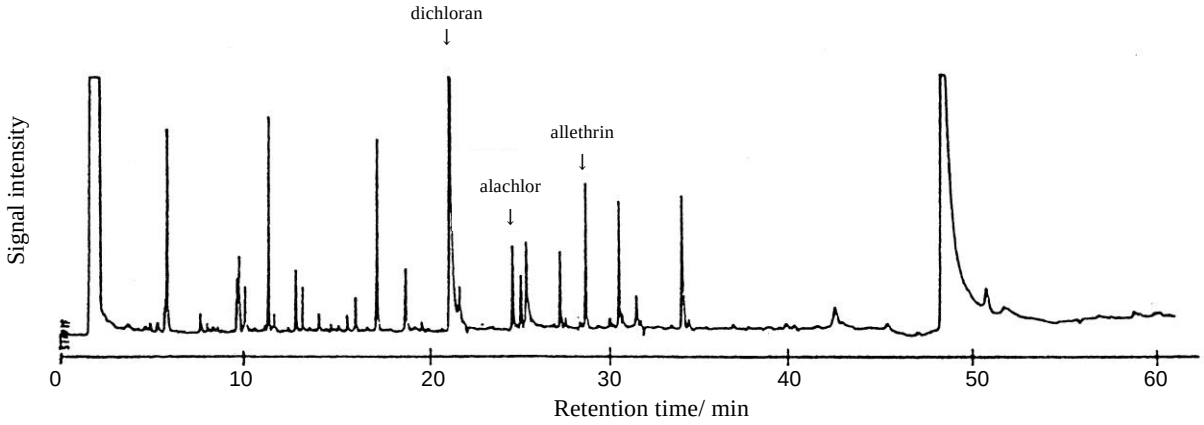


Figure 6.2.5-2. Chromatogram of formula feed spiked with dichloran equivalent to 100 µg/kg, allethrin equivalent to 50 µg/kg and alachlor equivalent to 50 µg/kg

Measurement conditions

Detector: Electron capture detector (ECD)

Column: DB-1701 (0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)

Carrier gas: He (1.6 mL/min, initial flow rate)

Make up gas: N_2 (59 mL/min)

Sample injection: Splitless mode

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 2 min) → ramp 30 °C/min → 140 °C → ramp 3°C/min → 220 °C

Detector temperature: 300 °C

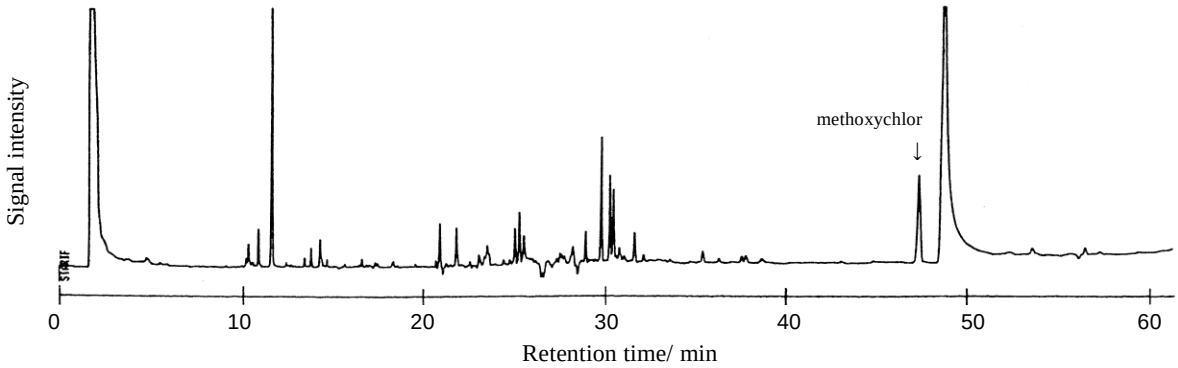


Figure 6.2.5-3. Chromatogram of formula feed spiked with methoxychlor equivalent to 100 µg/kg

For measurement conditions, see Figure 6.2.5-2.

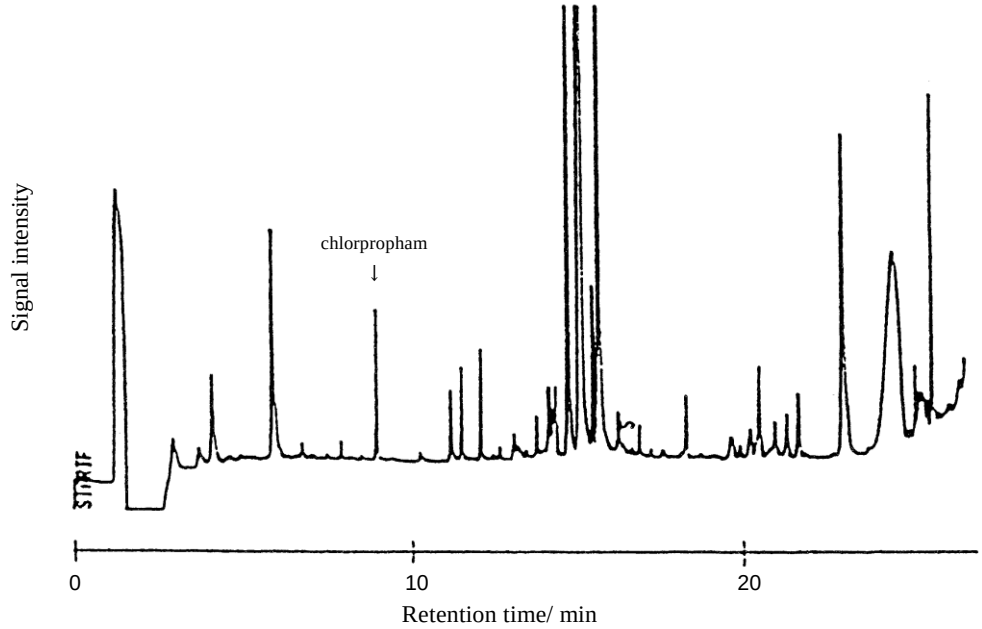


Figure 6.2.5-4. Chromatogram of formula feed spiked with chlorpropham equivalent to 200 µg/kg

Measurement conditions

Detector: Flame thermionic detector

Column: DB-1 (0.32 mm in inner diameter, 30 m in length, film thickness 0.25 µm)

Carrier gas: He (1.7 mL/min, initial flow rate)

Make up gas: N_2 (30 mL/min)

Hydrogen : 3.5 mL/min

Dry air : 100 mL/min

Sample injection: Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 80 °C (hold 2 min) → ramp 30 °C/min → 140 °C → ramp 3 °C/min → 80 °C

Detector temperature: 280 °C

6. Systematic analysis methods for glyphosate, glufosinate and related compounds by gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 2, Article 6]

Target Analytes:

Group A: Glyphosate and 3-(methylphosphinico)propionic acid[1] (2 compounds)

Group B: Glufosinate[1] (1 compound)

A. Reagent Preparation

1) Mixed standard stock solution. Weigh accurately 0.1 g each of glyphosate [$C_3H_8NO_5P$], glufosinate [$C_5H_{15}N_2O_4P$] and 3-(methylphosphinico)propionic acid [$C_4H_9O_4P$]. Transfer each of them to a 100 mL volumetric flask and dissolve by adding water respectively. Further, add water up to the graduation line of the respective flasks (Each 1 mL of these solutions contains 1 mg each of glyphosate, glufosinate and 3-(methylphosphinico)propionic acid.). Then, mix a certain amount of the solutions and dilute the mixture accurately by adding water to prepare mixed standard stock solution containing 100 μg each of glyphosate, glufosinate and 3-(methylphosphinico)propionic acid per 1 mL.

2) Strongly basic anion exchange resin (acetate form). Weigh 100 g of strongly basic anion exchange resin[*1] and transfer to a 500 mL Erlenmeyer flask. Add it 300 mL of water, stir and discard the supernatant. Repeat this procedure twice. Further, add 300 mL of water, remove bubbles, allow the resin to swell and discard the supernatant. Add this resin 200 mL of sodium hydroxide solution (1 mol/L), stir and discard the supernatant. Repeat this procedure twice. Then, add 200 mL of sodium hydroxide solution (1 mol/L), leave to stand overnight and discard the supernatant. Add this resin 300 mL of water, stir and discard the supernatant. Repeat this procedure until the pH of the supernatant decrease to 9 or less. Then, add 200 mL of acetic acid (1+9) solution, stir and discard the supernatant. Repeat this procedure twice. Add 200 mL of acetic acid (1+9) solution, leave to stand overnight and store the resin in this solution.

B. Quantification

Extraction. Weigh 10 g of the sample, transfer it to a stoppered 300 mL Erlenmeyer flask, add 200 mL of water, and extract by shaking for 30 minutes. Transfer the extract to a 50 mL centrifuge tube and centrifuge at 1,500×*g* for 10 minutes. Use the supernatant as a sample solution for column treatment I.

Column treatment I. Pour the strongly basic anion exchange resin (acetate form) into the column (10 mm inner diameter) up to the height of 7 cm from the bottom, add 5 mL of acetic acid (1+9) solution and elute so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Pack the upper end of the column packing material with glass wool loosely, add 100 mL of water and elute so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Repeat this procedure to prepare the column.

Load 25 mL of the sample solution accurately on the column and elute[*2] so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Further, add 10 mL of water and elute in the similar way.

Place a 200 mL recovery flask under the column and pour 100 mL of acetic acid (1+1) solution to the column. Elute[*2] glyphosate, glufosinate and 3-(methylphosphinico)propionic acid so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material and use the eluate as a

sample solution to be subjected to derivatization.

Derivatization. Concentrate the sample solution under reduced pressure[*3] in a water bath at 50 °C or lower to almost dryness, and then dry up by nitrogen gas flow. Dissolve the residue by adding 1 mL of acetic acid and 4 mL of trimethyl orthoacetate accurately[*4]. Put an airtight stopper on the container, heat at 100 °C for 2 hours[*5] and allow to cool. Concentrate the content under reduced pressure in a water bath at 50 °C or lower to almost dryness, and then dry up by nitrogen gas flow.

Dissolve the residue by adding 5 mL of ethyl acetate accurately[*4]. Transfer the solution to a 10 mL centrifuge tube and centrifuge at 650×*g* for 5 minutes. Use the supernatant as a sample solution for column treatment II.

Column treatment II. Connect a silica gel minicolumn (690 mg) (A) to the bottom of an aminopropylsilylated silica gel minicolumn (360 mg) and wash with 10 mL of ethyl acetate.

Load 4 mL of the sample solution accurately on the minicolumn and elute so that the liquid level reaches the upper end of the column packing material. Add 10 mL of ethyl acetate to the minicolumn and repeat the procedure twice.

Disconnect the aminopropylsilylated silica gel minicolumn and place a 50 mL recovery flask under the silica gel minicolumn (A).

Add 10 mL of acetone – water (19 : 1) to the silica gel minicolumn (A) and elute derivatives of glyphosate and 3-(methylphosphinico)propionic acid so that the liquid level reaches the upper end of the column packing material. Concentrate the eluate under reduced pressure in a water bath at 50 °C or lower to almost dryness, and then dry up by nitrogen gas flow.

Dissolve the residue by adding 1 mL of ethyl acetate accurately[*4]. Transfer the solution to a plastic[*6] centrifuge tube (capacity: 1.5 mL) and centrifuge at 5,000×*g* for 5 minutes. Use the supernatant as a sample solution (A) for gas chromatography.

The aminopropylsilylated silica gel minicolumn above is to be subjected to column treatment III.

Column treatment III. Connect a silica gel minicolumn (690 mg) (B) washed with 10 mL of ethyl acetate to the bottom of an aminopropylsilylated silica gel minicolumn used for column treatment II.

Add 10 mL of acetone to the minicolumn and elute so that the liquid level reaches the upper end of the column packing material to transfer the glufosinate derivative to the silica gel minicolumn (B).

Disconnect the aminopropylsilylated silica gel minicolumn, add 10 mL of acetone to the silica gel minicolumn (B), elute so that the liquid level reaches the upper end of the column packing material and wash the silica gel minicolumn (B). Place a 50 mL recovery flask under the silica gel minicolumn (B). Add 10 mL of acetone – water (19 : 1) to the minicolumn to elute glufosinate derivative. Concentrate the eluate under reduced pressure in a water bath at 50 °C or lower to almost dryness, and then dry up by nitrogen gas flow.

Dissolve the residue by adding 1 mL of ethyl acetate accurately[*4]. Transfer the solution to a plastic[*6] centrifuge tube (capacity: 1.5 mL) and centrifuge at 5,000×*g* for 5 minutes. Use the supernatant as a sample solution (B) for gas chromatography.

Derivatization of the standard stock solution. Transfer 200 µL of the mixed standard stock solution to a 200 mL recovery flask and dry up by the flow of nitrogen gas. Dissolve the residue by adding 1 mL of

acetic acid and 4 mL of trimethyl orthoacetate accurately. Put an airtight stopper on the recovery flask, heat at 100 °C for 2 hours[*5] and allow to cool. Concentrate the solution under reduced pressure in a water bath at 50 °C or lower to almost dryness, and then dry up by nitrogen gas flow.

Dissolve the residue by adding of ethyl acetate. Further, dilute with the same solvent to prepare standard solution containing 0.05 – 2μg each of glyphosate, glufosinate and 3-(methylphosphinico)-propionic acid per 1 mL.

Gas chromatography. Inject 2 μL each of the sample solution (A), the sample solution (B) and the respective standard solutions[*7] to obtain chromatograms.

Example of measurement conditions

Detector: Flame photometric detector (Filter for phosphorus detection)

Column: Fused silica capillary column (polyethylene glycol (average molecular weight 1,500) / diepoxide coating, 0.32 mm in inner diameter, 15 m in length, film thickness 0.25 μm)[2]

Carrier gas: He(8.4 mL/min)

Make up gas: N_2(30 mL/min)

Hydrogen : 75 mL/min

Dry air : 100 mL/min

Sample injection: Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: 100 °C (hold 1 min) → ramp 20 °C/min → 250 °C (hold 5 min)

Detector temperature: 275 °C

Calculation. Obtain the peak height of the glyphosate derivative from the resulting chromatograms[3] to prepare a calibration curve and calculate the amount of glyphosate present in the sample.

Similarly, obtain each peak height of the derivative of glufosinate and 3-(methylphosphinico)propionic acid to prepare respective calibration curve and calculate their respective amount in the sample. Then, calculate the amount of glufosinate in the sample using the following equation.

The amount of glufosinate in the sample (μg/kg) $= A + B \times 1.3$

A : the concentration (μg/kg) of glufosinate obtained from the calibration curve

B : the concentration (μg/kg) of 3-(methylphosphinico)propionic acid obtained from the calibration curve

* 1. Muromac 1×2 Cl^-(100 – 50 mesh) (Murotec) or equivalents.

2. The flow rate shall be 3 - 5 mL/min.

3. Because of its susceptibility to bumping, reduce the pressure at first gently, and then incrementally.

4. If necessary, the residue shall be subjected to supersonic treatment for enough diffusion.

5. Heat in a desiccators and the like. When a desiccators is used, provide adequate ventilation.

6. Chemically resistant to ethyl acetate.

7. Use a silanized inlet insert without glass wool.

«Summary of analysis method»

This method is a systematic analysis method for glyphosate, glufosinate and 3-(methylphosphinico)propionic acid (MPPA).

In this method, each agricultural chemical in feeds is extracted with water, purified by an anion exchange resin column, derivatizated, further purified by an aminopropylsilylated silica gel minicolumn as well as a silica gel minicolumn, and quantified by a gas chromatograph equipped with a flame photometric detector (filter for phosphorus detection).

The flow sheet of the analysis method is shown in Figure 6.2.6-1.

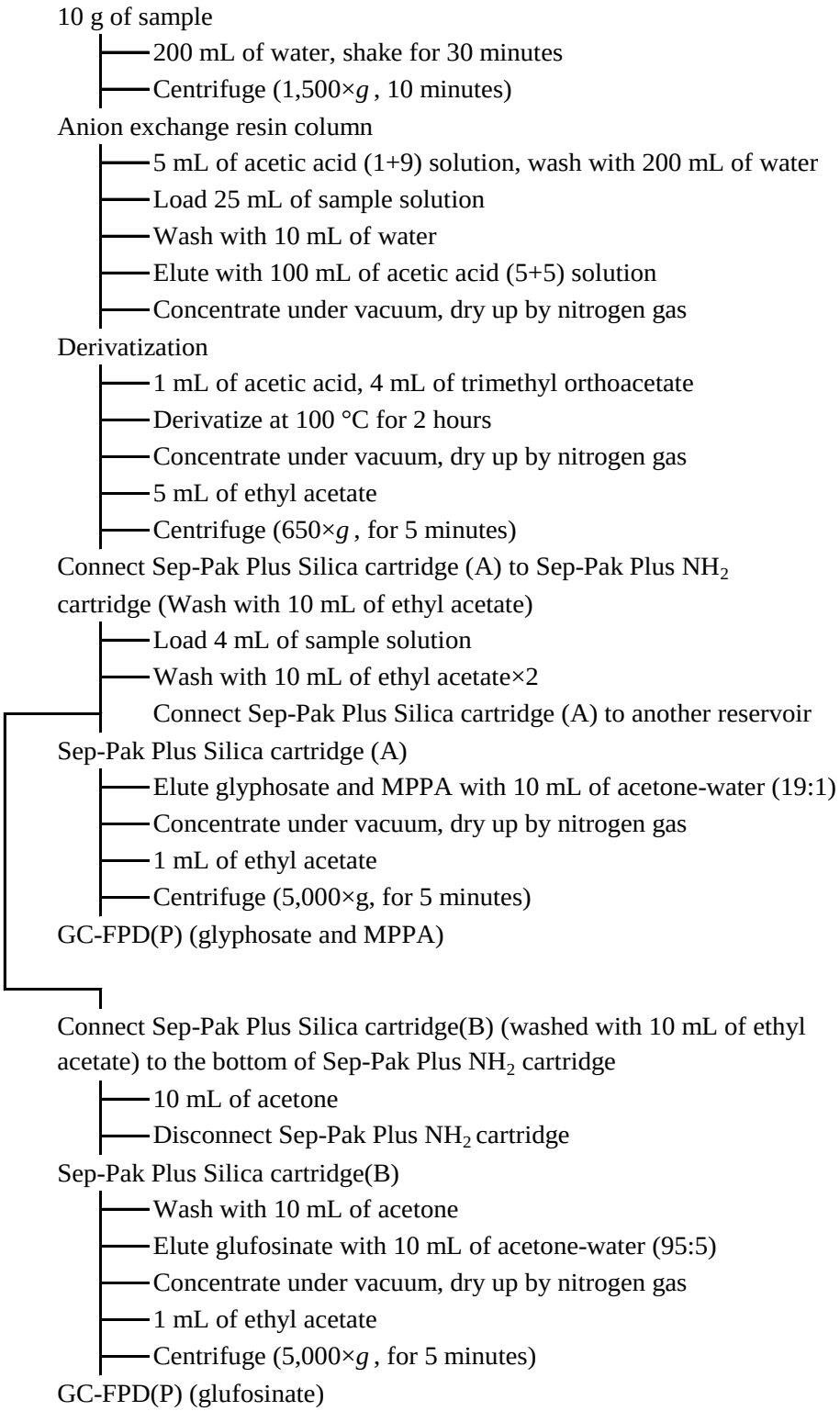


Figure 6.2.6-1. Flow sheet of the systematic analysis method for glyphosate, glufosinate and 3-(methylphosphinico)propionic acid

Reference: Yuji Shirai, Yuzo Ono, Koji Fujihara, Koji Aoyama : Research Report of Animal Feed, 27, 13 (2002).

«Method validation»

• Spike recovery and repeatability

(Group A)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Glyphosate	corn	100~1,000	3	85.0~91.7	10.6
	soybean meal	100~1,000	3	85.6~97.6	12.4
	adult hen formula feed	100~1,000	3	86.9~106.6	4.2
	finishing pig formula feed	100~1,000	3	93.1~94.6	10.4
3-(Methylphosphinico) propionic acid	corn	100~1,000	3	91.0~93.9	3.0
	soybean meal	100~1,000	3	96.9~99.8	3.3
	adult hen formula feed	100~1,000	3	95.6~99.6	8.5
	finishing pig formula feed	100~1,000	3	92.2~97.7	5.2

(Group B)

Component	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
Glufosinate	corn	100~1,000	3	85.8~90.4	12.4
	soybean meal	100~1,000	3	80.8~81.9	4.5
	adult hen formula feed	100~1,000	3	92.1~103.8	3.2
	finishing pig formula feed	100~1,000	3	93.5~100.3	4.5

• Collaborative study

(Group A)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%) (measured value(μg/kg))	intra-laboratory repeatability RSD_r (%)	inter-laboratory reproducibility RSD_R (%)	HorRat
Glyphosate	milo	6	mination	(977)	8.5	11.0	0.69
	finishing pig formula feed	6	500	94.2	9.2	10.4	0.58
3-(Methylphosphinico)propionic acid	finishing pig formula feed	6	500	99.7	6.4	10.5	0.59

(Group B)

Component	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
Glufosinate	finishing pig formula feed	6	500	103.0	7.4	14.0	0.79

• Lower limit of quantification: 20 μg/kg each in sample

«Notes and precautions»

[1] As to glufosinate, glufosinate and its metabolite, 3-(methylphosphonico)propionic acid are divided into group A and group B.

[2] For example, SUPELCOWAX 10 (Supelco).

[3] Examples of chromatogram are shown in Figure 6.2.6-2.

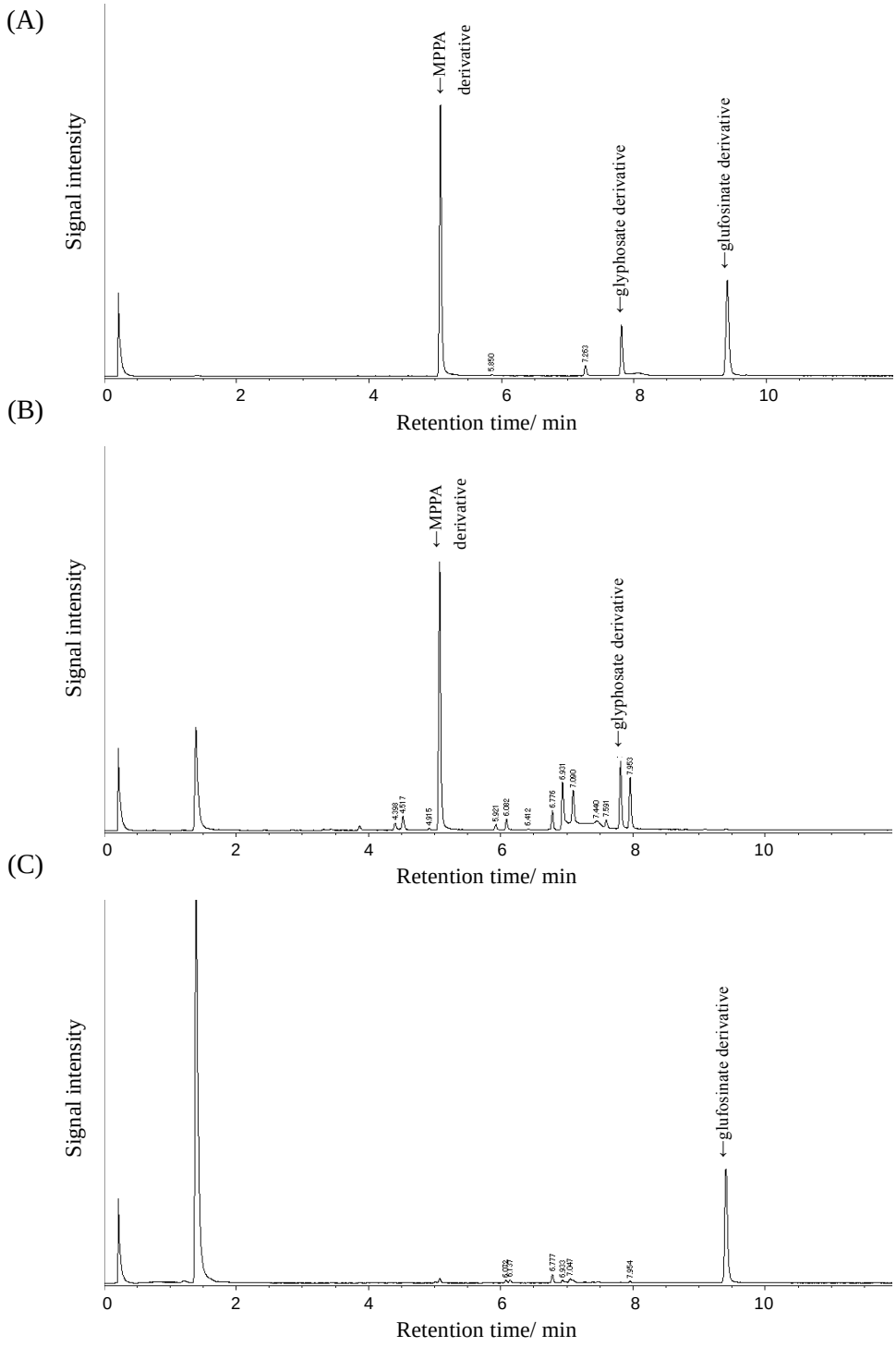


Figure 6.2.6-2. Chromatogram of adult hen formula feed spiked with glyphosate, glufosinate and MPPA equivalent to 1 mg/kg each

(A) Mixed standard solution (as glyphosate, glufosinate and MPPA equivalent to 2 ng each)

(B) Sample solution (group A)

(C) Sample solution (group B)

Section 3. Simultaneous Analysis Methods for Agricultural Chemicals

1. Simultaneous analysis method of agricultural chemicals by gas chromatograph-mass spectrometer [Analytical Standards of Feeds, Chapter 6, Section 3, Article 1]

Target Analytes[*1]: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o*,*p*'-DDD, *p*,*p*'-DDD, *o*,*p*'-DDE, *p*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDT, EPN, acetochlor, atrazine, anilofos, ametryn, alachlor, allidochlor, aldrin, allethrin, isazophos, isofenphos, isoprothiolane, iprobenfos, ethalfluralin, ethion, edifenphos, etofenprox, ethofumesate, ethoprophos, etridiazole, etrimphos, endrin, oxadiazon, oxychlordane, cadusafos, carfentrazone-ethyl, quintozene, kresoxim-methyl, chlorthal-dimethyl, *cis*-chlordane, *trans*-chlordane, chlorpyrifos, chlorpyrifos-methyl, chlorfenapyr, chlorfenvinphos (*E*-isomer), chlorfenvinphos (*Z*-isomer), chlorpropham, chlorobenzilate, diclofop-methyl, dichloran, cyhalothrin, diphenamid, difenoconazole, dimethenamid, dimethoate, dimepiperate, silafluofen, diazinon, terbacil, thiobencarb, dieldrin, tecnazene, tetrachlorvinphos, tetraconazole, tetradifon, tebuconazole, tebufenpyrad, tefluthrin, deltamethrin, terbutryn, terbufos, tralomethrin[*2], triadimefon, tri-allate, trifluralin, trifloxystrobin, tolylfluanid, napropamide, parathion, parathion-methyl, halfenprox, bifenthrin, piperophos, pyridaphenthion, pyridaben, pyriproxyfen, pirimiphos-methyl, vinclozolin, fipronil, fenarimol, fenitrothion, fenothiocarb, phenothrin, fenthion, phenthoate, fenvalerate, fenbuconazole, fenpropathrin, butamifos, flamprop-methyl, fulcythrinate, flutolanil, flutriafol, fluvalinate, flumioxazin, flumiclorac-pentyl, procymidone, propachlor, propazine, propanil, propargite, propiconazole, propham, profenofos, propetamphos, bromobutide, bromopropylate, bromophos, hexaconazole, benoxacor, heptachlor, heptachlor epoxide, *cis*-permethrin, *trans*-permethrin, penconazole, pendimethalin, benfluralin, phosalone, fosthiazate, phosmet, phorate, malathion, methacrifos, methidathion, methoxychlor, metominostorbin (*E*-isomer), metolachlor and mevinphos (138 compounds).

A. Reagent Preparation

Mixed standard solution[1]. Weigh accurately 25 mg each of the respective agricultural chmeical standards. Transfer each of them to a 50 mL brown volumetric flask and dissolve by adding 10 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks to prepare the standard stock solutions of respective agricultural chemicals (1 mL each of these solutions contains 0.5 mg respectively as each agricultural chemical.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare the mixed standard solutions containing 0.02 – 0.5 μg each of the respective agricultural chemicals per 1 mL.

Separately, add fipronil to the mixed standard solutions above to prepare the fipronil standard solutions containing 0.005 – 0.01 μg as fipronil per 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of the sample (as for grass hay, accurately 5 g), transfer it to a stoppered 200 mL Erlenmeyer flask, add 15 mL of water and leave to stand for 30 minutes. Then, add it 100 mL of acetonitrile and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask

and the residue with 50 mL of acetonitrile sequentially, and filter the wash by suction in the similar way.

Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to be about 15 mL[2] and use it as a sample solution for column treatment I.

Column treatment I. Load the sample solution on the porous-diatomite column (for 20 mL retention). Wash the recovery flask that had contained the sample solution with 5 mL of water, add the wash to the column and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that had contained the sample solution with 20 mL each of ethyl acetate – hexane (1 : 1) three times, add the wash to the column in order of precedence and elute respective agricultural chemicals to be quantified by natural flow until the liquid level reaches the upper end of the column packing material[3]. Further, add 40 mL of the same solvent to the column to elute in the similar way and add 1 mL of acetone – diethylene glycol (49 : 1) to the eluate. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding accurately weighed 10 mL of cyclohexane – acetone (4 : 1)[4], transfer the solution to a 10 mL centrifuge tube and centrifuge by 1,000×*g* for 5 minutes. Filter the supernatant through a membrane filter (pore size: 0.5 μm or less) and use the filtrate as a sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph. Dispense each elution fraction into a 200 mL recovery flask. Add a drop of acetone – diethylene glycol (49 : 1) to the eluate, concentrate it under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 2 mL of ethyl acetate and use this solution as a sample solution for column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 300 mm in length, particle size 15 μm)

Guard column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 100 mm in length, particle size 15 μm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 60-150 mL[5]

Column treatment II. Wash a graphite carbon/aminopropylsilanized silica gel layered minicolumn (500 mg/500 mg)[*3] with 10 mL of ethyl acetate[6].

Place a 25 mL pear-shape flask[7] under the minicolumn and load the sample solution on the minicolumn. Wash the recovery flask which had contained the sample solution twice with 2 mL each of ethyl acetate, add the wash to the minicolumn in order of precedence and elute respective agricultural chemicals by natural flow until the liquid level reaches the upper end of the column packing material. Add 4 mL of ethyl acetate to the minicolumn and elute in the similar way. Add a drop of acetone/diethylene glycol (49 : 1) to the eluate, concentrate under reduced pressure on a water bath at

40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding exactly 10 mL (5 mL for grass hay) of hexane – acetone (7 : 3) and use this solution as a sample solution for column treatment III.

Column treatment III. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL each of acetone and hexane sequentially.

Place a 25 mL recovery flask under the minicolumn and load accurately 4 mL of the sample solution on the minicolumn. Elute respective agricultural chemicals at a flow rate 1 - 2 mL/min until the liquid level reaches the upper end of the column packing material[8]. Further, add 6 mL of hexane – acetone (7 : 3) to the minicolumn elute in the similar way and add a drop of acetone – diethylene glycol (49 : 1) to the eluate. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding exactly 2 mL of 2,2,4-trimethylpentane – acetone (4 : 1) and use this solution as a sample solution for measurement by gas chromatograph-mass spectrometer.

Measurement by gas chromatograph-mass spectrometer. Inject 1 µL each of the sample solution and respective mixed standard solution to a gas chromatograph-mass spectrometer to obtain selected ion monitoring chromatograms.

Example of measurement conditions[9]

Column: Fused silice capillary column (5 % diphenyl/ 95 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)

Carrier gas: He (1.0 mL/min, initial flow rate)

Sample injection: Splitless mode (60 s)

Injection port temperature: 280 °C

Column oven temperature: Initial temperature 70 °C (hold 1 min) → ramp 25 °C/min → 150 °C → ramp 3 °C/min → 200 °C → ramp 8 °C/min → 280 °C (hold 10 min)

Detector: Quadrupole mass spectrometer*4

Interface temperature: 250 °C

Ion source temperature: 230 °C

Ionizing voltage: 70 eV

Ionization method: Electron ionization (EI)

Monitor ion: See Table 1[10].

Table 1 Monitor ions for respective agricultural chemicals

Name	Quantification ion	Qualifier ion
α-BHC	181	219
β-BHC	181	219
γ-BHC	181	219
δ-BHC	181	219
o,*p*′-DDD	235	237
p,*p*′-DDD	235	237
o,*p*′-DDE	246	318
p,*p*′-DDE	246	318
o,*p*′-DDT	235	237
p,*p*′-DDT	235	237
EPN	157	169
acetochlor	162	223
atrazine	200	215
anilofos	226	184
ametryn	227	212
alachlor	160	188
allidochlor	132	138
aldrin	263	265
allethrin	123	136
isazophos	161	119
isofenphos	213	255
isoprothiolane	118	162
iprobenfos	204	91
ethalfluralin	276	316
ethion	231	384
edifenphos	173	109
etofenprox	163	183
ethofumesate	161	286
etnoprophos	158	200
etridiazole	211	213
etrimphos	292	181
endrin	263	281
oxadiazon	175	258
oxychlordane	115	387
cadusafos	159	158
carfentrazone-ethyl	312	340
quintozene	237	295
kresoxim-methyl	116	206
chlorthal-dimethyl	301	332
cis-chlordane	375	373
trans-chlordane	375	373
chlorpyrifos	199	314
chlorptrifos-methyl	286	288
chlorfenapyr	59	247
chlorfenviphos (*E*-isomer)	267	323

Name	Quantification ion	Qualifier ion
chlorfenvifos (*Z*-isomer)	267	323
terbutryn	226	241
terbufos	231	288
triadimefon	208	181
triallate	268	86
trifluralin	306	264
trifloxystrobin	116	131
tolylfluanid	238	137
napropamide	128	271
parathion	291	109
parathion methyl	263	246
halfenprox	263	265
bifenthrin	181	166
piperophos	122	320
pyridaphenthion	340	199
pyridaben	147	364
pyriproxyfen	136	96
pirimiphos-methyl	290	305
vinclozolin	212	285
fipronil	367	213
fenarimol	219	330
fenitrothion	277	260
fenothiocarb	160	72
phenothrin	123	183
fenthion	278	125
phenthoate	274	246
fenvalerate	125	167
fenbuconazole	129	198
fenpropathrin	97	181
butamifos	286	200
flamprop-methyl	105	77
flucythrinate	199	157
flutolanil	173	281
flutriafol	164	123
fluvalinate	250	252
flumioxazin	354	287
flumiclorac pentyl	423	308
procymidone	283	285
propachlor	120	176
propanil	161	163
propargite	135	173
propazine	214	229
propiconazole	173	259
propham	179	137
profenofos	208	337

Name	Quantification ion	Qualifier ion	Name	Quantification ion	Qualifier ion
propetamphos	138	194	deltamethrin	181	253
bromobutide	119	232	bromopropylate	341	183
chlorpropham	127	213	bromophos	331	125
chlorobenzilate	251	139	hexaconazole	214	83
diclofop-methyl	253	340	benoxacor	120	259
dicloran	176	206	heptachlor	272	237
cyhalothrin	197	208	heptachlor epoxide	353	237
diphenamid	167	239	*cis* -permethrin	183	163
difenoconazole	265	323	*trans* -permethrin	183	163
dimethenamid	154	230	penconazole	159	248
dimethoate	125	229	pendimethalin	252	281
dimepiperate	119	145	benfluralin	292	264
silafluofen	179	286	phosalone	182	121
diazinon	179	304	fosthiazate	195	104
terbacil	161	160	phosmet	160	317
thiobencarb	100	257	phorate	75	121
dieldrin	263	277	malathion	173	125
tecnazene	203	261	methacrifos	180	240
tetrachlorvinphos	331	329	methidathion	145	85
tetraconazole	336	159	methoxychlor	227	274
tetradifon	227	356	metominostrobin (*E* -isomer)	196	238
tebuconazole	250	125	metolachlor	162	238
tebufenpyrad	318	333	mevinphos	127	192
tefluthrin	177	197			

Calculation. Obtain the peak height from the resulting chromatograms [11] to prepare a calibration curve and subsequently calculate the amount of the respective agricultural chemicals present in the sample[12][13].

* 1. This method does not ensure a simultaneous analysis of all the compounds above. Because of the possibilities of decomposition or measurement interference caused by interaction among compounds, it is required to verify these points by combinations of the Target Analytes beforehand.

2. When injected to a gas chromatograph mass spectrometer, tralomethrin is converted to deltamethrin, so that it should be quantified as the content of deltamethrin.

3. For example, ENVI-Carb/LC-NH_2 (Supelco) or equivalents.

4. Example of measurement conditions for GCMS-QP2010 (Shimadzu Corporation).

«Summary of analysis method»

This method is a simultaneous analysis method for pesticides (138 compounds).

Each pesticide in feeds is extracted with acetonitrile/water, purified by a porous-diatomite column, a GPC, a graphite carbon/aminopropylsilanized silica gel layered minicolumn and a Florisil minicolumn and quantified by a gas chromatograph mass spectrometer.

The flow sheet of the analysis method is shown in Figure 6.3.1-1.

Sample 10 g (grass hay 5 g)
- Water 15 mL, leave to stand for 30 minutes
- Acetonitrile 100 mL, shake for 30 minutes
- Filter under suction (No.5B)
- Wash with 50 mL of acetonitrile
- Concentrate under vacuum (40 °C or less, to 15 mL or less)

Chem Elut cartridge (for 20 mL retention)
- Load sample solution, wash with 5 mL of water, leave to stand for 5 minutes)
- Elute with 100 mL of hexane/ethyl acetate (1:1)
- Acetone/diethylene glycol (49:1) 1 mL
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- cyclohexane/acetone (4:1) 10 mL
- Membrane filter (0.5 µm or less)

GPC (Load 5 mL of sample solution)
- Elution fraction 60~150 mL
- Acetone/diethylene glycol (49:1) a drop
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- Ethyl acetate 2 mL

ENVI-Carb/NH2cartridge (Washed by 10 mL of ethyl acetate)
- Load sample solution
- Ethyl acetate 8 mL
- Acetone/diethylene glycol (49:1) a drop
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- Hexane/acetone (7:3) 10 mL (grass hay 5 mL)

Sep-Pak Plus Florisil cartridge (Washed by 5 mL each of acetone and hexane)
- Load 4 mL of sample solution
- Hexane/acetone (7:3) 6 mL
- Acetone/diethylene glycol (49:1) a drop
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- 2,2,4-trimethylpentane/acetone (4:1) 2 mL

GC-MS

Figure 6.3.1-1. Flow sheet of the simultaneous analysis method for agricultural chemicals

Reference: Tomoharu Nozaki, Asuka Horigome, Chie Watanabe: Research Report of Animal Feed, 31, 39 (2006).

Tomoharu Nozaki: Research Report of Animal Feed, 32, 108 (2007).

Mitsunori Yakata: Research Report of Animal Feed, 32, 147 (2007).

«Method validation»

• Spike recovery and repeatability

1) Tralomethrin

Sample type	Spike concentration (µg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or
corn	120~1,200	3	99.0~115.1	16.0
ryegrass	600~6,000	3	101.5~112.8	14.9

2) The other agricultural chemicals

Spike concentration Equivalent to 50 µg/kg, 100 µg/kg and 500 µg/kg

Results are shown in Table 6.3.1-1 and 6.3.1-2.

• Collaborative study

1) Tralomethrin

Sample ty	No. (Spike conc	Spike recovery	laboratory	laboratory	HorRa
	labs	(µg/kg)	(%)	RSDr (%)	RSDR (%)	
corn	8	76	89.1	18.6	31.1	1.41
alfalfa	8	76	119.3	6.8	38.2	1.74

2) The other agricultural chemicals

Spike concentration Equivalent to 100 µg/kg

Results are shown in Table 6.3.1-3 and 6.3.1-4.

• Lower limit of quantification: 10 µg/kg for fipronil; 100 µg/kg for deltamethrin and tralomethrin (grass hay 150 µg/kg); 50 µg/kg each for the other pesticides (spike recovery and relative standard deviation)

• Lower limit of detection: 3 µg/kg in sample for fipronil; 30 µg/kg for deltamethrin and tralomethrin (grass hay 50 µg/kg); 20 µg/kg each for the other pesticides

Table 6.3.1-1. Results of recovery test (formula feed for layer, repeated 3 times each)

component	spike concentration					
	50 µg/kg		100 µg/kg		500 µg/kg	
	recovery (%)	repeatability RSD (%)	recovery (%)	repeatability RSD (%)	recovery (%)	repeatability RSD (%)
α-BHC	100.2	8.4	89.1	7.3	107.6	7.9
β-BHC	100.6	10.9	88.0	6.7	104.0	10.3
γ-BHC	122.2	10.0	96.6	6.3	103.6	10.2
δ-BHC	103.6	11.8	90.6	6.6	102.6	11.0
o,*p*′-DDD	105.2	6.8	97.5	6.6	104.7	14.6
p,*p*′-DDD	111.4	7.7	103.2	6.8	111.3	13.0
o,*p*′-DDE	104.4	6.9	94.7	6.2	96.6	12.1
p,*p*′-DDE	102.4	6.6	97.5	6.2	98.0	10.6
o,*p*′-DDT	101.0	8.3	91.5	6.7	116.5	13.7
p,*p*′-DDT	116.4	2.9	92.7	8.1	121.8	16.8
EPN	70.4	4.8	98.3	9.4	170.2	19.3
acetochlor	93.0	14.2	100.2	6.7	118.9	10.5
atrazine	105.6	5.9	88.9	11.9	115.6	8.3
anilofos	128.4	7.6	115.6	7.5	158.6	14.8
ametryn	105.2	8.4	70.8	14.1	*30.5*	*25.8*
alachlor	98.8	8.5	96.9	9.6	110.7	11.3
allidochlor	114.8	13.1	99.9	6.0	114.2	7.6
aldrin	98.0	6.3	84.1	3.6	103.2	12.9
allethrin	97.2	8.7	99.6	7.4	109.6	11.7
isazophos	98.4	7.3	126.2	7.9	118.7	7.5
isofenphos	80.6	6.2	111.5	6.2	107.7	12.1
isoprothiolane	104.4	7.7	102.4	6.8	118.5	7.7

Table 6.3.1-1. Results of recovery test (formula feed for layer, repeated 3 times each) [continued]

component	spike concentration 50 µg/kg recovery (%)	50 µg/kg repeatability RSD (%)	100 µg/kg recovery (%)	100 µg/kg repeatability RSD (%)	500 µg/kg recovery (%)	500 µg/kg repeatability RSD (%)
iprobenfos	124.0	8.1	123.3	7.9	132.7	7.9
ethalfluralin	92.0	7.6	100.5	5.5	138.6	13.0
ethion	107.6	8.6	106.2	8.2	161.9	17.2
edifenphos	168.0	6.3	126.0	8.4	155.1	11.3
etfenprox	111.4	7.7	105.1	8.8	114.0	12.6
ethofumesate	105.0	7.6	113.7	13.6	99.2	5.6
ethprophos	105.0	7.8	104.3	5.8	125.3	6.5
etridiazole	93.4	13.3	91.5	10.3	125.2	9.4
etrimphos	96.6	7.0	96.7	8.0	114.0	8.2
endrin	119.8	5.3	101.5	8.7	129.8	11.3
oxadiazon	100.6	8.7	97.6	6.3	108.0	14.9
oxychlordane	115.6	7.1	73.3	9.0		
cadusafos	115.8	3.3	105.9	5.9	127.3	7.0
carfentrazone-ethyl	107.2	1.7	105.5	8.1	122.0	11.5
quintozene	83.2	6.7	*49.3*	*6.3*	103.4	19.3
kresoxim-methyl	104.4	9.0	103.8	7.2	126.9	13.7
chlorthal-dimethyl	101.0	8.9	93.2	7.4	108.3	11.9
cis -chlordane	99.4	9.7	71.3	9.0		
trans -chlordane	99.2	9.7	69.9	8.6		
chlorpyrifos	84.6	9.0	83.5	11.7	103.5	10.1
chlorpyrifos-methyl	101.6	9.1	85.4	11.2	111.9	14.1
chlorfenapyr	122.2	9.5	99.6	7.7	111.8	14.7
chlorfenviphos (*E* -isomer)	116.8	7.4	96.7	6.9	134.0	12.1
chlorfenviphos (*Z*- isomer)	123.8	6.9	98.8	8.6	134.8	12.6
chlorpropham	154.2	8.9	116.4	11.2	122.0	7.1
chlorbenzilate	114.4	7.9	108.3	6.5	119.9	12.0
diclofop-methyl	113.2	5.7	103.2	7.1	115.8	11.6
dicloran	137.4	13.2	89.3	15.1	132.4	9.4
cyhalothrin	*217.8*	*68.6*	130.6	8.1	156.5	18.9
diphenamid	95.4	9.0	99.2	7.1	116.2	11.8
difenoconazole	162.4	5.9	119.4	15.2	*138.7*	*23.8*
dimethenamid	102.0	8.2	96.6	9.2	105.8	6.8
dimethoate	105.6	15.3	105.3	15.5	122.3	8.2
dimepiperate	140.8	8.8	117.2	7.8	137.9	10.8
silafluofen	128.4	12.8	107.3	7.4	115.1	8.6
diazinon	109.4	10.1	104.2	8.1	120.2	4.1
terbacil	194.4	8.7	171.3	10.6	155.3	8.9
thiobencarb	111.6	8.4	97.0	7.3	115.4	9.6
dieldrin	100.6	5.4	93.0	5.6	104.2	11.8
tecnazene	101.6	8.1	92.8	10.9	121.2	8.6
tetrachlorvinphos	121.4	8.1	106.8	8.1	129.1	13.4
tetraconazole	96.8	10.7	103.7	7.3	116.5	19.7
tetradifon	99.8	7.4	102.1	8.3	113.5	11.0
tebuconazole	117.8	8.0	95.7	8.7	*126.4*	*22.6*
tebufenpyrad	115.6	6.9	110.4	7.6	115.7	15.5

Table 6.3.1-1. Results of recovery test (formula feed for layer, repeated 3 times each) [continued]

component	spike concentration 50 µg/kg recovery (%)	50 µg/kg repeatability RSD (%)	100 µg/kg recovery (%)	100 µg/kg repeatability RSD (%)	500 µg/kg recovery (%)	500 µg/kg repeatability RSD (%)
tefluthrin	107.6	8.2	103.7	9.3	108.1	10.2
deltamethrin	102.6	8.0	97.9	8.2	148.5	16.8
terbutryn	107.6	9.7	96.3	10.0	-	-
terbufos	94.0	5.7	93.1	8.1	113.6	7.1
triadimefon	104.6	8.0	97.7	8.9	125.0	11.3
triallate	100.4	7.2	102.2	9.9	110.5	11.6
trifluralin	111.4	7.5	107.1	11.1	178.4	14.0
trifloxystrobin	102.8	8.4	107.6	6.5	133.6	9.1
tolylfluanid	67.4	6.2	99.1	5.2	128.9	14.8
napropamide	126.6	2.8	96.5	9.6	129.4	7.9
parathion	114.0	6.1	108.2	11.2	*214.8*	*15.7*
parathion-methyl	106.4	4.3	111.6	8.5	160.9	11.4
halfenprox	106.0	6.4	104.8	9.2	165.5	15.4
bifenthrin	112.6	7.1	101.8	7.2	112.8	15.6
piperophos	146.4	5.6	110.1	7.1	*213.4*	*13.5*
pyridaphenthion	121.0	7.9	110.3	6.6	152.6	14.1
pyridaben	119.6	7.4	105.1	7.9	128.0	15.8
pyriproxyfen	124.8	7.2	102.1	8.9	117.3	16.7
pirimiphos-methyl	112.0	9.3	105.7	9.8	130.3	8.4
vinclozolin	96.6	5.4	98.9	8.9	109.3	10.2
fipronil	86.4	8.3	85.8	7.1	110.7	12.7
fenarimol	119.2	8.4	110.3	7.1	121.9	15.3
fenitrothion	97.0	8.9	105.8	7.8	175.2	16.3
fenothiocarb	104.8	7.6	99.0	7.0	117.1	6.5
phenothrin	*370.2*	*6.7*	180.5	14.7	139.8	15.4
fenthion	77.6	5.7	90.6	7.4	82.6	8.7
phenthoate	90.0	7.3	103.2	7.5	121.3	12.2
fenvalerate	105.3	7.5	107.7	9.9	154.8	15.5
fenbuconazole	117.8	12.6	88.1	15.9	*94.2*	*41.3*
fenpropathrin	121.0	10.2	116.8	9.0	114.4	13.9
butamifos	109.0	5.7	104.3	7.6	*206.8*	*18.2*
flamprop-methyl	52.6	12.2	109.8	8.6	113.7	11.4
flucythrinate	148.2	9.3	111.4	7.8	152.4	16.7
flutolanil	108.6	8.1	107.0	6.1	129.4	8.2
flutriafol	97.0	13.2	79.2	15.2	*54.6*	*68.0*
fluvalinate	105.1	10.1	98.0	8.0	172.3	17.4
flumioxazin	196.0	5.8	124.9	9.1	184.0	15.9
flumiclorac pentyl	121.0	5.8	121.9	6.0	118.4	13.1
procymidone	104.4	8.0	105.2	6.3	110.9	13.4
propachlor	101.2	10.5	96.2	7.8	128.5	8.5
propazine	96.4	6.8	102.4	8.7	93.5	4.9
propanil	111.4	8.1	89.6	14.5	131.8	7.8
propargite	137.7	7.5	119.5	4.4	130.3	17.1
propiconazole	136.6	7.6	102.1	8.6	141.6	13.3
propham	128.2	12.6	107.7	7.9	137.1	8.1

Table 6.3.1-1. Results of recovery test (formula feed for layer, repeated 3 times each) [continued]

component	spike concentration 50 µg/kg recovery (%)	50 µg/kg repeatability RSD (%)	100 µg/kg recovery (%)	100 µg/kg repeatability RSD (%)	500 µg/kg recovery (%)	500 µg/kg repeatability RSD (%)
profenofos	116.4	4.5	100.5	9.4	114.1	15.5
propetamphos	133.2	6.3	113.4	6.1	125.0	8.9
bromobutide	94.2	9.6	95.1	8.7	111.5	8.2
bromopropylate	108.8	6.1	102.9	6.5	119.2	14.0
bromophos	97.2	9.1	84.5	12.2	113.5	10.8
hexaconazole	110.6	5.6	99.6	8.9	*112.5*	*25.5*
benoxacor	99.4	9.3	102.9	8.2	122.5	8.7
heptachlor	101.4	8.5	69.5	7.1	132.9	13.8
heptachlor epoxide	101.8	8.1	75.1	6.1	115.8	13.3
cis-permethrin	115.2	7.8	98.8	7.9	124.7	13.1
trans-permethrin	115.6	7.3	97.8	7.6	120.1	13.5
penconazole	108.2	4.6	107.8	8.3	121.8	13.2
pendimethalin	85.2	9.2	92.5	9.7	160.9	16.8
benfluralin	91.2	7.7	95.2	11.1	153.3	16.4
phosalone	145.2	10.5	93.4	14.3	152.8	15.9
fosthiazate	*210.5*	*6.2*	152.9	14.2	*174.5*	*25.3*
phosmet	122.4	9.8	102.6	11.5	138.9	11.5
phorate	83.0	9.9	87.5	9.6	98.2	7.5
malathion	104.4	8.2	105.2	7.6	138.3	12.8
methacrifos	114.2	12.4	103.9	4.9	134.3	9.1
methiathion	138.8	4.9	111.7	8.8	146.0	10.1
methoxychlor	109.8	7.8	92.6	8.3	130.8	18.9
metominostrobin (*E*-isomer)	113.2	5.8	96.2	7.7	135.4	10.3
metolachlor	99.0	9.1	109.3	6.3	121.6	9.8
mevinphos	138.8	1.0	85.1	15.0	*116.1*	*44.6*

* The values in italics represent the recovery outside the range from 50 to 200 % or RSD exceeding 20 %.

Table 6.3.1-2. Results of recovery test (alfalfa, repeated 3 times each)

component	spike concentration 50 µg/kg recovery (%)	50 µg/kg repeatability RSD (%)	100 µg/kg recovery (%)	100 µg/kg repeatability RSD (%)	500 µg/kg recovery (%)	500 µg/kg repeatability RSD (%)
α-BHC	108.4	1.5	102.7	2.6	101.7	2.1
β-BHC	141.8	9.6	114.5	6.6	91.6	2.5
γ-BHC	96.6	19.7	89.2	5.0	99.4	4.2
δ-BHC	165.2	3.5	116.2	2.5	102.9	4.4
o,*p*′-DDD	120.0	3.2	109.2	2.3	88.4	5.0
p,*p*′-DDD	129.4	4.3	114.3	0.5	95.4	5.3
o,*p*′-DDE	122.2	2.9	108.7	2.8	76.4	5.4
p,*p*′-DDE	116.2	3.8	109.2	2.7	78.0	3.7
o,*p*′-DDT	116.8	3.6	106.0	0.9	91.3	6.2
p,*p*′-DDT	181.6	3.4	117.6	5.1	95.5	6.9
EPN	159.2	6.7	122.6	10.2	115.0	3.7
acetochlor	112.8	2.8	112.6	4.6	111.2	0.7

Table 6.3.1-2. Results of recovery test (alfalfa, repeated 3 times each) [continued]

component	spike concentration 50 µg/kg recovery (%)	50 µg/kg repeatability RSD (%)	100 µg/kg recovery (%)	100 µg/kg repeatability RSD (%)	500 µg/kg recovery (%)	500 µg/kg repeatability RSD (%)
atrazine	124.2	1.4	110.9	4.7	104.8	0.8
anilofos	166.4	2.4	140.3	9.1	140.1	4.1
ametryn	113.2	3.5	90.4	4.5	*31.4*	*6.8*
alachlor	112.8	3.4	104.9	3.4	107.3	4.1
allidochlor	138.6	11.1	113.7	4.7	106.6	4.3
aldrin	113.4	9.7	93.4	0.4	80.0	6.8
allethrin	*205.6*	*6.4*	154.1	6.1	94.9	5.4
isazophos	137.8	15.8	112.7	4.7	106.4	1.7
isofenphos	118.6	1.7	119.7	5.3	102.5	6.6
isoprothiolane	120.6	1.5	116.1	6.0	108.3	1.0
iprobenfos	156.2	2.6	142.8	5.3	116.9	1.9
ethalfluralin	116.8	3.9	111.1	4.5	91.8	5.2
ethion	133.4	2.1	121.1	5.5	113.6	6.6
edifenphos	*210.0*	*0.7*	161.4	1.8	146.1	7.2
etofenprox	120.8	5.6	117.5	4.1	79.3	7.0
ethofumesate	128.8	0.9	115.9	6.8	97.2	4.5
ethoprophos	135.0	1.5	112.7	0.9	113.2	2.5
etridiazole	99.8	9.2	102.9	5.0	110.3	1.4
etrimphos	114.2	2.5	101.8	5.3	101.1	2.5
endrin	148.4	2.8	116.4	3.6	117.9	3.2
oxadiazon	113.2	5.8	107.7	2.0	90.5	7.2
oxychlordane	95.0	1.3	74.0	4.5		
cadusaphos	195.0	4.5	141.5	5.9	117.4	5.3
carfentrazone-ethyl	141.6	2.5	125.3	6.5	106.6	2.9
quintozene	99.2	11.9	*46.4*	*12.9*	*79.5*	*23.8*
kresoxim-methyl	115.8	3.8	118.7	4.7	105.6	3.3
chlorthal-dimethyl	123.8	2.7	105.8	2.6	95.1	2.9
cis -chlordane	108.8	1.1	73.7	18.5		
trans -chlordane	108.6	1.3	74.0	16.8		
chlorpyrifos	115.0	4.0	97.1	6.7	84.0	3.4
chlorpyrifos-methyl	115.8	2.6	96.2	8.2	96.4	2.2
chlorfenapyr	141.8	2.0	126.5	1.4	97.1	2.2
chlorfenviphos (*E*- isomer)	143.4	2.8	111.3	2.7	113.3	4.0
chlorfenviphos (*Z*- isomer)	94.8	4.6	111.8	2.1	116.9	5.6
chlorpropham	179.8	13.3	141.6	2.7	118.2	4.8
chlorobenzilate	140.4	0.6	123.0	5.7	105.2	1.9
diclofop-methyl	137.8	6.0	116.5	1.5	98.6	5.1
dicloram	184.0	6.6	114.0	6.7	127.4	5.8
cyhalothrin	*395.2*	*148.0*	*178.3*	*127.2*	157.3	10.8
diphenamid	114.6	2.6	112.3	5.6	105.1	0.9
difenoconazole	*249.1*	*2.7*	146.3	8.2	120.4	5.9
dimethenamid	125.8	4.0	107.8	1.6	102.0	5.2
dimethoate	*1842.2*	*38.6*	*648.8*	*20.7*	103.0	0.8
dimepiperate	150.4	4.3	127.8	1.1	128.9	2.4
silafluofen	166.2	3.7	130.0	4.6	82.5	15.1

Table 6.3.1-2. Results of recovery test (alfalfa, repeated 3 times each) [continued]

component	spike concentration 50 µg/kg		100 µg/kg		500 µg/kg	
	recovery (%)	repeatability RSD (%)	recovery (%)	repeatability RSD (%)	recovery (%)	repeatability RSD (%)
diazinon	162.0	6.4	*261.3*	*2.8*	144.7	3.6
terbacil	158.8	2.9	121.6	1.1	110.3	4.2
thiobencarb	134.6	3.1	110.1	1.3	102.1	1.2
dieldrin	122.2	3.4	106.4	8.0	86.4	2.9
tecnazene	117.8	3.1	108.3	7.4	110.3	2.5
tetrachorvinphos	147.6	2.3	123.5	5.3	112.7	4.3
tetraconazole	119.6	4.5	111.4	4.8	96.9	3.8
tetradifon	129.0	8.8	109.1	7.3	97.7	6.5
tebuconazole	141.8	0.7	111.2	5.3	108.2	7.4
tebufenpyrad	136.4	4.1	120.9	6.3	101.0	11.1
tefluthrin	113.8	6.2	108.9	2.4	72.5	9.7
deltamethrin	142.4	3.8	119.8	1.5	111.6	8.3
terbuthrin	123.8	2.3	105.5	2.8	-	-
terbufos	105.2	5.7	105.7	5.2	96.1	4.6
triadimefon	129.6	4.5	107.3	1.7	110.3	1.7
triallate	123.8	1.1	112.1	2.7	88.7	5.5
trifluralin	135.2	4.0	116.3	2.1	116.9	6.6
trifloxystrobin	129.8	2.3	127.5	5.7	108.8	6.8
tolylfluanid	*28.8*	*2.8*	60.8	4.9	70.8	12.3
napropamide	150.0	3.5	110.5	3.1	117.5	3.0
parathion	156.2	3.5	128.7	3.8	157.9	0.9
parathion-methyl	149.8	1.1	126.2	6.7	129.9	5.5
halfenprox	137.6	0.7	125.1	6.6	106.4	12.7
bifenthrin	117.8	2.7	106.6	1.7	79.0	9.2
piperophos	*428.2*	*2.4*	*211.5*	*6.4*	172.2	7.4
pyridaphenthion	155.6	1.8	127.6	6.7	131.2	3.2
pyridaben	*228.8*	*2.1*	145.8	1.7	107.6	15.4
pyriproxyfen	141.2	2.4	114.8	1.3	95.9	4.5
pirimiphos-methyl	127.6	4.5	108.2	3.1	100.7	3.8
vinclozolin	115.6	3.1	107.6	5.5	97.0	2.6
fipronil	122.4	3.1	106.3	6.4	111.7	3.1
fenarimol	155.6	6.0	106.6	2.1	119.3	3.6
fenitrothion	140.0	2.4	121.1	5.0	135.9	3.2
fenothiocarb	127.0	5.4	113.5	1.9	116.1	4.9
phenothrin	*680.1*	*59.2*	*218.7*	*40.1*	81.8	7.7
fenthion	83.2	7.0	98.3	6.2	80.8	4.6
phenthoate	119.4	1.0	130.6	5.7	99.7	4.7
fenvalerate	121.1	2.1	121.6	6.2	114.6	12.0
fenbuconazole	144.8	2.8	104.4	11.2	75.0	15.2
fenpropathrin	*70.8*	*26.8*	162.7	4.5	87.4	8.5
butamifos	156.0	5.9	129.5	2.2	156.6	4.8
flamprop-methyl	128.6	1.4	119.7	5.3	102.0	2.7
flucythrinate	131.1	2.3	123.1	3.4	118.9	9.7
flutolanil	121.8	3.8	117.7	4.3	116.7	3.5
flutriafol	122.0	6.7	92.8	12.4	*53.6*	*35.2*

Table 6.3.1-2. Results of recovery test (alfalfa, repeated 3 times each) [continued]

component	spike concentration 50 µg/kg		100 µg/kg		500 µg/kg	
	recovery (%)	repeatability RSD (%)	recovery (%)	repeatability RSD (%)	recovery (%)	repeatability RSD (%)
fluvalinate	129.4	2.1	*127.4*	*20.3*	122.2	11.0
flumioxazin	*334.6*	*4.4*	163.9	4.1	188.3	5.3
flumiclorac pentyl	146.6	4.0	122.7	5.5	100.1	0.4
procymidone	114.6	1.6	112.1	3.7	98.8	1.1
propachlor	117.2	2.0	108.4	1.8	118.7	1.9
propazine	113.4	2.8	113.7	4.7	94.9	1.3
propanil	142.0	3.7	113.5	2.6	121.4	1.2
propargite	136.3	3.5	133.3	3.1	106.0	12.0
propiconazole	*529.1*	*6.5*	191.2	0.4	123.9	5.9
propham	109.4	1.3	107.6	1.9	115.3	1.5
profenofos	153.8	3.5	117.8	11.9	96.4	5.1
propetamphos	*386.0*	*2.8*	*217.0*	*2.8*	119.0	1.1
bromobutide	108.0	4.8	101.8	2.0	104.4	4.3
bromopropylate	127.8	1.9	117.2	6.0	101.5	4.0
bromophos	115.8	4.0	98.2	8.7	90.4	8.7
hexaconazole	130.2	1.4	112.5	1.1	100.9	11.5
benoxacor	89.8	10.0	112.1	5.9	115.0	2.1
heptachlor	114.2	0.9	84.4	2.4	99.4	7.9
heptachlor epoxide	113.4	0.5	78.7	8.5	95.1	5.4
cis -permethrin	124.6	0.3	106.5	0.8	87.2	12.7
trans -permethrin	156.4	2.0	116.4	0.6	88.7	12.4
penconazole	121.4	4.0	121.2	4.0	103.8	3.0
pendimethalin	124.4	1.6	115.3	4.2	107.6	4.5
benfluralin	110.6	4.7	103.0	2.3	104.5	2.7
phosalone	178.8	3.8	112.8	3.0	129.5	13.0
fosthiazate	*432.9*	*4.0*	*248.3*	*4.2*	158.3	5.1
phosmet	162.8	2.8	131.0	7.8	121.6	6.4
phorate	85.8	2.6	102.2	7.1	92.6	3.2
malathion	137.8	6.8	120.8	4.2	109.6	1.2
methacrifos	113.4	6.3	109.7	4.7	112.2	0.9
methidathion	197.8	6.6	143.0	3.4	136.3	1.0
methoxychlor	128.6	1.4	108.9	1.2	107.6	10.4
metominostrobin (*E*- isomer)	138.6	2.2	111.8	1.5	121.7	1.8
metolachlor	113.2	3.5	118.9	4.3	97.6	0.6
mevinphos	69.2	6.9	78.7	3.4	57.4	14.1

* The values in italics represent the recovery outside the range from 50 to 200 % or RSD exceeding 20 %.

Table 6.3.1-3. Results of collaborative study (formula feed for layer, spike concentration of each agricultural chemical is 100 μg/kg)

component	number of laboratories	recovery	intra-laboratory repeatability	inter-laboratory reproducibility	HorRat
		(%)	RSD_r (%)	RSD_R (%)	
α-BHC	9	89.7	5.2	18.7	0.85
β-BHC	9	90.5	5.1	22.7	1.03
γ-BHC	9	92.6	5.7	24.9	1.13
δ-BHC	9	89.3	5.2	21.2	0.96
o,*p*′-DDD	7	86.0	11.7	20.5	0.93
p,*p*′-DDD	8	102.1	4.9	20.1	0.91
o,*p*′-DDE	7	93.6	6.6	10.0	0.45
p,*p*′-DDE	7	92.3	7.6	9.3	0.42
o,*p*′-DDT	9	111.7	7.1	29.9	1.36
p,*p*′-DDT	9	105.7	7.8	24.2	1.10
EPN	9	131.9	5.1	37.0	1.68
acetochlor	9	106.5	4.6	25.7	1.17
atrazine	9	87.6	9.5	27.9	1.27
anilofos	8	131.9	5.2	25.5	1.16
ametryn	6	59.9	15.8	37.6	1.71
alachlor	9	97.9	4.7	20.3	0.92
allidochlor	7	101.9	2.5	20.9	0.95
aldrin	9	86.6	8.9	27.3	1.24
allethrin	8	90.9	14.4	28.1	1.28
isazophos	9	109.9	7.8	21.4	0.97
isofenphos	8	93.8	8.7	21.7	0.99
isoprothiolane	8	99.5	6.5	33.2	1.51
iprobenfos	9	124.4	12.2	28.0	1.27
ethalfluralin	9	93.1	9.5	30.5	1.39
ethion	9	117.4	5.7	28.1	1.28
edifenphos	8	140.0	13.0	39.3	1.78
etofenprox	9	113.5	6.9	26.7	1.21
ethofumesate	8	102.3	4.6	30.8	1.40
ethoprophos	9	105.4	8.5	21.7	0.99
etridiazole	8	99.1	4.6	31.4	1.43
etrimphos	7	105.2	6.8	7.7	0.35
endrin	9	110.5	7.2	21.7	0.99
oxadiazon	7	93.6	5.7	17.6	0.80
oxychlordane	9	81.4	8.8	38.3	1.74
cadusafos	9	107.0	8.9	22.4	1.02
carfentrazone-ethyl	8	97.6	5.4	23.4	1.06
quintozene	8	82.0	7.9	43.3	1.97
kresoxim-methyl	9	107.2	4.0	21.2	0.96
chlorthal-dimethyl	9	88.2	5.9	23.8	1.08
cis-chlordane	9	82.3	6.3	34.0	1.55
trans-chlordane	7	98.2	6.1	11.5	0.52
chlorpyrifos	9	104.2	8.8	33.1	1.50
chlorpyrifos-methyl	7	100.6	7.4	8.3	0.38
chlorfenapyr	9	97.5	6.1	13.2	0.60
chlorfenviphos(*E*-isomer)	9	102.5	6.5	25.2	1.14
chlorfenviphos(*Z*-isomer)	9	100.2	6.9	22.7	1.03

Table 6.3.1-3. Results of collaborative study (formula feed for layer, spike concentration of each agricultural chemical is 100 μg/kg) [continued]

component	number of laboratories	recovery (%)	intra-laboratory repeatability RSD_r (%)	inter-laboratory reproducibility RSD_R (%)	HorRat
chlorpropham	9	106.6	6.1	16.0	0.73
chlorobenzilate	9	114.7	3.8	23.1	1.05
diclofop-methyl	9	99.3	6.8	19.4	0.88
dicloran	9	98.6	12.1	26.1	1.19
cyhalothrin	9	129.5	7.8	30.9	1.41
diphenamid	9	97.8	3.4	20.3	0.92
difenoconazole	9	112.8	7.6	27.1	1.23
dimethenamid	9	97.3	4.5	18.5	0.84
dimethoate	9	112.9	14.3	32.7	1.49
dimepiperate	9	113.3	6.1	18.2	0.83
silafluofen	9	103.7	7.0	23.2	1.06
terbacil	8	133.2	8.4	23.3	1.06
diazinon	9	98.9	5.2	19.0	0.86
thiobencarb	9	100.8	12.6	27.1	1.23
dieldrin	8	81.1	8.5	29.1	1.32
tecnazene	7	91.4	6.3	10.3	0.47
tetrachlorvinphos	8	116.8	3.4	22.4	1.02
tetraconazole	8	91.5	4.8	23.1	1.05
tetradifon	9	95.1	3.6	21.8	0.99
tebuconazole	7	113.4	5.9	25.2	1.15
tebufenpyrad	9	111.0	4.6	18.5	0.84
tefluthrin	8	91.0	3.0	20.7	0.94
deltamethrin	9	121.5	7.7	41.8	1.90
terbutryn	7	73.1	8.0	41.0	1.86
terbufos	9	85.1	7.6	26.6	1.21
triadimefon	8	101.6	6.5	19.4	0.88
triallate	9	90.3	4.6	19.1	0.87
trifluralin	9	98.5	9.0	30.1	1.37
trifloxystrobin	8	122.7	5.5	43.7	1.99
tolylfluanid	9	94.2	6.2	28.3	1.29
napropamide	7	103.6	6.1	16.6	0.75
parathion	9	120.6	9.7	33.9	1.54
parathion-methyl	9	114.5	12.4	34.2	1.55
halfenprox	8	108.4	7.4	20.1	0.91
bifenthrin	8	97.2	6.2	11.2	0.51
piperophos	9	163.0	7.1	38.3	1.74
pyridaphenthion	8	135.2	5.3	33.6	1.53
pyridaben	8	101.4	3.5	15.3	0.70
pyriproxyfen	9	110.8	6.6	17.0	0.77
pirimiphos-methyl	9	99.0	4.7	23.3	1.06
vinclozolin	9	97.3	5.3	24.3	1.10
fipronil	8	98.7	10.8	37.3	1.70
fenarimol	8	110.6	4.5	9.2	0.42
fenitrothion	9	126.7	8.7	41.7	1.90
fenothiocarb	9	108.4	5.0	13.9	0.63
phenothrin	7	93.0	5.5	20.2	0.92

Table 6.3.1-3. Results of collaborative study (formula feed for layer, spike concentration of each agricultural chemical is 100 µg/kg) [continued]

component	number of laboratories	recovery (%)	intra-laboratory repeatability RSD_r (%)	inter-laboratory reproducibility RSD_R (%)	HorRat
fenthion	9	75.4	3.2	30.4	1.38
phenthoate	8	92.3	4.0	27.2	1.23
fenvalerate	9	128.9	9.3	35.2	1.60
fenbuconazole	8	96.5	7.0	14.1	0.64
fenpropathrin	8	105.6	5.6	21.8	0.99
butamifos	8	121.6	7.6	43.2	1.96
flamprop-methyl	8	94.1	4.1	38.0	1.73
flucythrinate	9	132.3	6.6	27.2	1.23
flutolanil	8	83.9	8.1	27.3	1.24
flutriafol	4	70.4	19.1	37.2	1.69
fluvalinate	9	126.4	7.7	33.1	1.51
flumioxazin	9	134.3	10.4	43.6	1.98
flumiclorac pentyl	7	120.2	5.3	21.1	0.96
procymidone	8	99.4	5.6	8.8	0.40
propachlor	9	101.6	4.3	18.2	0.83
propazine	9	83.0	7.1	28.0	1.27
propanil	9	107.8	6.4	20.4	0.93
propargite	8	111.5	9.2	15.3	0.69
propiconazole	9	116.9	16.4	24.1	1.09
propham	8	105.0	3.8	17.9	0.81
profenofos	8	94.0	11.5	32.7	1.49
propetamphos	9	98.5	7.4	24.3	1.10
bromobutide	8	100.7	4.2	19.0	0.86
bromopropylate	8	107.7	3.7	21.9	1.00
bromophos	9	94.4	5.7	22.3	1.01
hexaconazole	7	78.7	11.8	20.5	0.93
benoxacor	9	106.4	7.5	22.4	1.02
heptachlor	9	87.8	6.3	34.5	1.57
heptachlor epoxide	9	82.6	5.9	29.6	1.35
cis -permethrin	8	99.8	5.5	14.7	0.67
trans -permethrin	9	106.9	3.5	24.4	1.11
penconazole	8	92.0	3.9	20.3	0.92
pendimethalin	9	107.3	8.1	39.1	1.78
benfluralin	9	91.4	8.1	33.4	1.52
phosalone	7	124.6	7.3	13.5	0.62
fosthiazate	9	137.3	11.3	36.6	1.66
phosmet	8	119.6	4.4	26.2	1.19
phorate	9	80.1	8.0	30.5	1.39
malathion	8	108.0	4.9	24.0	1.09
methacrifos	9	94.4	5.0	20.8	0.94
methidathion	8	115.3	11.7	19.0	0.87
methoxychlor	9	107.7	6.3	24.2	1.10
metominostrobin (*E*- isomer)	7	98.3	6.5	29.2	1.33
metolachlor	9	100.6	3.1	21.0	0.95
mevinphos	9	83.5	10.8	40.7	1.85

Table 6.3.1-4. Results of collaborative study (alfalfa, spike concentration of each agricultural chemical is 100 μg/kg)

component	number of laboratories	recovery (%)	intra-laboratory repeatability RSD_r (%)	inter-laboratory reproducibility RSD_R (%)	HorRat
α-BHC	9	103.1	4.4	10.0	0.45
β-BHC	9	96.1	4.2	22.0	1.00
γ-BHC	8	77.7	6.3	32.1	1.46
δ-BHC	8	102.7	6.4	10.3	0.47
o,*p*′-DDD	9	91.8	4.9	16.9	0.77
p,*p*′-DDD	9	102.7	5.3	16.6	0.76
o,*p*′-DDE	8	93.1	4.5	9.7	0.44
p,*p*′-DDE	9	87.1	5.6	18.9	0.86
o,*p*′-DDT	9	104.4	6.9	23.5	1.07
p,*p*′-DDT	7	98.9	4.9	23.7	1.08
EPN	7	121.1	5.0	29.8	1.35
acetochlor	7	114.9	5.5	7.7	0.35
atrazine	7	109.0	6.6	7.7	0.35
anilofos	8	140.3	4.7	18.0	0.82
ametryn	8	88.5	10.8	18.6	0.84
alachlor	8	112.2	4.6	6.2	0.28
allidochlor	8	110.7	15.9	26.5	1.21
aldrin	9	82.9	4.8	19.4	0.88
allethrin	8	111.1	9.8	27.7	1.26
isazophos	9	131.4	6.8	25.9	1.18
isofenphos	9	105.5	9.2	23.5	1.07
isoprothiolane	7	118.6	5.0	7.7	0.35
iprobenfos	8	132.2	4.6	13.4	0.61
ethalfluralin	9	96.6	8.4	23.8	1.08
ethion	8	111.5	4.9	24.6	1.12
edifenphos	7	163.8	5.8	14.2	0.65
etofenprox	8	109.9	5.7	24.8	1.13
ethofumesate	8	107.4	3.7	8.6	0.39
ethoprophos	7	121.8	5.7	8.0	0.36
etridiazole	9	99.4	20.2	39.7	1.81
etrimphos	7	107.8	5.8	6.7	0.30
endrin	8	129.9	5.6	38.3	1.74
oxadiazon	8	96.3	2.6	15.5	0.71
oxychlordane	7	91.1	4.6	10.1	0.46
cadusafos	9	165.8	7.6	33.6	1.53
carfentrazone-ethyl	9	127.9	6.5	15.4	0.70
quintozene	8	71.5	10.3	31.0	1.41
kresoxim-methyl	7	113.8	4.9	9.1	0.41
chlorthal-dimethyl	9	97.3	4.9	19.1	0.87
cis-chlordane	7	92.9	4.8	9.3	0.42
trans-chlordane	9	86.8	6.9	26.6	1.21
chlorpyrifos	8	93.4	4.8	21.5	0.98
chlorpyrifos-methyl	8	93.3	5.0	19.8	0.90
chlorfenapyr	9	102.2	4.4	16.1	0.73
chlorfenviphos (*E*-isomer)	9	121.9	6.0	9.3	0.42
chlorfenviphos (*Z*-isomer)	9	121.5	5.3	10.2	0.46

Table 6.3.1-4. Results of collaborative study (alfalfa, spike concentration of each agricultural chemical is 100 µg/kg) [continued]

component	number of laboratories	recovery (%)	intra-laboratory repeatability RSD_r (%)	inter-laboratory reproducibility RSD_R (%)	HorRat
chlorpropham	9	122.0	4.3	16.0	0.73
chlorobenzilate	8	113.0	4.7	17.4	0.79
diclofop-mathyl	8	114.2	4.8	16.5	0.75
dicloran	9	119.0	7.4	22.0	1.00
cyhalothrin	7	108.5	6.5	22.1	1.01
diphenamid	8	114.2	3.8	10.3	0.47
difenoconazole	9	144.8	13.9	23.9	1.08
dimethenamid	8	114.1	4.7	6.0	0.27
dimethoate	6	129.0	21.2	20.4	0.93
dimepiperate	9	135.9	7.4	21.1	0.96
silafluofen	9	106.5	6.2	19.1	0.87
terbacil	6	172.3	3.2	8.9	0.40
diazinon	7	125.8	4.1	7.8	0.35
thiobencarb	9	110.1	5.2	17.6	0.80
dieldrin	8	95.4	5.3	9.2	0.42
tecnazene	9	91.9	10.4	24.7	1.12
tetrachlorvinphos	8	123.0	5.4	17.1	0.78
tetraconazole	9	104.7	7.0	17.8	0.81
tetradifon	9	99.6	6.7	21.9	0.99
tebuconazole	8	126.2	9.8	15.5	0.71
tebufenpyrad	9	118.2	4.8	13.8	0.63
tefluthrin	9	89.3	5.5	18.1	0.82
deltamethrin	9	123.6	11.7	29.2	1.33
terbutryn	7	112.2	6.7	8.2	0.37
terbufos	9	99.4	7.7	28.4	1.29
triadimefon	9	123.4	6.7	17.7	0.80
triallate	9	95.1	4.6	17.3	0.79
trifluralin	9	108.7	7.7	17.1	0.78
trifloxystrobin	8	115.4	4.9	23.9	1.08
tolylfulanid	9	59.8	9.4	34.9	1.59
napropamide	9	124.3	6.5	14.7	0.67
parathion	7	133.6	8.9	7.5	0.34
parathion-methyl	9	130.8	7.5	26.0	1.18
halfenprox	8	113.9	6.5	35.2	1.60
bifenthrin	9	101.5	5.9	20.3	0.92
piperophos	9	179.6	5.4	29.9	1.36
pyridaphenthion	8	137.2	4.7	24.1	1.10
pyridaben	9	125.7	5.3	22.5	1.02
pyriproxyfen	9	124.2	5.4	18.9	0.86
pirimiphos-methyl	8	112.2	5.0	8.1	0.37
vinclozolin	7	105.0	4.9	6.1	0.28
fipronil	8	120.0	5.5	15.3	0.69
fenarimol	9	135.4	7.3	15.6	0.71
fenitrothion	9	134.9	7.8	33.2	1.51
fenothiocarb	9	117.8	6.3	13.4	0.61
phenothrin	8	103.1	7.1	26.3	1.20

Table 6.3.1-4. Results of collaborative study (alfalfa, spike concentration of each agricultural chemical is 100 µg/kg) [continued]

component	number of laboratories	recovery	intra-laboratory repeatability	inter-laboratory reproducibility	HorRat
		(%)	RSD_r (%)	RSD_R (%)	
fenthion	9	78.9	14.2	28.9	1.31
phenthoate	7	103.9	6.4	10.6	0.48
fenvalerate	8	123.9	13.4	20.4	0.93
fenbuconazole	9	123.8	11.8	32.8	1.49
fenpropathrin	8	114.2	13.0	33.6	1.53
butamifos	9	137.5	7.3	17.5	0.80
flamprop-methyl	7	113.3	3.5	7.8	0.35
flucythrinate	9	152.1	11.0	29.5	1.34
flutolanil	8	120.8	4.5	23.8	1.08
flutriafol	6	73.4	20.0	29.3	1.33
fluvalinate	9	136.4	10.1	29.2	1.33
flumioxazin	8	147.7	5.2	22.3	1.01
flumiclorac pentyl	7	125.9	4.5	12.2	0.55
procymidone	8	106.1	4.5	8.5	0.39
propachlor	8	125.5	5.0	8.9	0.40
propazine	9	103.8	4.8	26.5	1.21
propanil	7	122.6	6.1	6.6	0.30
propargite	8	111.2	10.8	20.4	0.93
propiconazole	9	177.1	13.4	28.6	1.30
propham	7	111.5	5.3	7.5	0.34
profenofos	8	119.0	6.2	14.9	0.68
propetamphos	8	115.0	6.7	35.5	1.62
bromobutide	8	113.5	3.9	7.6	0.35
bromopropylate	8	113.4	4.2	18.5	0.84
bromophos	8	93.2	4.7	18.3	0.83
hexaconazole	8	126.0	6.7	17.1	0.78
benoxacor	9	126.7	8.2	32.2	1.46
heptachlor	9	93.1	9.9	23.3	1.06
heptachlor eopxide	9	87.9	5.6	21.4	0.97
cis -permethrin	8	104.0	6.9	11.1	0.51
trans -permethrin	9	111.0	5.2	18.2	0.83
penconazole	9	110.8	6.2	21.5	0.98
pendimethalin	9	112.7	8.3	28.1	1.28
benfluralin	9	102.5	6.9	17.9	0.81
phosalone	8	131.4	6.7	21.2	0.96
fosthiazate	9	182.0	6.1	14.3	0.65
phosmet	8	131.6	6.4	22.1	1.01
phorate	9	86.2	11.9	34.0	1.55
malathion	8	113.6	6.2	19.2	0.87
methacrifos	9	101.9	6.6	20.0	0.91
methidathion	8	158.7	12.5	29.4	1.34
methoxychlor	9	122.0	8.7	29.6	1.34
metomistrobin (*E*- isomer)	9	126.4	6.3	15.2	0.69
metolachlor	9	109.4	5.3	21.8	0.99
mevinphos	9	60.8	10.1	26.6	1.21

«Notes and precautions»

[1] The respective agricultural chemical standards are available from Kanto Chemical, Wako Pure Chemical Industries and other manufacturers. According to this method, the standards are to be dissolved with acetone followed by dilution with 2,2,4-trimethylpentane, but dissolving with acetone alone will do.

As for mixed standard solutions, those which are commercially available can also be used. Further, some services to prepare standard solutions by using specified agricultural chemicals are available.

When agricultural chemicals are mixed to prepare a standard solution, it is required to confirm beforehand that interaction among compounds will not affect the measurements. In addition, due to the nature of GC-MS, monitoring of a number of quantifying ions simultaneously may often cause a decrease in the sensitivity of GC-MS.

However, because of time required per measurement of almost one hour, it takes long time for a laboratory having only small number of GC-MSs to perform SIM chromatography of mixed standards divided into many groups.

Therefore, in this method, standards are divided into two groups to monitor about 10 ions each simultaneously. For the division of standards into groups and the example of grouping of ions for SIM, see the footnotes of Figures 6.3.1-2 and 6.3.1-3.

[2] Caution is demanded against bumping. It tends to occur at the beginning and the end of the concentration. The smaller the amount of the concentrated residue is, the more smoothly the next operation will go.

[3] Even in cases the flow rate is low, it must be natural flow, because no study under conditions other than natural has not been made., If increase of the flow rate by applying pressure is needed, for example, each laboratory must confirm the validity of the treatment prior to the analysis.

[4] There is no need to dissolve the residue by ultrasound treatment and the like.

[5] The elution fraction should be determined after being identified in each laboratory. It only needs to contain analyte agricultural chemicals. A rough guide is that from the peak elution of acrinathrin to before eluting tricyclazole, although they are not the subjects of this method.

[6] The flow rate during the conditioning of the minicolumn does not affect the recovery etc., applying pressure will not harm.

[7] In this method, a 25 mL pear-shape flask is used because of low elution volume, but other containers, such as a 50 mL recovery flask, can be used depending on circumstances in each laboratory.

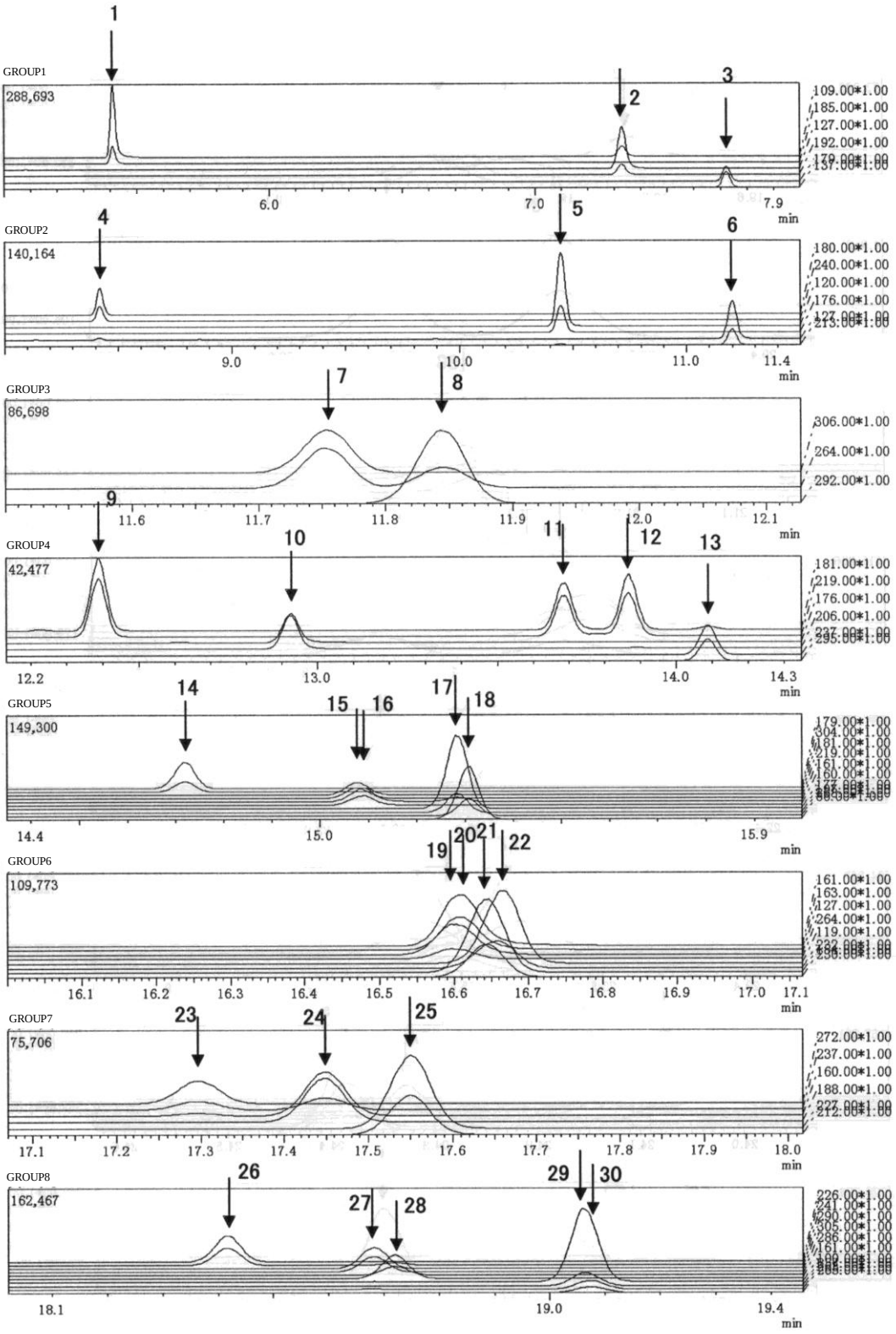
[8] Natural flow will also do.

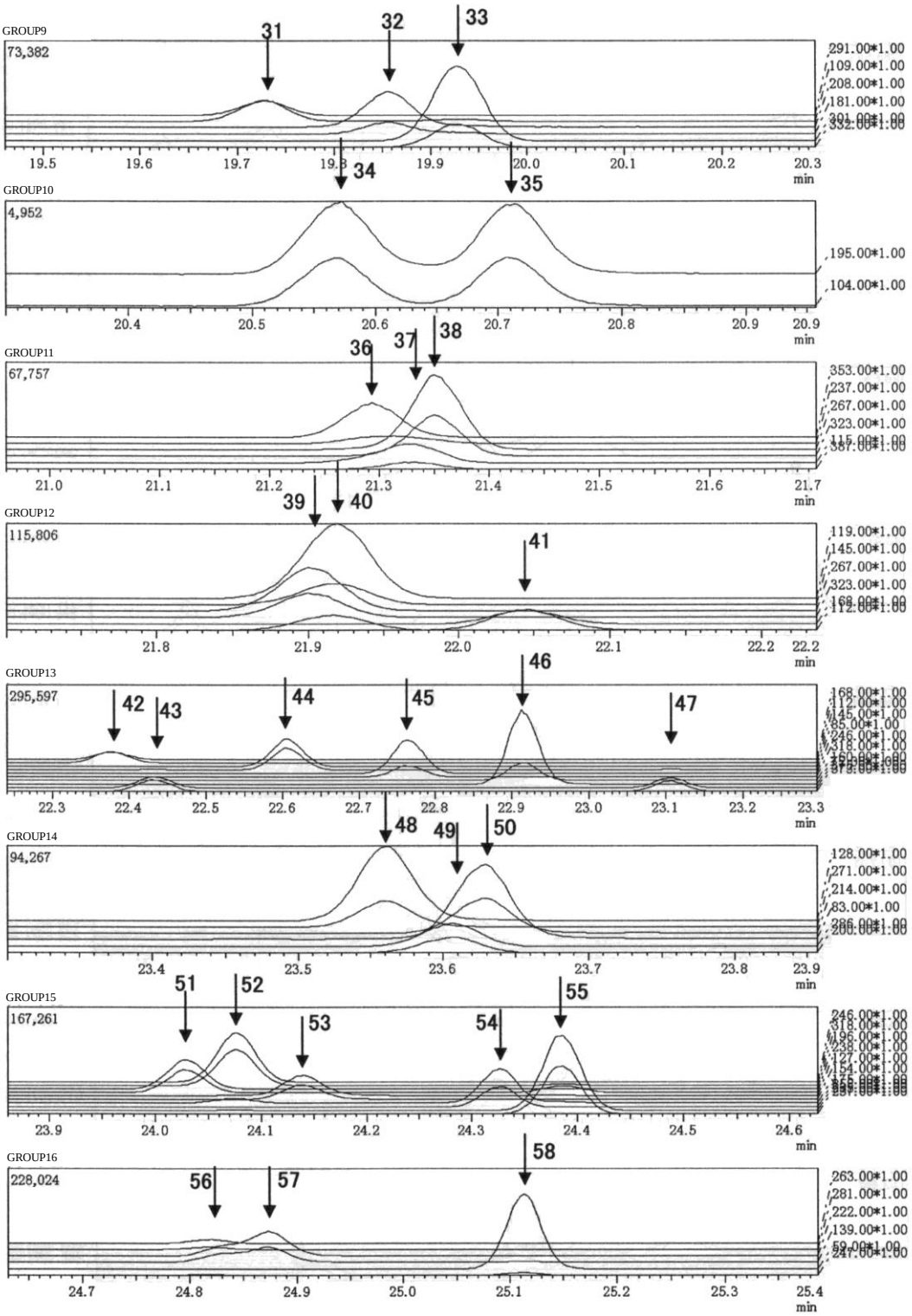
[9] Because this method is a simultaneous analysis for agricultural chemical multiresidue, interfering peaks that overlap with those of quantification ions of each agricultural chemical may be detected depending on samples. In such cases, the presence or absence of a agricultural chemical should be determined based on the ratio of the peak of an interfering ion to that of the monitored ion. Generally, the acceptable relative range for this ratio is from 30 % to 50 %. But this range may vary according to the type of each agricultural chemical, it should be identified in each laboratory. When it is difficult to confirm whether an agricultural chemical is present or not even by comparison with the monitored ions, its presence or absence can be determined based on the mass spectrum obtained by GC-MS in

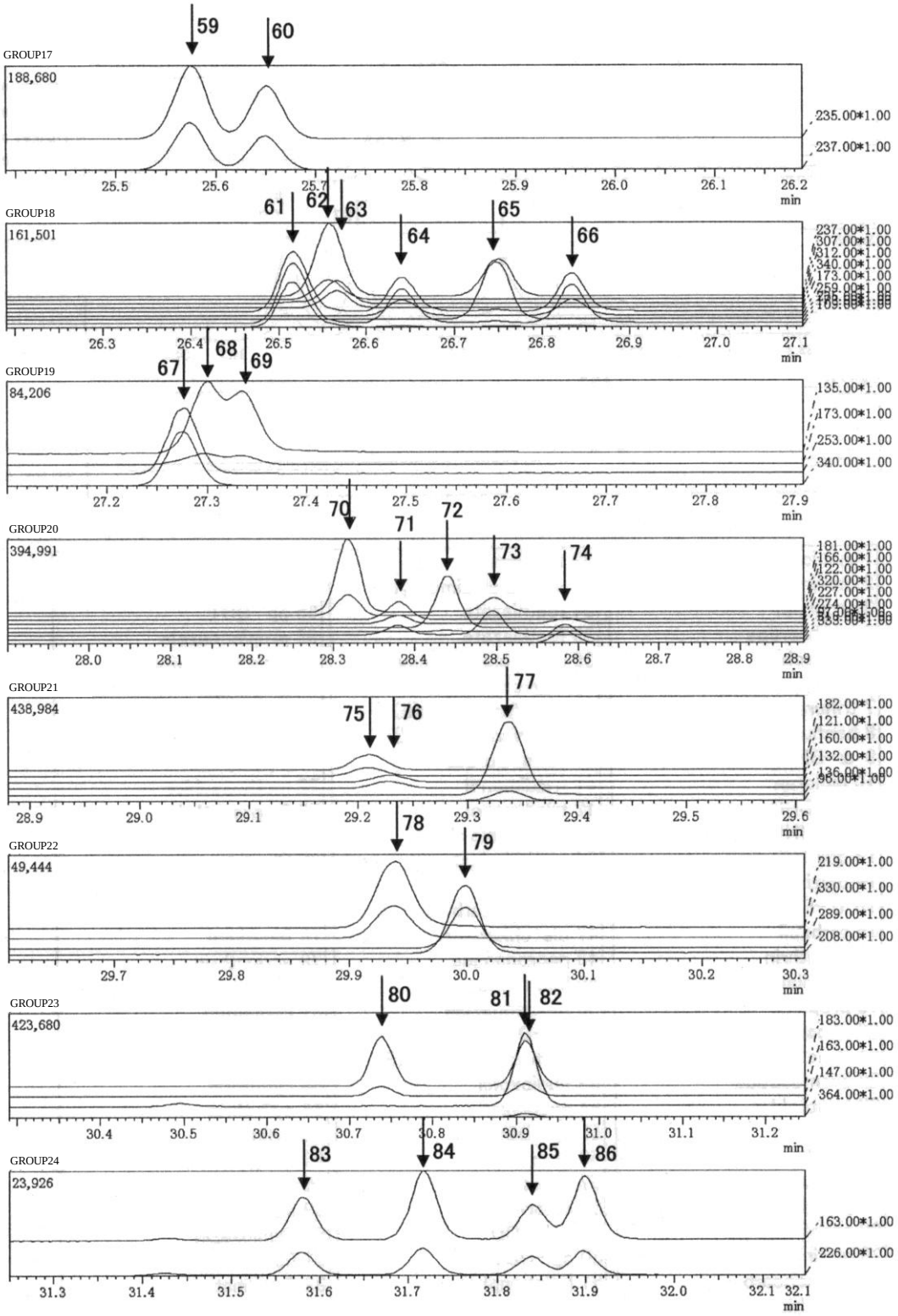
scan mode (*m*/*z* 50-500) instead of in SIM mode, provided the content of the pesticide is high (about 100 μg/kg or more, depending on the type of pesticides). When, despite these operations, there is doubt about the presence of a pesticide, it should be confirmed by modifying the temperature rising program or replacing the column.

[10] Ions to be monitored can be change according to the presence of interfering substances or the sensitivity.

[11] Examples of chromatogram are shown in Figure 6.3.1-2 and 6.3.1-3. The retention time of respective agricultural chemicals may vary from manufacturer to manufacturer of column, etc., so, where necessary, it should be measured for each agricultural chemical.


GROUP1
288,693
109.00*1.00
185.00*1.00
127.00*1.00
192.00*1.00
179.00*1.00
137.00*1.00
1
2
3
6.0
7.0
7.9
min
GROUP2
140,164
180.00*1.00
240.00*1.00
120.00*1.00
176.00*1.00
127.00*1.00
215.00*1.00
4
5
6
9.0
10.0
11.0
11.4
min
GROUP3
86,698
306.00*1.00
264.00*1.00
292.00*1.00
7
8
9
11.6
11.7
11.8
11.9
12.0
12.1
min
GROUP4
42,477
181.00*1.00
219.00*1.00
176.00*1.00
206.00*1.00
237.00*1.00
295.00*1.00
10
11
12
13
12.2
13.0
14.0
14.3
min
GROUP5
149,300
179.00*1.00
304.00*1.00
181.00*1.00
219.00*1.00
161.00*1.00
160.00*1.00
14
15
16
17
18
14.4
15.0
15.9
min
GROUP6
109,773
161.00*1.00
163.00*1.00
127.00*1.00
264.00*1.00
119.00*1.00
19
20
21
22
16.1
16.2
16.3
16.4
16.5
16.6
16.7
16.8
16.9
17.0
17.1
min
GROUP7
75,706
272.00*1.00
237.00*1.00
160.00*1.00
188.00*1.00
227.00*1.00
212.00*1.00
23
24
25
17.1
17.2
17.3
17.4
17.5
17.6
17.7
17.8
17.9
18.0
min
GROUP8
162,467
226.00*1.00
241.00*1.00
290.00*1.00
305.00*1.00
286.00*1.00
161.00*1.00
26
27
28
29
30
18.1
19.0
19.4
min


GROUP9
73,382
31
32
33
291.00*1.00
109.00*1.00
208.00*1.00
181.00*1.00
19.5
19.6
19.7
19.8
19.9
20.0
20.1
20.2
20.3
min
34
35
GROUP10
4,952
195.00*1.00
104.00*1.00
20.4
20.5
20.6
20.7
20.8
20.9
min
36
37
38
GROUP11
67,757
353.00*1.00
237.00*1.00
267.00*1.00
323.00*1.00
21.0
21.1
21.2
21.3
21.4
21.5
21.6
21.7
min
39
40
GROUP12
115,806
41
119.00*1.00
145.00*1.00
267.00*1.00
323.00*1.00
21.8
21.9
22.0
22.1
22.2
min
46
GROUP13
295,597
42
43
44
45
47
168.00*1.00
145.00*1.00
246.00*1.00
318.00*1.00
22.3
22.4
22.5
22.6
22.7
22.8
22.9
23.0
23.1
23.2
23.3
min
48
49
50
GROUP14
94,267
128.00*1.00
271.00*1.00
214.00*1.00
83.00*1.00
23.4
23.5
23.6
23.7
23.8
23.9
min
51
52
55
GROUP15
167,261
53
54
246.00*1.00
318.00*1.00
196.00*1.00
127.00*1.00
154.00*1.00
23.9
24.0
24.1
24.2
24.3
24.4
24.5
24.6
min
58
GROUP16
228,024
56
57
263.00*1.00
281.00*1.00
222.00*1.00
139.00*1.00
24.7
24.8
24.9
25.0
25.1
25.2
25.3
25.4
min


GROUP17
188,680
235.00*1.00
237.00*1.00
GROUP18
161,501
GROUP19
84,206
GROUP20
394,991
GROUP21
438,984
GROUP22
49,444
GROUP23
423,680
GROUP24
23,926
163.00*1.00
226.00*1.00
min

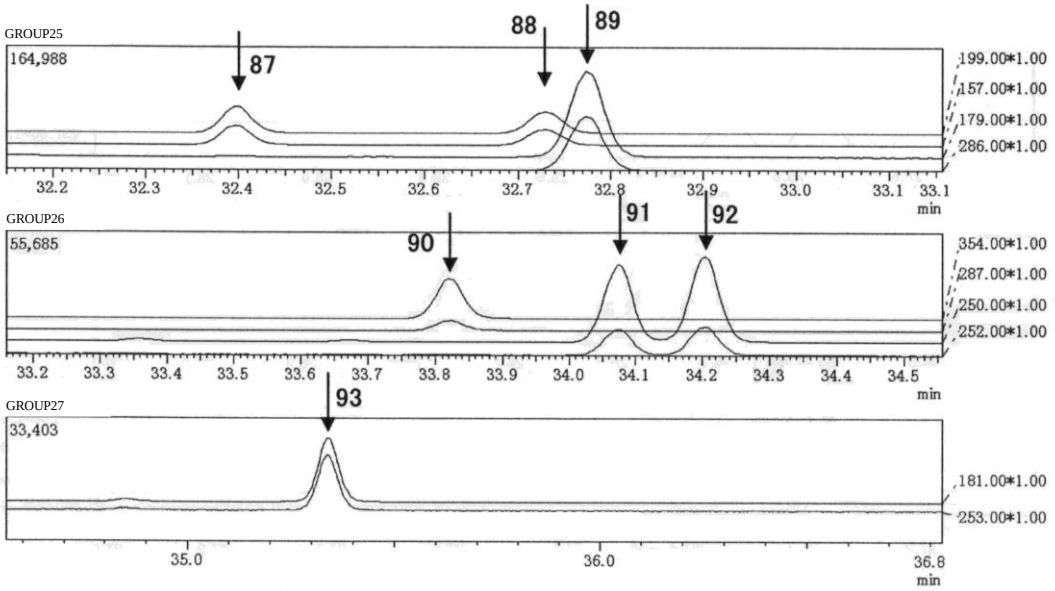
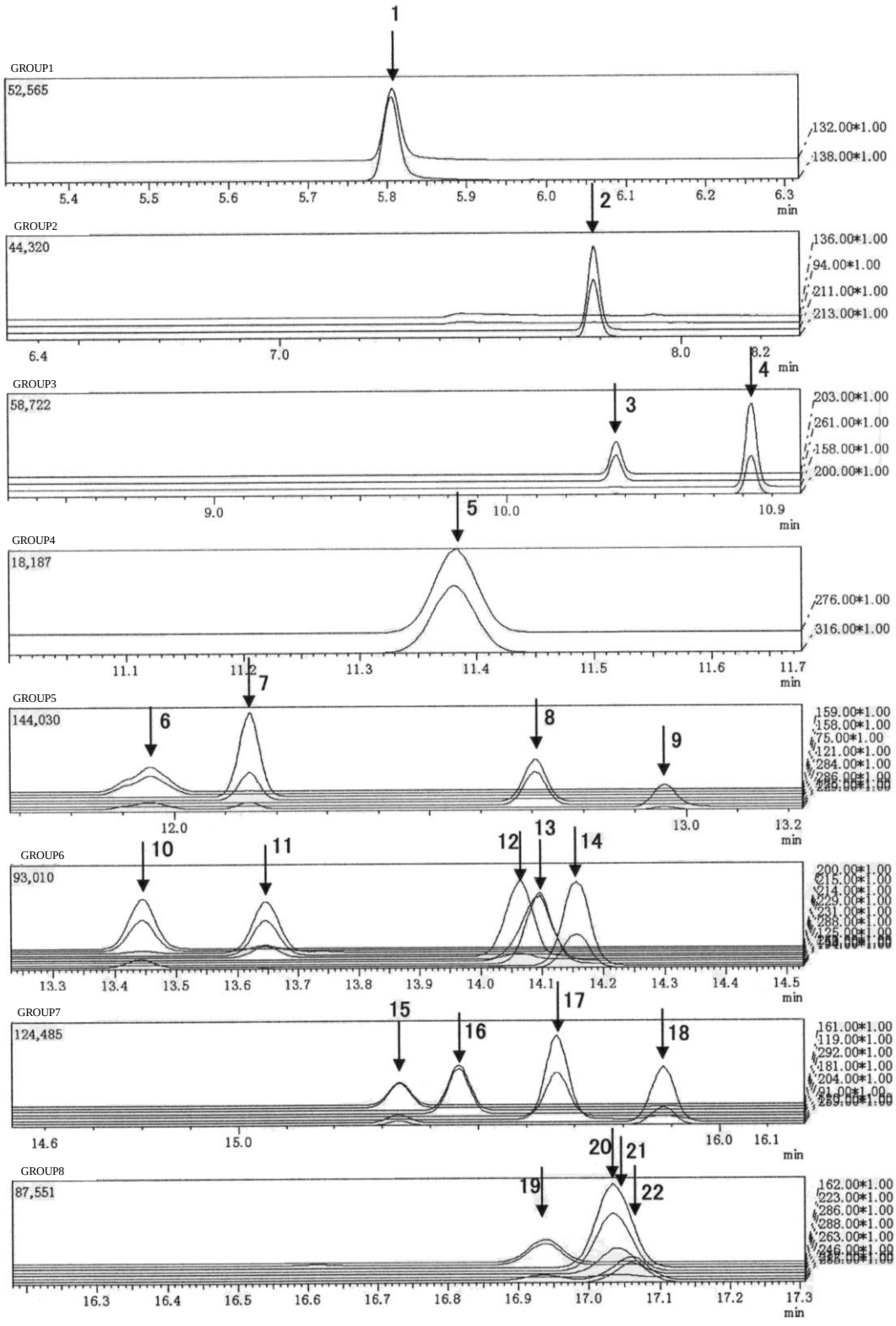
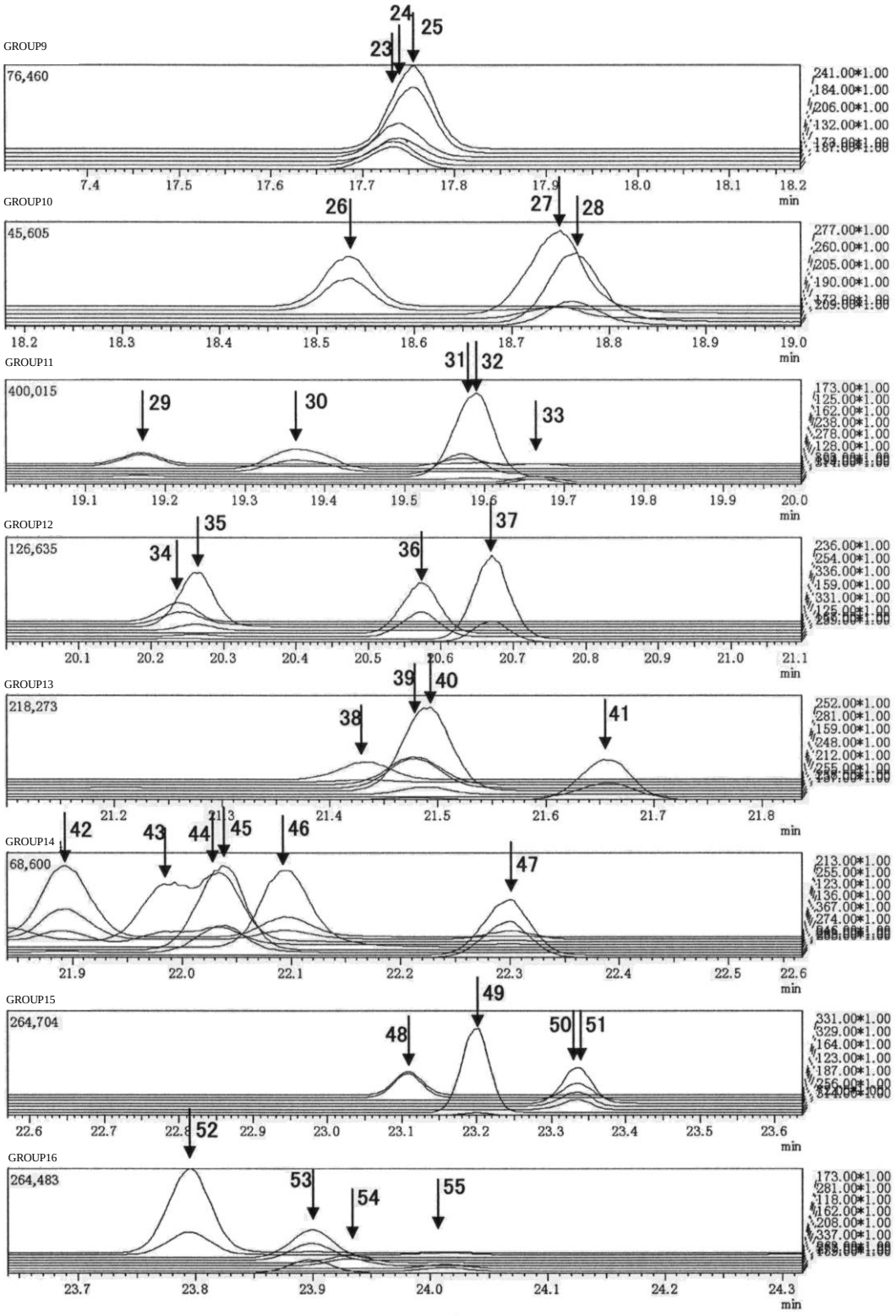
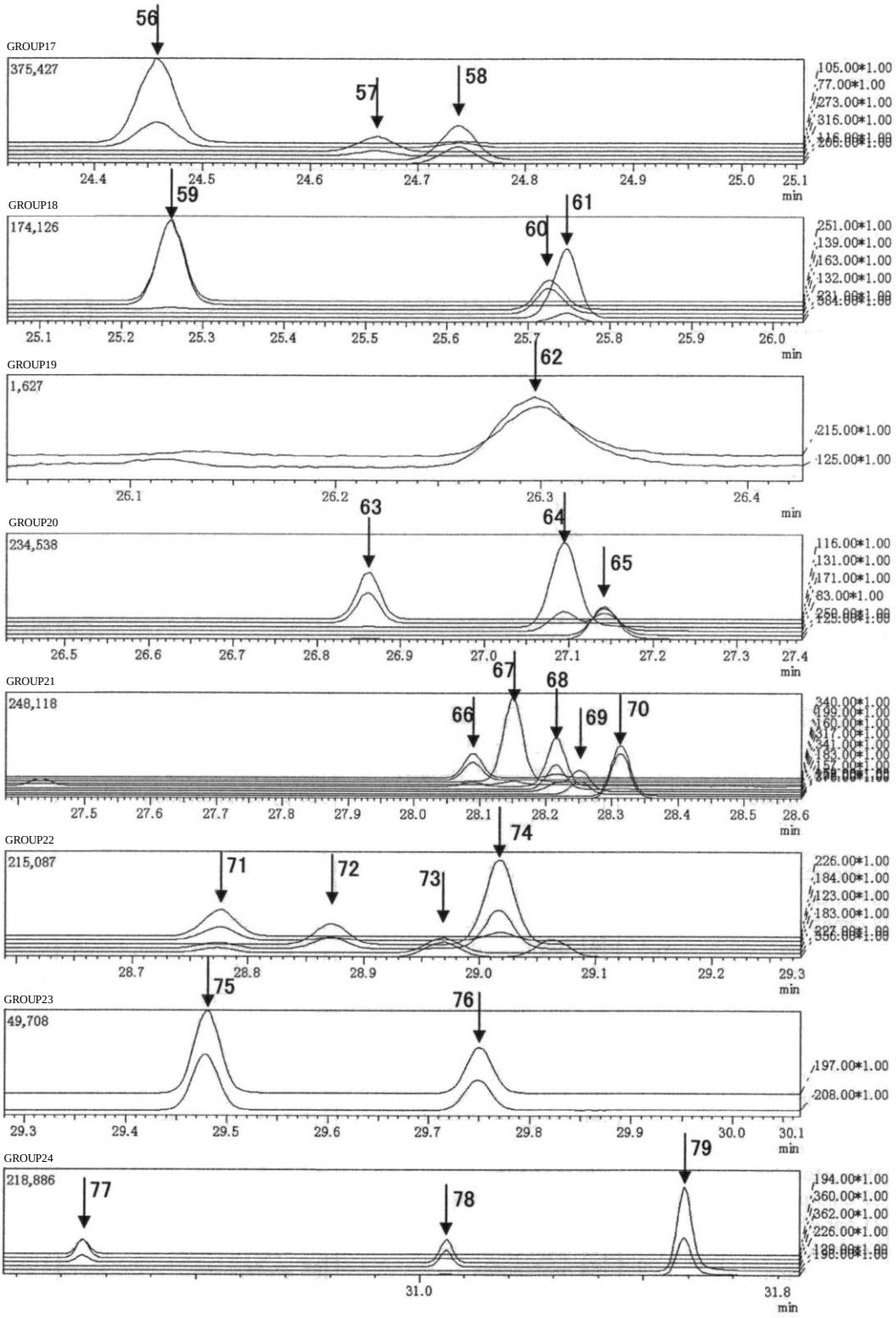


Figure 6.3.1-2. SIM chromatogram of group A agricultural chemicals standard solution (100 µg/mL of each agricultural chemical)
(Y-axis: Signal intensity, X-axis: Retention time.
Including compounds that are not listed in the Analytical Standards of Feeds)

1	dichlorvos	25	ametryn	48	napropamide	71	piperophos
2	mevinphos	26	terbutryn	49	butamifos	72	methoxychlor
3	propham	27	pirimiphos-methyl	50	hexaconazole	73	fenpropathrin
4	methacrifos	28	ethofumesate	51	*p*,*p*'-DDE	74	tebufenpyrad
5	propachlor	29	aldrin	52	metominostrobin (*E*- isome	75	phosalone
6	chlorpropham	30	thiobencarb	53	fludioxonil	76	azinphos-methyl
7	trifluralin	31	parathion	54	oxadiazon	77	pyriproxyfen
8	benfluralin	32	triadimefon	55	*o*,*p*'-DDD	78	fenarimol
9	α-BHC	33	chlorthal-dimethyl	56	endrin	79	acrinathrin
10	dicloran	34	fosthiazate	57	cyproconazole	80	*cis*-permethrin
11	β-BHC	35	fosthiazate	58	chlorfenapyr	81	pyridaben
12	γ-BHC	36	heptachlor epoxide	59	*p*,*p*'-DDD	82	*trans*-permethrin
13	quintozene	37	oxychlordane	60	*o*,*p*'-DDT	83	cyfluthrin
14	diazinon	38	chlorfenvifos (*E*- isomer)	61	edifenfos	84	cyfluthrin
15	δ-BHC	39	chlorfenvifos (*Z*- isomer)	62	quinoxyfen	85	cyfluthrin
16	terbacil	40	dimepiperate	63	carfentrazone-ethyl	86	cyfluthrin
17	tefluthrin	41	triadimenol	64	propiconazole	87	flucythrinate
18	triallate	42	triadimenol	65	*p*,*p*'-DDT	88	flucythrinate
19	propanil	43	*trans*-chlordane	66	propiconazole	89	silafluofen
20	phosphamidon	44	methidathion	67	diclofop-methyl	90	flumioxazin
21	dimethenamid	45	*o*,*p*'-DDE	68	propargite	91	fluvarinate
22	bromobutide	46	fenothiocarb	69	propargite	92	fluvarinate
23	heptachlor	47	*cis*-chlordane	70	bifenthrin	93	deltamethrin
24	alachlor						


GROUP1
52,565
132.00*1.00
138.00*1.00
5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 min
1
2
GROUP2
44,320
136.00*1.00
94.00*1.00
211.00*1.00
213.00*1.00
6.4 7.0 8.0 8.2 min
4
GROUP3
58,722
3
203.00*1.00
261.00*1.00
158.00*1.00
200.00*1.00
9.0 10.0 10.9 min
5
GROUP4
18,187
276.00*1.00
316.00*1.00
11.1 11.2 11.3 11.4 11.5 11.6 11.7 min
7
GROUP5
144,030
6
8
9
159.00*1.00
158.00*1.00
75.00*1.00
121.00*1.00
284.00*1.00
12.0 13.0 13.2 min
GROUP6
93,010
10
11
12
13
14
200.00*1.00
215.00*1.00
214.00*1.00
229.00*1.00
231.00*1.00
288.00*1.00
13.3 13.4 13.5 13.6 13.7 13.8 13.9 14.0 14.1 14.2 14.3 14.4 14.5 min
GROUP7
124,485
15
16
17
18
161.00*1.00
119.00*1.00
292.00*1.00
181.00*1.00
204.00*1.00
14.6 15.0 16.0 16.1 min
20
21
GROUP8
87,551
19
22
162.00*1.00
223.00*1.00
286.00*1.00
288.00*1.00
263.00*1.00
16.3 16.4 16.5 16.6 16.7 16.8 16.9 17.0 17.1 17.2 17.3 min


GROUP9
76,460
24
25
23
241.00*1.00
184.00*1.00
206.00*1.00
132.00*1.00
7.4 17.5 17.6 17.7 17.8 17.9 18.0 18.1 18.2 min
GROUP10
45,605
26
27
28
277.00*1.00
260.00*1.00
205.00*1.00
190.00*1.00
18.2 18.3 18.4 18.5 18.6 18.7 18.8 18.9 19.0 min
GROUP11
400,015
31
32
29
30
33
173.00*1.00
125.00*1.00
162.00*1.00
236.00*1.00
278.00*1.00
128.00*1.00
19.1 19.2 19.3 19.4 19.5 19.6 19.7 19.8 19.9 20.0 min
GROUP12
126,635
35
37
34
36
236.00*1.00
254.00*1.00
336.00*1.00
159.00*1.00
331.00*1.00
20.1 20.2 20.3 20.4 20.5 20.6 20.7 20.8 20.9 21.0 21.1 min
GROUP13
218,273
39
40
38
41
252.00*1.00
281.00*1.00
159.00*1.00
248.00*1.00
212.00*1.00
21.2 21.3 21.4 21.5 21.6 21.7 21.8 min
GROUP14
68,600
42
43
44
45
46
47
213.00*1.00
255.00*1.00
123.00*1.00
136.00*1.00
367.00*1.00
274.00*1.00
21.9 22.0 22.1 22.2 22.3 22.4 22.5 22.6 min
GROUP15
264,704
49
48
50
51
331.00*1.00
329.00*1.00
164.00*1.00
123.00*1.00
187.00*1.00
22.6 22.7 22.8 22.9 23.0 23.1 23.2 23.3 23.4 23.5 23.6 min
52
GROUP16
264,483
53
54
55
173.00*1.00
281.00*1.00
118.00*1.00
162.00*1.00
208.00*1.00
337.00*1.00
23.7 23.8 23.9 24.0 24.1 24.2 24.3 min


GROUP17
375,427
105.00*1.00
77.00*1.00
273.00*1.00
316.00*1.00
56
57
58
min
GROUP18
174,126
251.00*1.00
139.00*1.00
163.00*1.00
132.00*1.00
59
60
61
min
GROUP19
1,627
215.00*1.00
125.00*1.00
62
min
GROUP20
234,538
116.00*1.00
131.00*1.00
171.00*1.00
83.00*1.00
63
64
65
min
GROUP21
248,118
66
67
68
69
70
min
GROUP22
215,087
226.00*1.00
184.00*1.00
123.00*1.00
183.00*1.00
71
72
73
74
min
GROUP23
49,708
197.00*1.00
208.00*1.00
75
76
min
GROUP24
218,886
194.00*1.00
360.00*1.00
362.00*1.00
226.00*1.00
77
78
79
min

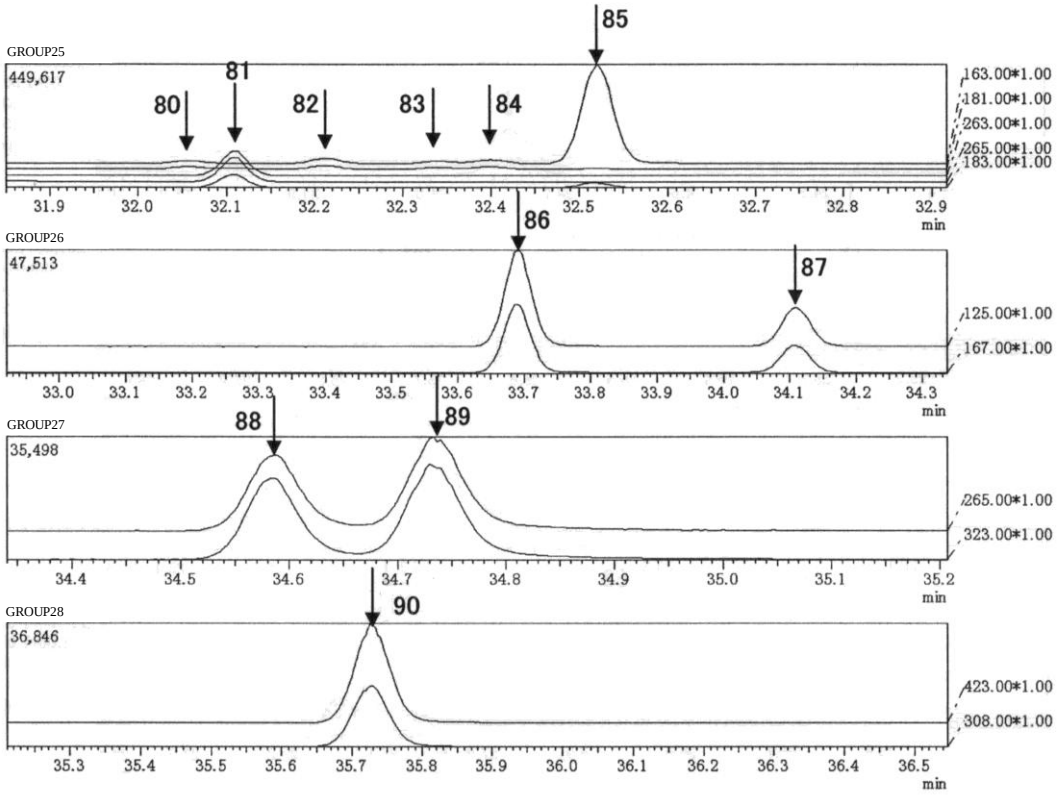


Figure 6.3.1-3. SIM chromatogram of group B agricultural chemicals standard solution (100 µg/mL of each agricultural chemical)
(Y-axis: Signal intensity, X-axis: Retention time.
Including compounds that are not listed in the Analytical Standards of Feeds)

1	allidochlor	24	prometryn	47	procymidone	69	EPN
2	etridiazole	25	metalaxyl	48	tetrachlorvinphos	70	picolinafen
3	tecnazene	26	fenitrothion	49	triazamate(reference)	71	anilofos
4	ethoprophos	27	bromacil	50	flutriafol	72	phenothrin
5	ethalfluralin	28	quinoclamine	51	imazamethabenz-methyl ester	73	tetradifon
6	cadusafos	29	malathion			74	phenothrin
7	phorate	30	metolaclor	52	flutolanil	75	cyhalothrin
8	hexachlorobenzene	31	fenthion	53	isoprothiolane	76	cyhalothrin
9	dimethoate	32	fenpropimorph	54	profenofos	77	pyraclofos
10	atrazine	33	chlorpyrifos	55	dieldrin	78	coumaphos
11	propazine	34	nitrothal-isopropyl	56	flamprop-methyl	79	fenbucobazole
12	terbufos	35	tetraconazole	57	bupirimate	80	cypermethrin
13	cyanophos	36	bromophos	58	kresoxim-methyl	81	halfenprox
14	propetamphos	37	diphenamid	59	chlorobanzilate	82	cypermethrin
15	isazophos	38	pendimethalin	60	oxadixyl	83	cypermethrin
16	etrimphos	39	dimethametryn	61	ethion	84	cypermethrin
17	iprobenfos	40	penconazole	62	azamethiphos	85	etfenprox
18	benoxacor	41	tolyfluanid	63	tryfluoxystrobin	86	fenvalerate
19	acetochlor	42	isofenphos	64	hexazinone	87	fenvalerate
20	chlorpyrifos-methyl	43	allethrin	65	tebuconazole	88	difenoconazole
21	parathion-methyl	44	fipronil	66	pyridaphenthion	89	difenoconazole
22	vinclozolin	45	allethrin	67	phosmet	90	flumiclorac pentyl
23	tridiphane (reference)	46	phenthoate	68	bromopropylate		

[12] In this method, as the wide acceptable range was defined, there are possibilities that the detected values deffer from the actual contents. When a detected agricultural chemical component can be analyzed by some other methods, it is advisable to try the other methods. In this regard, possible differences in the lower limit of detection as well as that of quantification among analysis methods should be considered.

[13] In this methid, peak height is used for quantification not to be affected by the peaks of interfering substances.

«Reference method»

During the development of this method, a laboratory conducted experiments by the use of Supercritical Fluid Extraction (SFE), which is as follows. However, this reference method has not yet been properly validated and the sample amount is small, so, it should be evaluated in each laboratory before use.

Quantification

Formula feed

Weigh 2 g of sample, add 250 µL of acetone as modifier and elute by SFE using hexane as a collecting solvent. Concentrate the collecting solvent, add acetonitrile and subject to liquid-liquid distribution. Concentrate the acetonitrile layer and dry up. Dissolve the residue by adding 1 mL of acetonitrile – toluene (3 : 1), load the solution on the Envi-carb/NH_2 cartridge and elute by adding 20 mL of acetonitrile – toluene (3 : 1), concentare and dry up. Dissolve the residue by adding 1 mL of 2,2,4-trimethylpentane – acetone (4 : 1) and subject to GC-MS.

Grass hay

Weigh 1 g of sample, add 250 µL of acetone as modifier and elute by SFE using acetone as a collecting solvent. Concentrate the collecting solvent and dry up. Dissolve the residue by adding 1 mL of acetonitrile – toluene (3 : 1), load the solution on the Envi-carb/NH_2 cartridge and elute by adding 20 mL of acetonitrile – toluene (3 : 1), concentrate and dry up. Dissolve the residue by adding 1 mL of 2,2,4-trimethylpentane – acetone (4 : 1) and subject to GC-MS.

Results of Analysis

Results are shown in the following Table.

		formula feed		grass hay	
		first	second	first	second
1	dichlorvos	95.5	86.5	95.6	100.6
2	mevinphos	124.5	118.5	111.0	111.0
3	propham	124.4	122.6	118.2	116.3
4	methacrifos	113.9	103.9	105.5	108.0
5	propachlor	114.8	107.8	104.0	107.4
6	chlorpropham	115.6	108.8	119.4	121.2
7	trifluralin	108.4	101.1	102.8	106.9
8	benfluralin	103.3	95.3	97.6	102.1
9	α-BHC	110.8	102.5	122.7	124.4
10	dicloran	104.9	98.2	67.4	67.6
11	β-BHC	105.9	98.3	101.7	97.8
12	γ-BHC	99.0	91.2	92.1	97.6
13	quintozene	100.3	92.1	99.4	104.4
14	diazinon	111.4	114.5	105.1	111.0
15	δ-BHC	114.5	89.2	86.2	78.4
16	terbacil	128.5	117.2	55.9	51.5
17	triallate	99.3	92.3	116.2	115.8
18	tefluthrin	117.1	110.5	120.1	124.1
19	propanil	98.2	96.2	42.9	41.5
20	phosphamidon	120.1	107.8	87.6	102.1
21	dimethenamid	117.0	110.7	109.9	115.4
22	bromobutide	111.8	112.2	99.2	111.1
23	heptachlor	98.6	87.0	92.2	97.6
24	alachlor	115.5	108.0	108.0	113.0
25	ametryn	109.0	102.8	90.5	86.4
26	terbutryn	108.3	102.4	98.7	102.6
27	pirimiphos-methyl	115.7	108.0	104.4	109.1
28	ethofumesate	123.4	127.6	106.6	107.5
29	aldrin	75.6	70.5	107.2	110.4
30	benthiocarb	119.7	117.3	123.4	127.9
31	parathion	111.5	99.0	95.6	100.6
32	triadimefon	115.6	111.3	103.3	109.6
33	chlorthal-dimethyl	112.1	105.6	109.8	113.3
34	fosthiazate	115.0	106.7	97.5	104.4
35	fosthiazate	138.2	113.9	124.8	126.9
36	heptachlor epoxyde	110.6	102.5	103.9	107.5
37	oxychlordane	111.7	107.9	100.6	116.6
38	chlorfenviphos (*E*- isomer)	123.9	113.2	119.7	121.0
39	dimepiperate	118.7	108.2	115.1	119.3
40	chlorfenvifos (*Z*- isomer)	118.4	109.5	105.0	111.7
41	triadimenol	144.5	138.9	115.2	112.4
42	triadimenol	103.4	93.0	100.4	92.7
43	*trans*-chlordane	111.2	103.5	106.3	110.0
44	methidathion	120.2	103.5	83.5	81.9
45	*o*,*p*'-DDE	102.4	94.2	105.5	108.8
46	fenothiocarb	121.3	113.6	117.0	128.0
47	*cis*-chlordane	108.0	100.6	103.1	107.5
48	napropamide	108.7	102.6	96.3	182.7
49	hexaconazole	127.7	111.2	90.3	94.7
50	butamifos	117.6	107.3	102.5	106.6
51	*p*,*p*'-DDE	108.7	93.6	111.4	114.0
52	metominostrobin (*E*- isomer)	120.7	110.9	99.3	101.0
53	fludioxonil	63.4	68.7	48.3	45.7
54	*o*,*p*'-DDD	109.6	104.3	105.1	109.7
55	oxadiazon	119.5	111.8	111.6	117.3
56	endrin	120.8	103.7	106.8	116.5
57	cyproconazole	128.5	124.9	110.3	117.2
58	chlorfenapyr	104.8	97.5	101.2	101.2
59	*p*,*p*'-DDD	109.6	100.8	110.5	114.0
60	*o*,*p*'-DDT	126.1	108.9	85.9	84.3
61	edifenphos	126.4	113.1	109.1	119.3
62	quinoxyfen	111.6	99.1	92.5	90.3
63	carfentrazone-ethyl	124.5	113.0	106.7	111.1
64	propiconazole	118.1	107.5	86.8	88.8
65	*p*,*p*'-DDT	126.7	110.0	107.6	110.2
66	propiconazole	118.6	107.3	148.4	161.5
67	methoxychlor	132.9	107.2	94.4	96.8
68	diclofop-methyl	114.5	119.2	106.6	109.8
69	propargite	117.3	101.4	83.0	82.1
70	piperophos	121.3	113.3	103.8	105.4
71	bifenthrin	111.4	102.9	111.5	114.5
72	fenpropathrin	118.4	112.2	108.9	108.5
73	tebufenpyrad	117.8	108.6	106.8	109.7
74	azinphos-methyl	146.6	113.7	81.7	74.0
75	phosalone	167.6	141.0	95.9	102.1
76	pyriproxyfen	127.0	117.7	124.2	125.7
77	fenarimol	124.0	111.9	79.5	71.8
78	acrinathrin	121.1	95.0	99.3	92.4
79	*cis*-permethrin	119.8	103.8	108.8	109.6
80	pyridaben	115.5	96.1	109.1	101.3
81	*trans*-permethrin	119.0	108.6	111.4	111.4
82	cyfluthrin	126.0	126.8	161.8	158.8
83	cyfluthrin	142.4	130.9	123.5	110.0
84	cyfluthrin	177.1	163.0	168.2	186.4
85	cyfluthrin	144.0	125.5	117.3	106.9
86	flucythrinate	141.5	124.1	135.0	132.5
87	flucythrinate	142.5	122.8	125.6	123.5
88	silafluofen	114.8	101.9	117.5	112.6
89	flumioxazin	122.9	102.0	86.0	81.3
90	fluvalinate	163.6	122.3	131.5	126.1
91	fluvalinate	139.5	97.5	105.1	97.2
92	deltamethrin	81.6	63.1	66.4	61.9

		formula feed		grass hay	
		first	second	first	second
1	allidochlor	130.0	125.4	124.3	126.3
2	etridiazole	96.7	93.5	94.6	97.5
3	tecnazene	103.3	98.6	103.9	107.6
4	ethoprophos	117.1	111.3	136.6	132.8
5	ethalfluralin	103.0	96.0	102.2	106.8
6	cadusafos	127.2	119.2	174.7	178.5
7	phorate	93.4	91.6	120.5	123.0
8	dimethoate	155.5	100.4	62.0	48.0
9	atrazine	119.2	114.6	105.6	103.0
10	propazine	111.6	112.7	116.6	115.3
11	terbufos	103.8	101.9	118.8	122.0
12	cyanophos	121.4	114.2	103.9	103.1
13	propetamphos	118.9	113.6	132.5	145.9
14	isazophos	145.4	138.5	159.1	171.9
15	etrimphos	132.2	125.2	132.0	136.0
16	iprobenfos	129.4	124.1	126.9	131.1
17	benoxacor	113.2	107.5	112.6	116.7
18	acetochlor	132.5	125.1	132.3	138.5
19	parathion-methyl	102.6	93.7	92.8	90.6
20	vinclozolin	110.7	105.0	112.7	117.7
21	prometryn	114.4	109.8	115.7	105.4
22	chlorpyrifos-methyl	110.0	103.8	107.3	109.6
23	metalaxyl	149.0	142.3	138.6	178.4
24	fenitrothion	110.1	107.1	93.5	96.2
25	quinoclamine	73.4	68.2	23.3	17.9
26	bromacil	114.6	120.1	52.5	53.2
27	malathion	127.9	108.6	115.7	111.5
28	metolachlor	121.6	116.4	121.4	123.2
29	fenthion	98.1	93.3	109.3	110.3
30	fenpropimorph	53.6	45.5	107.7	100.2
31	chlorpyrifos	114.4	109.6	115.4	118.3
32	tetraconazole	119.0	124.1	123.9	121.8
33	nitrothal-isopropyl	102.5	102.4	99.0	99.9
34	bromophos	114.2	104.7	115.0	115.6
35	diphenamid	118.7	112.8	114.1	111.9
36	pendimethalin	105.2	99.1	109.8	112.7
37	dimethametryn	122.7	124.6	118.4	123.0
38	penconazole	132.5	128.9	123.4	121.1
39	tolylfluanid	112.4	104.2	99.0	101.1
40	allethrin	197.1	194.5	-	-
41	allethrin	112.9	122.3	81.4	75.5
42	isofenphos	116.9	112.3	118.6	123.7
43	phenthoate	109.7	103.0	124.6	124.8
44	fipronil	107.9	103.3	79.4	83.7
45	procymidone	111.8	106.5	106.5	109.7
46	tetrachlorvinphos	121.7	117.7	115.2	118.5
47	flutriafol	94.8	-	105.9	94.4
48	imazamethabenz-methyl ester	51.6	-	62.2	52.3
49	flutolanil	124.7	122.3	122.5	118.8
50	isoprothiolane	114.8	125.8	115.4	109.7
51	profenofos	131.7	120.6	124.8	129.5
52	diedrin	108.4	116.0	120.6	122.9
53	flamprop-methyl	118.5	113.8	107.3	108.1
54	bupirimate	114.2	114.2	113.8	107.3
55	kresoxim-methyl	119.9	113.3	125.3	117.4
56	chlorobenzilate	124.8	120.0	119.9	123.4
57	oxadixyl	151.7	138.8	79.7	62.2
58	ethion	120.9	111.9	133.1	127.2
59	azamethiphos	141.0	116.1	87.4	57.7
60	trifloxystrobin	121.6	119.9	113.0	122.1
61	hexazinone	109.1	106.2	62.9	57.6
62	tebuconazole	139.5	135.9	100.5	95.5
63	pyridaphenthion	121.3	110.4	101.8	93.2
64	phosmet	118.3	97.1	93.9	82.3
65	bromopropyrate	121.5	117.2	114.1	116.6
66	EPN	112.6	116.0	87.4	94.1
67	picolinafen	114.6	111.6	121.0	122.6
68	anilofos	111.3	111.1	99.4	92.8
69	tetradifon	108.1	110.9	106.9	110.0
70	phenothrin	113.8	118.9	120.8	121.7
71	phenothrin	114.4	100.8	128.5	135.1
72	cyhalothrin	115.3	104.8	112.6	111.0
73	cyhalothrin	153.4	160.4	124.9	129.6
74	pyraclofos	132.2	119.9	135.3	130.4
75	coumaphos	124.8	113.3	118.9	97.6
76	fenbuconazole	123.8	121.9	228.4	262.7
77	halfenprox	98.4	96.9	106.2	100.7
78	cypermethrin	132.4	93.8	80.0	90.3
79	cypermethrin	150.0	134.9	158.5	114.5
80	cypermethrin	114.0	110.3	342.0	225.4
81	cypermethrin	238.4	141.5	226.7	294.6
82	etofenprox	118.9	115.6	116.5	122.2
83	fenvalerate	115.0	104.2	134.2	130.8
84	fenvalerate	186.1	193.5	170.1	179.2
85	difenoconazole	158.5	153.9	118.8	117.8
86	difenoconazole	148.2	141.4	97.4	97.8
87	flumiclorac pentyl	115.3	110.1	113.2	113.5

2. Simultaneous analysis method of organochlorine agricultural chemicals by gas chromatography

[Analytical Standards of Feeds, Chapter 6, Section 3, Article 2]

Target Analytes: *α*-BHC, *β*-BHC, *γ*-BHC, *δ*-BHC, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, aldrin, endrin, dieldrin, heptachlor and heptachlor epoxide (15 compounds)

A. Reagent Preparation

1) Mixed standard solution. Weigh accurately 20 mg each of *α*-BHC [$C_6H_6Cl_6$][1], *β*-BHC [$C_6H_6Cl_6$][1], *γ*-BHC [$C_6H_6Cl_6$][1], *δ*-BHC [$C_6H_6Cl_6$][1], *o,p'*-DDD [$C_{14}H_{10}Cl_4$][1], *p,p'*-DDD [$C_{14}H_{10}Cl_4$][1], *o,p'*-DDE [$C_{14}H_8Cl_4$][1], *p,p'*-DDE [$C_{14}H_8Cl_4$][1], *o,p'*-DDT [$C_{14}H_9Cl_5$][1], *p,p'*-DDT [$C_{14}H_9Cl_5$][1], aldrin [$C_{12}H_8Cl_6$][1], endrin [$C_{12}H_8Cl_6O$][1], dieldrin [$C_{12}H_8Cl_6O$][1], heptachlor [$C_{10}H_5Cl_7$][1] and heptachlor epoxide [$C_{10}H_5Cl_7O$][1]. Transfer each of them to a 100 mL volumetric flask and dissolve by adding 20 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks to prepare the standard stock solutions (Each 1 mL of these solutions contains 0.2 mg of the respective agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare the mixed standard solutions containing 0.05 – 0.2 μg each of the respective agricultural chemicals per 1 mL.

2) Magnesium silicate. Dry synthetic magnesium silicate (particle size 149-250 μm (100-60 mesh)) at 130 °C for 5 hours.

B. Quantification

Extraction. Weigh 10.0 – 50.0 g of the sample, transfer it to a 500 mL separating funnel. Add it 300 mL of acetonitrile – water (13 : 7) and extract by shaking for 30 minutes. Place a beaker under a Büchner funnel[2] and filter the extract through a filter paper (No. 5B) by suction.

Purification. Transfer 150 mL of the extract[3] to a 1 L separating funnel A already containing 600 mL of sodium chloride solution (5 w/v%) and 100 mL of hexane, shake vigorously for 5 minutes and leave to stand. Transfer the water layer (lower layer) to a 1 L separating funnel B, add 50 mL of hexane, shake gently and leave to stand. Discard the water layer and transfer the hexane layer (upper layer) to the separating funnel A. Then, add 100 mL of water to the separating funnel A, shake gently and leave to stand. Transfer the water layer to a 500 mL separating funnel C. Add 100 mL of water to the separating funnel A and operate in the similar way. Transfer the water layer to the separating funnel C, and the hexane layer to a 500 mL Erlenmeyer flask, respectively. Add 100 mL of hexane to the separating funnel C, shake gently and leave to stand. Discard the water layer and combine the hexane layer (upper layer) with the content of the Erlenmeyer flask. Dehydrate the hexane layer with a suitable amount of sodium sulfate (anhydrous)[4] and filter into a 500 mL recovery flask through filter paper (No. 2S). Wash the Erlenmeyer flask and the filter paper with a small amount of hexane sequentially, filter the washings through the filter paper and combine with the filtrate. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to be around 5 mL to be subjected to column treatment.

Column treatment. Suspend 9 g of magnesium silicate and 3 g of sodium sulfate (anhydrous) respectively in hexane, pour the suspensions into a column (15 mm inner diameter) sequentially, add 40 mL of hexane and elute so that the liquid level reaches to the height of 3 mm from the upper end of the column

packing material to prepare a column.

Place a 300 mL recovery flask under the column and load the sample solution on the column. Wash the recovery flask that had contained the sample solution with a small amount of hexane, add the washings to the column and elute agricultural chemicals by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Further, add 150 mL of hexane – diethyl ether (17 : 3) to the column to elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 3 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution for gas chromatography.

Gas chromatography. Inject 1 μL each of the sample solution and respective mixed standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Electron capture detector

Column: Fused silica capillary column (100 % cyanopropylphenyl polysiloxane coating, 0.32 mm in inner diameter, 30 m in length, film thickness 0.25 μm)[5]

Carrier gas: He (1.5 mL/min)

Make up gas: N_2 (60 mL/min)

Sample injection: Cool on column

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 60 °C (hold 1 min) → ramp 20 °C/min → 180 °C (hold 1 min) → ramp 2 °C/min → 220 °C (hold 1 min) → ramp 1 °C/min → 250 °C

Detector temperature: 280 °C

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[6] to prepare a calibration curve and subsequently calculate the amount of the respective agricultural chemicals in the sample.

«Summary of analysis method»

This method is a simultaneous analysis method for organochlorine agricultural chemicals such as BHC, DDT and the like.

Each agricultural chemical in feeds is extracted with acetonitrile/water, purified by liquid-liquid extraction and a magnesium silicate column and quantified by a gas chromatograph equipped with an electron capture detector.

The flow sheet of the analysis method is shown in Figure 6.3.2-1.

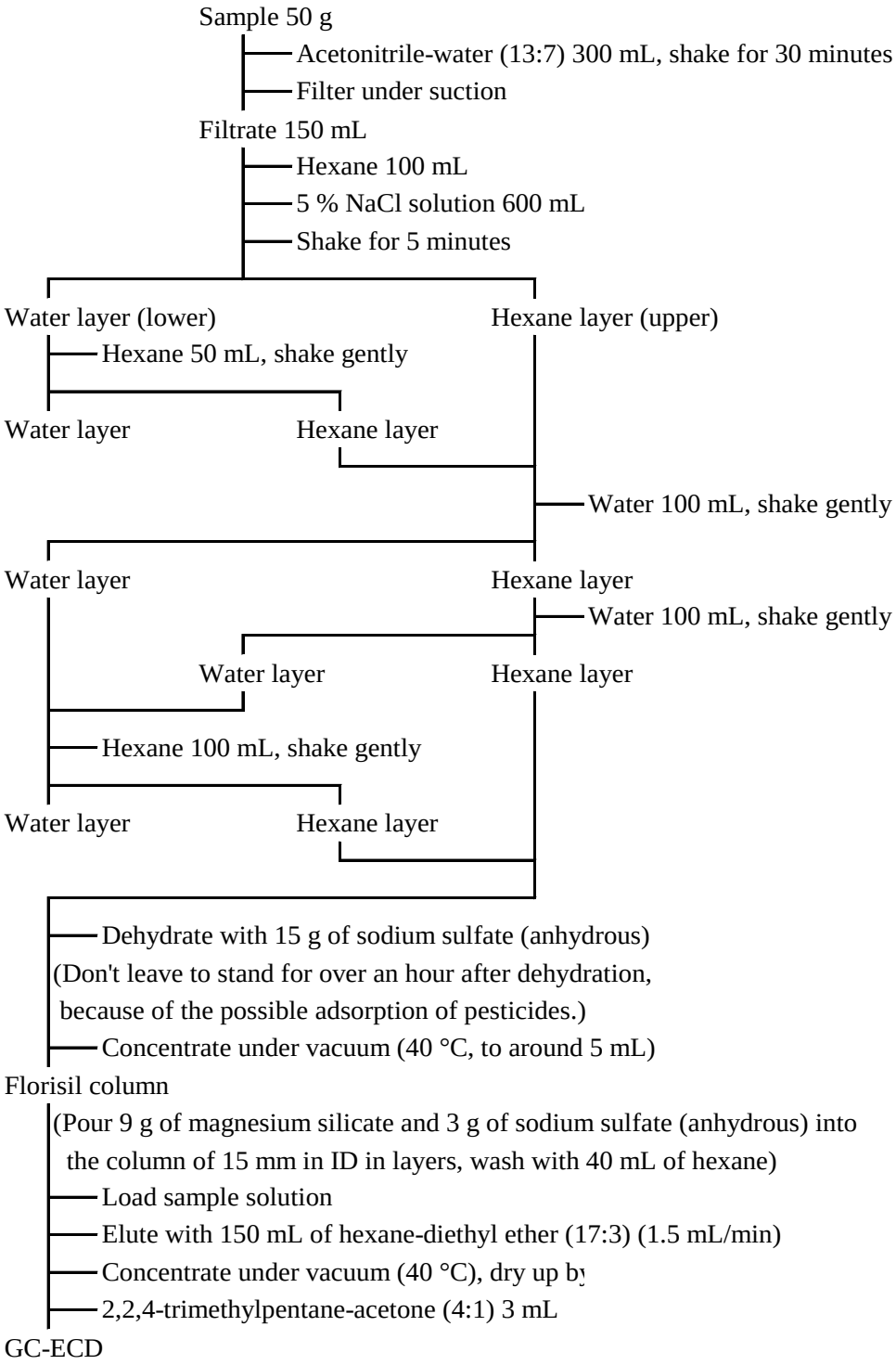


Figure 6.3.2-1. Flow sheet of the simultaneous analysis method for organochlorine agricultural chemicals

Reference: Yukinobu Nakamura, Soichiro Matsumura, Takeo Kozu, Yuji Fukumoto: Research Report of Animal Feed, 13, 15 (1988).

Yuji Shirai, Reiko Kazama: Research Report of Animal Feed, 22, 48 (1997).

«Method validation»

• Spike recovery and repeatability

Compound	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
α-BHC	corn	10~100	3	93.3~96.1	19.8
	formula feed	10~100	3	81.7~88.8	10.1
β-BHC	corn	10~100	3	104.5~112.8	13.1
	formula feed	10~100	3	103.4~106.1	11.7
γ-BHC	corn	10~100	3	97.0~105.7	14.1
	formula feed	10~100	3	81.1~92.5	11.6
δ-BHC	corn	10~100	3	110.6~118.1	7.4
	formula feed	10~100	3	92.6~103.1	6.6
o,*p*'-DDD	corn	10~100	3	115.9~117.4	6.6
	formula feed	10~100	3	101.6~109.1	7.5
p,*p*'-DDD	corn	10~100	3	90.9~99.8	24.8
	formula feed	10~100	3	86.6~90.1	7.3
o,*p*'-DDE	corn	10~100	3	100.1~104.2	9.6
	formula feed	10~100	3	88.9~94.1	8.9
p,*p*'-DDE	corn	10~100	3	87.9~97.2	12.1
	formula feed	10~100	3	77.9~86.1	9.0
o,*p*'-DDT	corn	10~100	3	91.7~97.3	14.5
	formula feed	10~100	3	85.8~87.9	6.4
p,*p*'-DDT	corn	10~100	3	103.5~117.8	16.4
	formula feed	10~100	3	101.4~107.5	6.3
aldrin	corn	10~100	3	79.6~84.4	14.9
	formula feed	10~100	3	73.7~74.2	4.2
endrin	corn	10~100	3	81.1~87.8	15.5
	formula feed	10~100	3	98.0~103.9	8.0
dieldrin	corn	10~100	3	105.6~109.4	9.0
	formula feed	10~100	3	99.4~105.8	4.9
heptachlor	corn	10~100	3	80.5~86.6	11.4
	formula feed	10~100	3	82.5~84.2	16.8
heptachlor epoxide	corn	10~100	3	83.3~99.3	8.9
	formula feed	10~100	3	81.8~89.5	7.0

• Collaborative study

Compound	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
α-BHC	adult hen formula feed	3	50	94.1	2.8	8.2	0.37
β-BHC	adult hen formula feed	3	50	104.6	4.7	9.4	0.43
γ-BHC	adult hen formula feed	3	50	98.0	4.9	3.6	0.16
δ-BHC	adult hen formula feed	3	50	99.8	8.0	5.7	0.26
o,*p*'-DDD	adult hen formula feed	3	50	89.1	4.8	7.7	0.35
p,*p*'-DDD	adult hen formula feed	3	50	88.7	4.7	11.0	0.50
o,*p*'-DDE	adult hen formula feed	3	50	80.4	4.8	4.4	0.20
p,*p*'-DDE	adult hen formula feed	3	50	73.3	6.2	12.2	0.56
o,*p*'-DDT	adult hen formula feed	3	50	74.4	5.9	7.1	0.32
p,*p*'-DDT	adult hen formula feed	2	50	81.7	5.2	10.3	0.47
aldrin	adult hen formula feed	3	50	77.5	10.2	13.8	0.63
endrin	adult hen formula feed	3	50	89.8	5.9	16.9	0.77
dieldrin	adult hen formula feed	3	50	93.7	5.9	13.6	0.62
heptachlor	adult hen formula feed	2	50	80.8	6.3	12.2	0.55
heptachlor epoxide	adult hen formula feed	3	50	91.1	4.8	11.4	0.52

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers.

[2] Use a Kiriyama funnel of about 9 cm bore.

[3] When the extract is likely to form into an emulsion during the liquid-liquid extraction in the purification process, transfer 150 mL of the extract to a 500 mL recovery flask and concentrate under reduced pressure on a water bath at 40 °C or lower to remove most of acetonitrile in the extract before use, so that the extract becomes less likely to emulsify.

In this case, the procedures to "add 100 mL of water to the separating funnel A and operate in the similar way" in the quantification process can be skipped.

[4] Leaving the hexane layer mixed with sodium sulfate for over an hour after dehydration may lead to adsorption of BHC on sodium sulfate particles.

[5] For example, DB-1701 (Agilent Technologies).

[6] Example of chromatogram is shown in Figure 6.3.2-2.

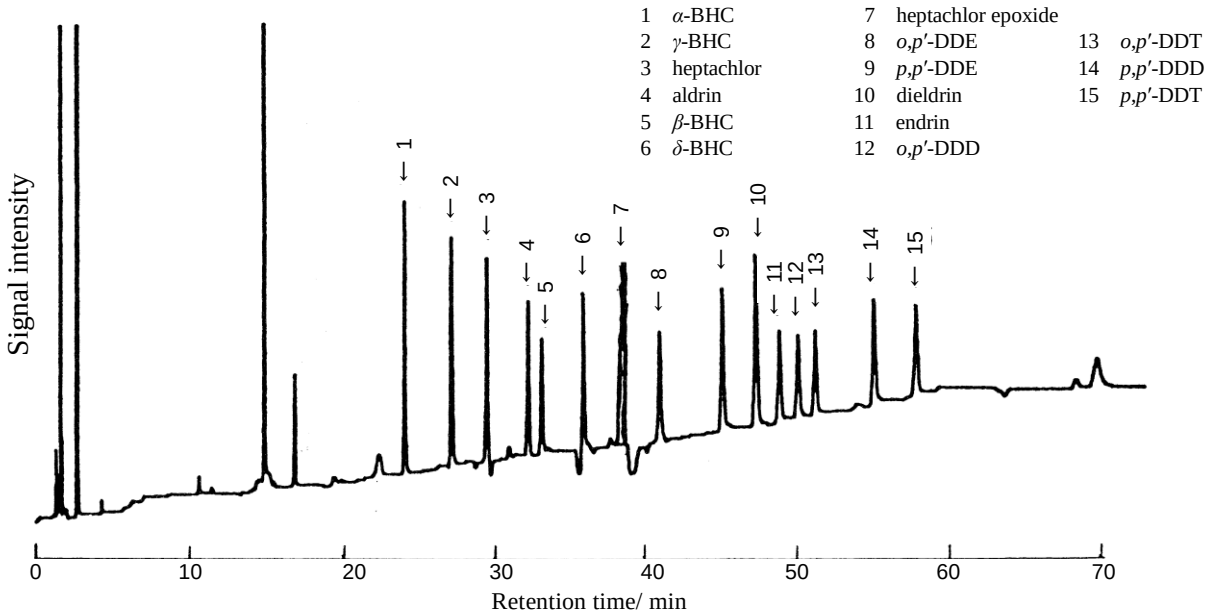


Figure 6.3.2-2. Chromatogram of corn spiked with various organochlorine agricultural chemicals equivalent to 10 µg/kg

Measurement conditions

Detector: Electron capture detector (ECD)

Column: DB-5 (0.32 mm in inner diameter, 30 m in length, film thickness 0.25 µm)

Carrier gas: He (0.7 kg/cm^2)

Make up gas: N_2 (60 mL/min)

Sample injection: Cool on

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 60 °C (hold 1 min) → ramp 20 °C/min → 180 °C (hold 1 min) → ramp 2 °C/min → 220 °C(hold 1 min) → ramp 1 °C/min→250 °C

Detector temperature: 280 °C

3. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)

[Analytical Standards of Feeds, Chapter 6, Section 3, Article 3]

Target Analytes: XMC, aldicarb (including aldicarb sulfoxide and aldicarb sulfone[*1]), isoprocarb, carbaryl, carbofuran, xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb (12 compounds)

A. Reagent Preparation

Mixed standard solution. Weigh accurately 20 mg each of XMC [$C_{10}H_{13}NO_2$][1], aldicarb [$C_7H_{14}N_2O_2S$][1], aldicarb sulfoxide [$C_7H_{14}N_2O_3S$][1], aldicarb sulfone [$C_7H_{14}N_2O_4S$][1], isoprocarb [$C_{11}H_{15}NO_2$][1], carbaryl [$C_{12}H_{11}NO_2$][1], carbofuran [$C_{12}H_{15}NO_3$][1], xylylcarb [$C_{10}H_{13}NO_2$][1], fenobucarb [$C_{12}H_{17}NO_2$][1], propoxur [$C_{11}H_{15}NO_3$][1], bendiocarb [$C_{11}H_{13}NO_4$][1] and metolcarb [$C_9H_{11}NO_2$][1]. Transfer each of them to a 100 mL volumetric flask and dissolve by adding acetonitrile respectively. Further, add the same solvent up to the graduation line of the respective flasks to prepare the standard stock solutions (Each 1 mL of these solutions contains 0.2 mg of the respective agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding acetonitrile to prepare the mixed standard solutions containing 0.1 – 3 µg each of the respective agricultural chemicals per 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 15 mL of water to moisten and leave to stand for 30 minutes. Further, add it 100 mL of acetonitrile and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 40 mL of acetonitrile sequentially, and filter the washings by suction in the similr way.

Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to be around 15 mL and add 5 g of sodium chloride to prepare a sample solution to be subjected to column treatment I.

Column treatment I. Load the sample solution on a porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes.

Place a 300 mL recovery flask under the column, wash the recovery flask that had contained the sample solution three times with 10 mL each of ethyl acetate, add the wash to the column in order of precedence and elute by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 120 mL of ethyl acetate to the column and elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue with 10 mL of cyclohexane – acetone (7 : 3) accurately, transfer the solution to a 10 mL centrifufe tube and centrifuge at 1,500×g for 5 minutes. Filter the supernatant through a membrane filter (pore size: 0.5 µm or less) and use the filtrate as sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph[2]. Dispense each elution fraction to be quantified into a 200 mL recovery flask, concentrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of ethyl acetate – methanol (99 : 1) accuratelyand use this solution as a sample solution for column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 300 mm in length, particle size 15 µm)

Guard column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 100 mm in length, particle size 15 µm)

Eluent: Cyclohexane – acetone (7 : 3)

Flow rate: 5 mL/min

Fraction collection: 65-115 mL

Column treatment II. Connect a graphite carbon minicolumn (250 mg) to the bottom of an aminopropylsilylated silica gel minicolumn (360 mg)[*2] and wash with 10 mL of ethyl acetate – methanol (99 : 1).

Place a 50 mL recovery flask under the minicolumn and load accurately 2 mL of the sample solution on the minicolumn and elute the agricultural chemicals by positive pressure[*3] so that the liquid level reaches the upper end of the column packing material. Further add 20 mL of ethyl acetate – methanol (99 : 1) to the minicolumn and elute by positive pressure[*3] in the similar way. Concentrate the effluent under reduced pressure in a water bath at 40 °C or lower to almost dryness, and then dry up by nitrogen gas flow.

Dissolve the residue by adding 1 mL of acetonitrile accurately, filter the solution through a membrane filter (pore size: 0.5 µm or less), and use the filtrate as a sample solution for liquid chromatography.

Liquid chromatography[3]. Inject 20 µL each of the sample solution and respective mixed standard solutions into a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Fluorescence detector (excitation wavelength: 340 nm,emission wavelength: 445 nm)

Column: Octadecylsilylated silica gel column (3.9 mm in inner diameter, 150 mm in length, particle size 4 µm)[*4] [4]

Eluent: Water – methanol (22 : 3) → 0.1 min → water – tetrahydrofuran (9 : 1) → 29.9 min → water – tetrahydrofuran (7 : 3) → 10 min → water – methanol (22 : 3)[5]

Reaction solutions[*5]: Solution I (alkaline solution)[6]: Dissolve 1 g of sodium hydroxide in water to be 500 mL.

Solution II (OPA reagent)[7]: Dissolve 50 mg of *o*-phthalaldehyde with 5 mL of methanol, add sodium borate solution (dissolve 19.1 g of sodium tetraborate decahydrate in water to be 1 L) to be 500 mL and mix with 50 µL of 2-mercaptoethanol (prepare before use).

Flow rate: Eluent 1.0 mL/min, Reaction solutions 0.3 mL/min respectively.

Temperature: Column oven 40 °C, Reaction chamber 80 °C

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[8] to prepare a calibration curve and subsequently calculate the amount of the respective agricultural chemicals in the sample.

The amount of aldicarb in the sample is calculated from the respective calculated amount of aldicarb, aldicarb sulfoxide and aldicarb sulfone using the following equation.

The amount of aldicarb in sample (μg/kg) $= (A + B \times 0.922 + C \times 0.856) \times 25$ [9][10]

A : The weight of aldicarb determined from the calibration curve (ng)

B : The weight of aldicarb sulfoxide determined from the calibration curve (ng)

C : The weight of aldicarb sulfone determined from the calibration curve (ng)

* 1. Aldicarb sulfoxide and aldicarb sulfone are oxidative metabolites of aldicarb.

2. Supelclean ENVI-Carb (reservoir volume 6 mL, Supelco) or equivalents.

3. Flow rate should be 1-2 mL/min.

4. Carbamate Analysis (Waters) or equivalents.

5. Combine the reaction solution I with the eluate from the column and hydrolyze agricultural chemicals within a reaction coil in the reaction chamber. Let the hydrolyzed solution cool to the room temperature, combine with the reaction solution II for fluorescence derivatizing and transfer to the fluorescence detector immediately.

Use a reaction coil, RXN 1000 Coil (0.5 mm in inner diameter, about 5 m in length; injection volume 1 mL, Teflon, Waters) or equivalents.

«Summary of analysis method»

This method is a simultaneous analysis method for XMC, aldicarb (including its oxidative metabolites), isoprocarb, carbaryl, carbofuran, xylylcarb, bendiocarb, metolcarb, fenobucarb and propoxur.

Each agricultural chemical in sample is extracted with acetonitrile/water, purified by the use of a porous diatomite column, GPC, an aminopropylsilylated silica gel minicolumn and a graphite carbon minicolumn and quantified by gradient elution liquid chromatography with post-column fluorescence derivatization using OPA reagent.

The flow sheet of the analysis method is shown in Figure 6.3.3-1.

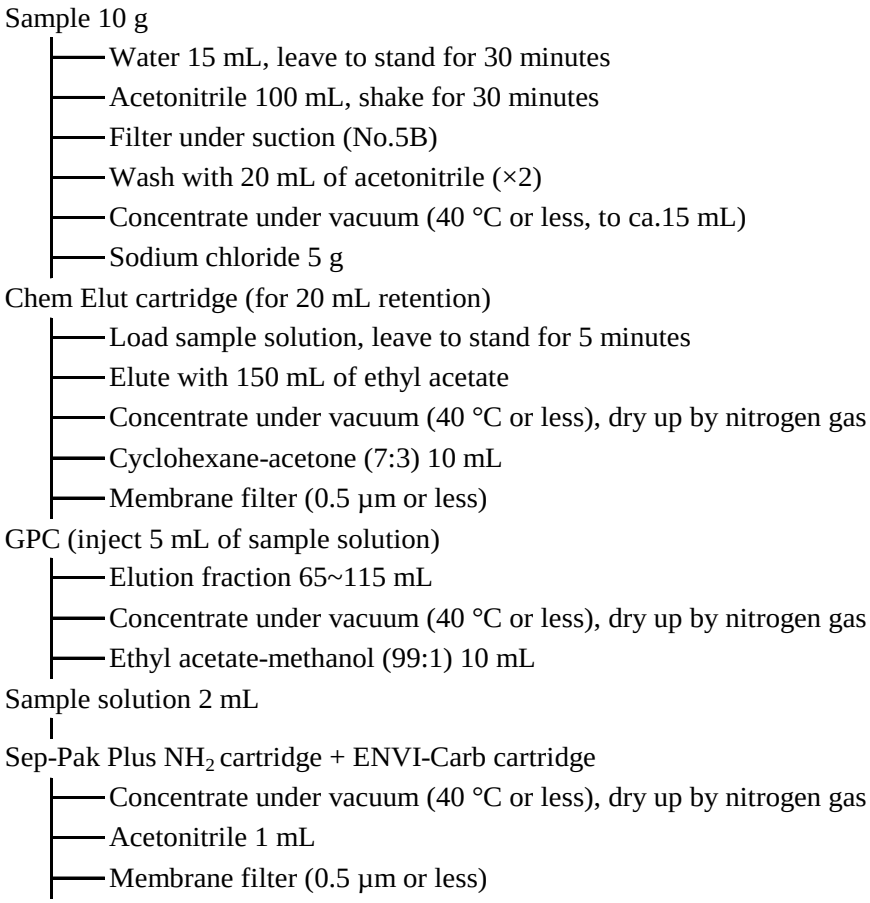


Figure 6.3.3-1. Flow sheet of the simultaneous analysis method for carbamate agricultural chemicals by LC (1)

Reference: Norio Aita: Research Report of Animal Feed, 23, 1 (1998).

«Method validation»

• Spike recovery and repeatability

Compound	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
XMC	growing chick formula feed	200~1,000	3	94.7~103.3	13.0
	dairy cattle formula feed	200~1,000	3	97.7~102.3	4.7
	oats hay	200~1,000	3	90.3~93.7	13.2
aldicarb	growing chick formula feed	200~1,000	3	87.0~96.0	13.5
	dairy cattle formula feed	200~1,000	3	90.0~92.7	5.7
	oats hay	200~1,000	3	46.0~63.0	28.2
aldicarb sulfoxide	growing chick formula feed	200~1,000	3	74.0~79.3	21.1
	dairy cattle formula feed	200~1,000	3	68.7~74.0	15.6
	oats hay	200~1,000	3	78.7~91.0	20.1
aldicarb sulfone	growing chick formula feed	200~1,000	3	78.7~86.0	14.1
	dairy cattle formula feed	200~1,000	3	79.3~82.0	14.2
	oats hay	200~1,000	3	79.0~88.3	15.9
isoprocarb	growing chick formula feed	200~1,000	3	96.7~103.3	15.0
	dairy cattle formula feed	200~1,000	3	100.7~105.3	3.5
	oats hay	200~1,000	3	84.7~96.3	15.0
carbaryl	growing chick formula feed	200~1,000	3	95.3~102.7	12.5
	dairy cattle formula feed	200~1,000	3	97.7~105.0	1.6
	oats hay	200~1,000	3	87.0~99.3	9.4
carbofuran	growing chick formula feed	200~1,000	3	97.7~105.0	14.5
	dairy cattle formula feed	200~1,000	3	93.0~103.7	2.8
	oats hay	200~1,000	3	88.7~90.7	10.4
xylylcarb	growing chick formula feed	200~1,000	3	94.7~107.3	8.2
	dairy cattle formula feed	200~1,000	3	101.3~102.3	5.6
	oats hay	200~1,000	3	88.7~93.7	8.0
fenobucarb	growing chick formula feed	200~1,000	3	91.7~102.0	12.4
	dairy cattle formula feed	200~1,000	3	99.0~103.0	3.0
	oats hay	200~1,000	3	87.0~91.7	11.1
propoxur	growing chick formula feed	200~1,000	3	93.3~102.3	13.8
	dairy cattle formula feed	200~1,000	3	100.3~101.3	10.7
	oats hay	200~1,000	3	84.0~91.3	8.7
bendiocarb	growing chick formula feed	200~1,000	3	99.3~104.3	13.7
	dairy cattle formula feed	200~1,000	3	98.3~103.3	5.2
	oats hay	200~1,000	3	87.0~95.0	9.1
metolcarb	growing chick formula feed	200~1,000	3	87.3~100.0	16.0
	dairy cattle formula feed	200~1,000	3	97.7~100.0	6.1
	oats hay	200~1,000	3	86.7~91.0	9.8

• Collaborative study

Compound	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
XMC	adult hen formula feed	3	500	106.2	1.9	3.4	0.19
	alfalfa	3	500	98.4	5.9	5.4	0.30
aldicarb	adult hen formula feed	3	500	108.2	2.2	17.9	1.02
	alfalfa	3	500	66.2	15.5	36.4	1.93
aldicalb sulfoxide	adult hen formula feed	3	500	102.0	2.0	18.1	1.02
	alfalfa	3	500	107.3	21.2	22.9	1.30
aldicarb sulfone	adult hen formula feed	3	500	105.6	8.3	12.4	0.70
	alfalfa	3	500	90.4	3.6	15.4	0.86
isoprocarb	adult hen formula feed	3	500	104.7	2.1	4.2	0.24
	alfalfa	3	500	97.3	3.1	9.9	0.56
carbaryl	adult hen formula feed	3	500	106.4	1.7	3.3	0.19
	alfalfa	3	500	97.3	3.1	7.8	0.44
carbofuran	adult hen formula feed	3	500	107.6	0.6	1.6	0.09
	alfalfa	3	500	97.8	5.9	7.5	0.42
xylylcarb	adult hen formula feed	3	500	105.1	1.4	3.8	0.22
	alfalfa	3	500	96.7	5.4	5.9	0.33
fenocarb	adult hen formula feed	3	500	105.3	1.8	4.1	0.23
	alfalfa	3	500	96.2	4.4	7.0	0.39
propoxur	adult hen formula feed	3	500	106.4	1.8	3.0	0.17
	alfalfa	3	500	100.0	3.3	10.1	0.57
bendiocarb	adult hen formula feed	3	500	106.2	1.5	3.3	0.19
	alfalfa	3	500	96.0	3.9	7.3	0.41
metolcarb	adult hen formula feed	3	500	104.0	2.1	3.2	0.18
	alfalfa	3	500	96.0	3.9	6.6	0.37

• Lower limit of quantification: 25 μg/kg each in sample for XMC, aldicarb, isoprocarb, carbaryl and carbofuran; 10 μg/kg each in sample for xylylcarb, fenobucarb, propoxur, bendiocarb and metolcarb

«Notes and precautions»

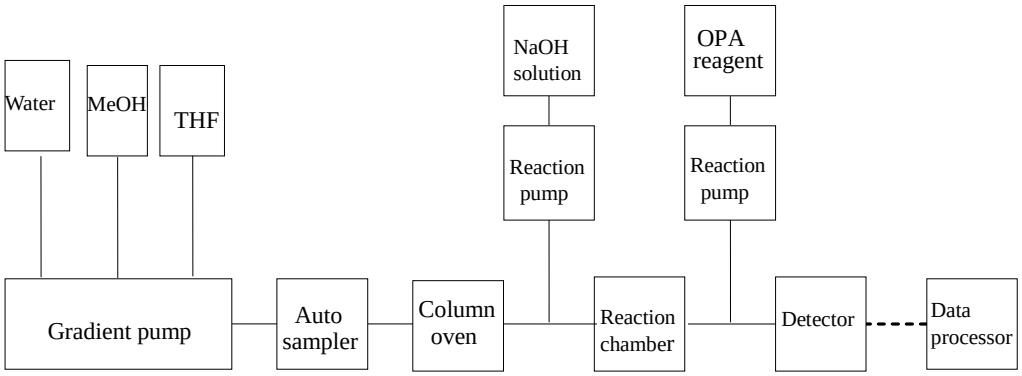
[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers.

[2] For gel permeation chromatography (GPC), see also [6] and [7] of **«Notes and precautions»** in Article 1, Section 2 “Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography” in this chapter.

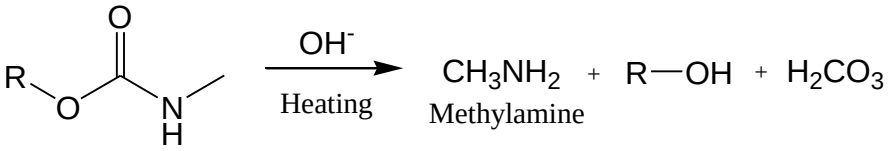
[3] XMC etc. separately eluted from the dedicated column using gradient elution are hydrolyzed by heating in the presence of sodium hydroxide to produce methylamines, which are then converted to fluorescent derivatives by reaction with OPA reagent and 2-mercaptoethanol. The obtained fluorescent derivatives are quantified by a fluorescence detector.

As the fluorescent derivatives are unstable at high temperature, it is required to let them cool to room temperature. This method requires a complex system which consists of a gradient pump, an auto sampler, an on-line degasser, a column oven, two reaction pumps, a reaction chamber, a reaction coil (column), a system control unit and a data processor. It is convenient to use a commercially available *N*-methylcarbamate analysis system.

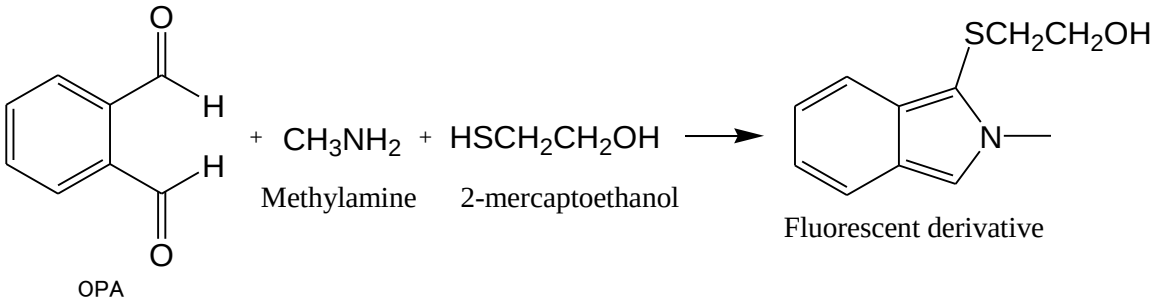
Summary of apparatus



Hydrolysis



Derivatization



[4] Any end-capped columns equivalent to this one can be used.

[5] Because of gradient elution, equilibration time of more than 15 minutes is required between injections.

[6] It is better to filter the solution with the use of a membrane filter having pore size of 0.5 µm or less after preparation.

[7] After adding sodium borate solution to be 500 mL, the solution should be filtered through a membrane having pore size of 0.5 µm or less or degassed before adding 2-mercaptoethanol. Membrane filtration or degassing after the preparation causes volatilization of 2-mercaptoethanol. Reagent should be used under shielded from light, in an amber bottle, for example.

[8] Example of chromatogram is shown in Figure 6.3.2-2.

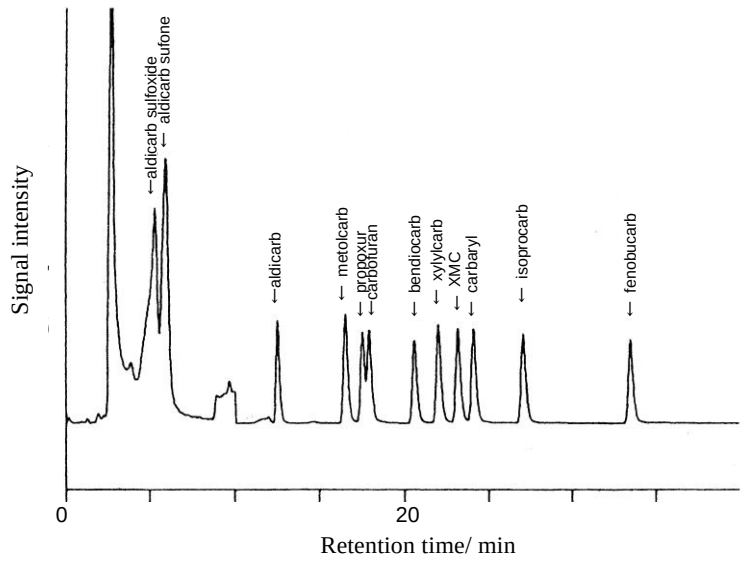


Figure 6.3.3-2. Chromatogram of formula feed spiked with 12 *N*-methylcarbamate agricultural chemicals equivalent to 0.5 mg/kg

Measurement conditions

Detector: Fluorescence detector (Ex: 340 nm, Em: 445 nm)

Column: Carbamate Analysis Column from Waters (3.9 mm in inner diameter, 150 mm in length, particle size 4 μm)

Eluent: Time (minute)	:	0	0.1	30	40
Water	:	88	90	70	88
Methanol	:	12			12
Tetrahydrofuran	:		10	30	

Reaction solutions : Solution I sodium hydroxide solution

Solution II OPA reagent

Flow rate: Eluent 1.0 mL/min, Reaction solutions 0.3 mL/min respectively.

Temperature: Column oven 40 °C, Reaction chamber 80 °C

[9] The value 0.922 is the conversion factor from aldicarb sulfoxide to aldicarb (190.3/206.3).

[10] The value 0.856 is the conversion factor from aldicarb sulfone to aldicarb (190.3/222.3).

4. Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (2)

[Analytical Standards of Feeds, Chapter 6, Section 3, Article 4]

Target Analytes: Ethiofencarb (including ethiofencarb sulfoxide and ethiofencarb sulfone[*1]), bendiocarb and methiocarb (including methiocarb sulfoxide and methiocarb sulfone[*2]) (3 compounds)

A. Reagent Preparation

1) Ethiofencarb sulfone standard stock solution. Weigh accurately 10 mg of ethiofencarb sulfone [$C_{11}H_{15}NO_4S$][1]. Transfer it to a 50 mL volumetric flask and dissolve by adding acetonitrile. Further, add the same solvent up to the graduation line of the flask to prepare the ethiofencarb sulfone standard stock solution (Each 1 mL of this solution contains 0.2 mg of ethiofencarb sulfone.).

2) Bendiocarb standard stock solution. Weigh accurately 10 mg of bendiocarb [$C_{11}H_{13}NO_4$][1]. Transfer it to a 50 mL volumetric flask and dissolve by adding acetonitrile. Further, add the same solvent up to the graduation line of the flask to prepare the bendiocarb standard stock solution (Each 1 mL of this solution contains 0.2 mg of ethiofencarb sulfone.).

3) Methiocarb sulfone standard stock solution. Weigh accurately 10 mg of methiocarb sulfone [$C_{11}H_{15}NO_4S$][1]. Transfer it to a 50 mL volumetric flask and dissolve by adding acetonitrile. Further, add the same solvent up to the graduation line of the flask to prepare the methiocarb sulfone standard stock solution (Each 1 mL of this solution contains 0.2 mg of methiocarb sulfone.).

4) Mixed standard solution. Before use, mix a certain amount of the respective standard stock solutions of ethiofencarb sulfone, bendiocarb and methiocarb sulfone, dilute the mixture accurately by adding acetonitrile to prepare the pesticides mixed standard solutions containing 0.1 – 3 μg each of the respective agricultural chemicals per 1 mL.

B. Quantification

Extraction. Weigh 5 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 15 mL of water to moisten and leave to stand for 30 minutes. Further, add it 80 mL of acetonitrile and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to be around 15 mL and add 5 g of sodium chloride to prepare a sample solution to be subjected to column treatment.

Column treatment. Load the sample solution on a porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that had contained the sample solution three times with 10 mL each of ethyl acetate, add the washings to the column in order of precedence and elute ethiofencarb, ethiofencarb oxidative metabolites, bendiocarb, methiocarb and methiocarb oxidative metabolites by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 70 mL of ethyl acetate to the column and elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 10 mL of cyclohexane – ethyl acetate (1 : 1) accurately, filter the solution through a membrane filter (pore size: 5.0 μm) and use the filtrate as sample solution for gel

permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph. Dispense each elution fraction[*3] of ethiofencarb, ethiofencarb oxidative metabolites, bendiocarb, methiocarb and methiocarb oxidative metabolites into a 200 mL recovery flask, concentrate under reduced pressure on a water bath at 40 °C or below to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue with 2 mL of acetone and use this solution as a sample solution for oxidation.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer (particle size 37.5-75 µm (400-200 mesh)[*4]) packed column (packing volume 60 g, 30 mm in inner diameter)[*5] [2]

Eluent: Cyclohexane – ethyl acetate (1 : 1)

Flow rate: 5 mL/min

Oxidation[3]. Add 3 mL of magnesium sulfate heptahydrate solution (20 w/v%) and 15 mL of potassium permanganate solution (1.6 w/v%) to the sample solution[4], leave to stand for 30 minutes and further add 3 g of sodium chloride. Load this solution to a porous diatomite column (for 20 mL retention) connected to the bottom of an aminopropylsilylated silica gel minicolumn (360 mg) and leave to stand for 5 minutes.

Place a 300 mL recovery flask under the minicolumn, wash the recovery flask that had contained the sample solution three times with 10 mL each of ethyl acetate, add the washings to the column in order of precedence and elute ethiofencarb sulfone, bendiocarb and methiocarb sulfone by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 70 mL of ethyl acetate to the minicolumn and elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 1 mL of acetonitrile accurately, filter the solution through a membrane filter (pore size: 0.5 µm or less) and use the filtrate as sample solution for liquid chromatography.

Liquid chromatography[5]. Inject 20 µL each of the sample solution and respective mixed standard solutions into a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Fluorescence detector (excitation wavelength: 340 nm, emission wavelength: 445 nm)

Column: Octadecylsilylated silica gel column (4.6 mm in inner diameter, 150 mm in length, particle size 5 µm)[*6] [6]

Eluent: Water – methanol (41 : 9) → 30 min → (3 : 7) → 3min → (1 : 9)[7]

Reaction solutions[*7]: Solution I (alkalinesolution)[8]: Dissolve 1 g of sodium hydroxide in water to be 500 mL.

Solution II (OPA reagent)[9]: Dissolve 50 mg of *o*-phthalaldehyde with 5 mL of methanol, add sodium borate solution (dissolve 19.1 g of sodium tetraborate decahydrate in water to be 1 L) to be 500 mL and mix with 50 µL of mercaptoethanol (prepare before use).

Flow rate: Eluent 1.0 mL/min, Reaction solutions 0.3 mL/min

Temperature: Column oven 40 °C, Reaction chamber 80-90 °C[10]

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[11] to prepare a calibration curve and subsequently calculate the amount of ethiofencarb [$C_{11}H_{15}NO_2S$], bendiocarb and methiocarb [$C_{11}H_{15}NO_2S$].

Ethiofencarb in sample (μg/kg) =E×20×0.876[12]

E : The weight of ethiofencarb sulfone determined from the calibration curve (ng)

Bendiocarb in sample (μg/kg) =B×20×1

B : The weight of bendiocarb determined from the calibration curve (ng)

Methiocarb in sample (μg/kg) =M×20×0.876[13]

M : The weight of methiocarb determined from the calibration curve (ng)

* 1. Ethiofencarb sulfoxide and ethiofencarb sulfone are oxidative metabolites of ethiofencarb.

2. Methiocarb sulfoxide and methiocarb sulfone are oxidative metabolites of methiocarb.

3. Confirm the fraction of ethiofencarb and its oxidative metabolite, bendiocarb as well as methiocarb and its oxidative metabolite eluted from the column prepared.

4. Bio-Beads S-X3 Beads (Bio Rad) or equivalents.

5. 60 g of packing material is moistened with cyclohexane – ethyl ethyl acetate (1 : 1) overnight, and then pack into the column (the height of the packing material should be ca. 360 mm).

6. STR ODS-II (Shinwa Chemical Industries) or equivalents.

7. Combine the reaction solution I to the eluate from the column and hydrolyze pesticides within a reaction coil in the reaction chamber. Let the hydrolyzed solution cool to the room temperature, combine with the reaction solution II for fluorescence derivatizing and transfer to the fluorescence detector immediately.

Use a reaction coil, RXN 1000 Coil (0.5 mm in inner diameter, about 5 m in length; injection volume 1 mL, Teflon, Waters) or equivalents.

«Summary of analysis method»

This method is a simultaneous analysis method for bendiocarb, ethiofencarb, ethofencarb oxidative metabolite, methiocarb and methiocarb oxidative metabolite.

Each agricultural chemical in feed is extracted with acetonitrile/water and purified by a porous diatomite column and GPC. Then, ethiofencarb, ethiofencarb sulfoxide, methiocarb and methiocarb sulfoxide are oxidized by potassium permanganate solution to stable ethiofencarb sulfone and methiocarb sulfone respectively. The oxidative metabolites are purified by a porous diatomite column connected to the bottom of an aminopropylsilylated silica gel minicolumn and quantified by gradient elution liquid chromatography with post-column fluorescence derivatization using OPA reagent. Further, the weights of ethiofencarb sulfone and methiocarb sulfone are multiplied by respective conversion factor to convert to ethiofencarb and methiocarb.

The flow sheet of the analysis method is shown in Figure 6.3.4-1.

Sample 5 g
- Water 15 mL, leave to stand for 30 minutes
- Acetinitrile 80 mL, shake for 30 minutes
- Filter under suction (No. 5B)
- Wash with 50 mL of acetonitrile
- Concentrate under vacuum (40 °C or less, to ca. 15 mL)
- Sodium chloride 5 g

Chem Elut cartridge (for 20 mL retention)
- Load sample solution, leave to stand for 5 minutes
- Ethyl acetate 100 mL
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- Cyclohexane-ethyl acetate (1:1) 10 mL
- Membrane filter (pore size 5 μm or less)

GPC (inject 5 mL of sample solution)
- elution fraction 100-160 mL
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- Acetone 2 mL

Oxidation
- 20 % magnesium sulfate solution 3 mL
- 1.6 % potassium permanganate solution 15 mL
- Leave to stand for 30 minutes
- Sodium chloride 5 g

Chem Elut cartridge (for 20 mL retention) + Sep-Pak Plus NH_2cartridge
- Ethyl acetate 100 mL
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- Acetonitrile 1 mL
- Membrane filter (pore size: 0.5 μm or less)

LC- post-column derivatization -FL (Ex: 340 nm, Em: 445 nm)

Figure 6.3.4-1. Flow sheet of the simultaneous analysis method for carbamate agricultural chemicals by LC (2)

Reference: Japan Food Research Laboratories: 1994 Emergency projects to prevent residual harmful substances: Development of analysis methods for antimicrobials and the like in animal feed, 46 (1995)

Norio Aita, Susumu Yoshinaga: Research Report of Animal Feed, 22, 11 (1997).

«Method validation»

• Spike recovery and repeatability

Compound	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
ethiofencarb	adult hen formula feed	100~1,000	3	87.3~90.3	3.4
	corn	100~1,000	3	84.7~90.0	4.7
	alfalfa meal	100~1,000	3	81.7~84.0	6.7
bendiocarb	adult hen formula feed	100~1,000	3	96.7~101.3	5.2
	corn	100~1,000	3	97.0~100.3	5.7
	alfalfa meal	100~1,000	3	95.3~96.7	8.9
methicarb	adult hen formula feed	100~1,000	3	75.7~81.7	2.0
	corn	100~1,000	3	77.7~84.7	9.0
	alfalfa meal	100~1,000	3	84.7~90.7	10.4

• Collaborative study

Compound	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
ethiofencarb	adult hen formula feed	3	250	73.7	2.5	17.9	0.87
	oats hay	3	250	60.8	3.5	17.3	0.81
bendiocarb	adult hen formula feed	3	250	97.0	3.8	5.3	0.27
	oats hay	3	250	92.4	2.1	6.2	0.31
methiocarb	adult hen formula feed	3	250	86.6	6.8	13.7	0.68
	oats hay	3	250	83.4	3.0	9.7	0.48

• Lower limit of quantification: 20 μg/kg each in sample

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers.

[2] Commercial GPC columns can be used. In such cases, the operating conditions are as follows.

Column: Shodex CLNpack EV-2000 AC (20 mm in inner diameter, 300 mm in length, particle size 15 μm)

Guard column: Shodex CLNpack EV-G AC (20 mm in inner diameter, 100 mm in length, particle size 15 μm)

Eluent: cyclohexane – acetone (7 : 3)

Flow rate: 5 mL/min

Elution fraction: 65-110 mL

[3] Ethiofencarb, ethiofencarb sulfoxide, methiocarb and methiocarb sulfoxide are oxidized by potassium permanganate solution to ethiofencarb sulfone and methiocarb sulfone respectively. Then, the oxidative metabolites are transferred to an organic solvent on a porous diatomite column, and potassium permanganate etc. are removed using an aminopropylsilylated silica gel minicolumn.

[4] The GPC eluate described in Article 3 “Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)” of this section is concentrated under vacuum and dissolved with 5 mL of ethyl acetate – methanol (99 : 1), 2 mL of which can be used as a sample solution.

[5] Ethiofencarb sulfone etc. separately eluted using gradient elution are hydrolyzed by heating in the

presence of sodium hydroxide to produce methylamines, which are then converted to fluorescent derivatives by reaction with OPA reagent and 2-mercaptoethanol. The obtained fluorescent derivatives are quantified by a fluorescence detector.

As the fluorescent derivatives are unstable at high temperature, it is required to let them cool to room temperature. This method requires a complex system which consists of a gradient pump, an auto sampler, an on-line degasser, a column oven, two reaction pumps, a reaction chamber, a reaction coil (column), a system control unit and a data processor. It is convenient to use a commercially available *N*-methylcarbamate analysis system.

For summary of apparatus, hydrolysis and derivatization, see [3] of **«Notes and precautions»** in Article 3 "Simultaneous analysis method of carbamate agricultural chemicals by liquid chromatography (1)" of this section.

[6] Any end-capped columns equivalent to this one can be used.

[7] Because of gradient elution, equilibration time of more than 15 minutes is required among injections.

[8] It is better to filter the solution with the use of a membrane filter having pore size of 0.5 μm or less after preparation.

[9] After adding sodium borate solution to be 500 mL, the solution should be filtered through a membrane having pore size of 0.5 μm or less or degassed before adding 2-mercaptoethanol. Membrane filtration or degassing after the preparation causes volatilization of 2-mercaptoethanol. Reagent should be used under shielded from light, in an amber bottle, for example.

[10] The temperature of the reaction chamber of 80-90 °C is sufficient.

[11] Example of chromatogram is shown in Figure 6.3.4-2.

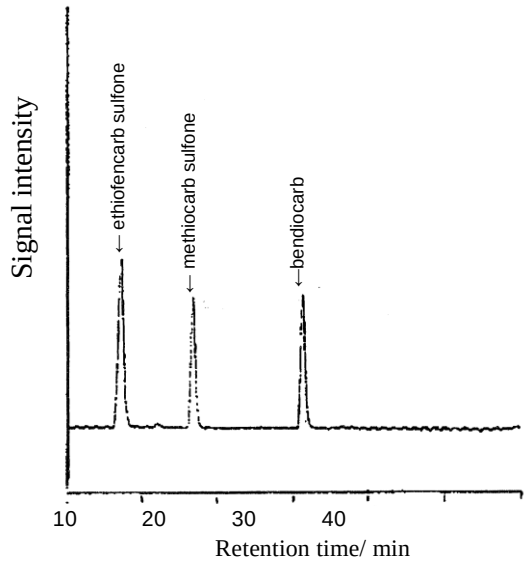


Figure 6.3.4-2. Chromatogram of bendiocarb, ethiofencarb sulfone and methiocarb sulfone (1 mg/kg each)

Measurement conditions

Detector: Fluorescence detector (Ex 340 nm, Em 445 nm)

Column: STR ODS-II

Eluent: Time (minute)	:	0	30	33
Water	:	82	30	10
Methanol	:	18	70	90

Reaction solutions : Solution I sodium hydroxide solution

Solution II OPA reagent

Flow rate: Eluent 1.0 mL/min, Reaction solutions 0.3 mL/min respectively.

Temperature: Column oven 40 °C, Reaction chamber 80 °C

[12] The value 0.876 is the conversion factor from ethiofencarb sulfone to ethiofencarb (225.3/257.3).

[13] The value 0.876 is the conversion factor from methiocarb sulfone to methiocarb (225.3/257.3).

5. Simultaneous analysis method of carbamate agricultural chemicals by gas chromatography

[Analytical Standards of Feeds, Chapter 6, Section 3, Article 5]

Target Analytes: XMC, isoprocarb, carbaryl, xylylcarb, fenobucarb, propoxur and metolcarb (7 compounds)

A. Reagent Preparation

1) Mixed standard solution. Weigh accurately 20 mg each of XMC [$C_{10}H_{13}NO_2$][1], isoprocarb [$C_{11}H_{15}NO_2$][1], carbaryl [$C_{12}H_{11}NO_2$][1], xylylcarb [$C_{10}H_{13}NO_2$][1], fenobucarb [$C_{12}H_{17}NO_2$][1], propoxur [$C_{11}H_{15}NO_3$][1] and metolcarb [$C_9H_{11}NO_2$][1]. Transfer each of them to a 100 mL volumetric flask and dissolve by adding 20 mL of acetone respectively. Further, add 2,2,4-trimethylpentane up to the graduation line of the respective flasks to prepare the standard stock solutions (Each 1 mL of these solutions contains 0.2 mg of the respective agricultural chemicals.).

Before use, mix a certain amount of the respective standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare the mixed standard solutions containing 0.5 – 4 μg each of the respective agricultural chemicals per 1 mL.

2) Coagulant. Dissolve 2 g of ammonium chloride in 400 mL of water and add 4 mL of phosphoric acid to prepare a coagulant.

3) Potassium permanganate/phosphoric acid solution. Dissolve 2.5 g of potassium permanganate in 1 L of phosphoric acid (1+400) solution.

4) Silver nitrate-coated alumina. To neutral alumina for column chromatograph (particle size: 63-200 μm (230-70 mesh))[*1] dried for 24 hours at 130 °C, add an appropriate amount of 50 w/v% silver nitrate solution equivalent to 5 v/w% and shake to mix.

5) Magnesium silicate. To synthetic magnesium silicate (particle size: 149-250 μm (100-60 mesh)) dried for 24 hours at 30 °C, add an appropriate amount of water equivalent to 5 v/w% and shake to mix.

6) Diatom earth. Wash diatom earth[*2] with warm water and methanol, and air-dry.

B. Quantification

Extraction. Weigh 20.0 g of the sample, transfer it to a 500 mL separating funnel, add 30 mL of water to moisten and leave to stand for 30 minutes. Further, add it 70 mL of acetone and extract by shaking for 30 minutes. Place a 500 mL recovery flask under a Büchner funnel[2] and filter the extract through a filter paper (No. 5B) by suction. Then, wash the separating funnel and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure[3] on a water bath at 40 °C or lower to be around 40 mL and use as a sample solution for purification.

Purification. Transfer sample solution to a 300 mL separating funnel A already containing 100 mL of sodium chloride solution (5 w/v%) and 50 mL of dichloromethane, shake vigorously for 3 minutes and leave to stand. Transfer the dichloromethane layer (lower layer) to a 300 mL recovery flask. Add 50 mL of dichloromethane to the residual solution, shake gently and leave to stand. Combine the dichloromethane layer with the recovery flask. Concentrate the dichloromethane layer under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flor of nitrogen gas.

Dissolve the residue by adding 20mL of acetone[4], add 2 g of diatom earth, 80 mL of coagulant[5] and

20 mL of potassium permanganate/phosphoric acid solution[6], swirl slightly and leave to stand for 5 minutes. Filter the supernatant through a filter paper (No. 5A) into the 300 mL separating funnel B. Add 10 mL of acetone to the recovery flask, swirl slightly, add 40 mL of coagulant and operate in the similar way. Further, wash the recovery flask and the filter paper with a small amount of coagulant – acetone (4 : 1) sequentially, filter the washings through the filter paper and combine with the content of the separating funnel B. Add 50 mL of dichloromethane to the separating funnel B, shake vigorously for 3 minutes and leave to stand. Transfer the dichloromethane layer (lower layer) to an Erlenmeyer flask. Add 50 mL of dichloromethane to the separating funnel B, repeat this procedure twice and combine each dichloromethane layer with the content of the Erlenmeyer flask. Dehydrate the dichloromethane layer with a suitable amount of sodium sulfate (anhydrous) and filter into a 500 mL recovery flask through a filter paper (No. 5A). Wash the Erlenmeyer flask and the filter paper with a small amount of dichloromethane sequentially, filter the washings through the filter paper and combine with the filtrate. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue with 5 mL of hexane – acetone (9 : 1) and use this solution as a sample solution for column treatment.

Column treatment. Suspend 3 g of sodium sulfate (anhydrous), 2 g of magnesium silicate, 3 g of sodium sulfate (anhydrous), 2 g of silver nitrate-coated alumina[7] and 3 g of sodium sulfate (anhydrous) respectively in hexane – acetone (9 : 1), pour the suspensions into a column (15 mm inner diameter) sequentially and elute so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Place a 500 mL recovery flask under the column and load the sample solution on the column. Wash the recovery flask that had contained the sample solution with 5 mL of hexane – acetone (9 : 1) five times, add the washings to the column in order of precedence and elute agricultural chemicals by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Further, add 200 mL of hexane – acetone (9 : 1) to the column to elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution for gas chromatography.

Gas chromatography. Inject 1 µL each of the sample solution and respective mixed standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame thermionic detector

Column: Fused silica capillary column (50 % diphenyl/50 % dimethyl-polysiloxane coating, 0.32 mm in inner diameter, 30 m in length, film thickness 0.25 µm)[8]

Carrier gas: He (4 mL/min)

Make up gas: He (26 mL/min)

Hydrogen: 4 mL/min

Dry air: 100 mL/min

Sample injection[9]: Cool on column

Injection port temperature: 280 °C

Column oven temperature: Initial temperature 50 °C (hold 1 min) → ramp 20 °C/min → 180 °C (hold 5 min) → ramp 2 °C/min → 190 °C (hold 2 min) → ramp 15 °C/min → 230 °C

Detector temperature: 300 °C

Calculation. Obtain respective the peak area from the resulting chromatograms[10] to prepare a calibration curve and subsequently calculate the amount of the respective agricultural chemicals in the sample.

* 1. Aluminiumoxid 90 Aktiv neutral Art. 1077 (Merck) or equivalents.

2. Hyflo Supercel (Celite Corporation) or equivalents.

«Summary of analysis method»

This method is a simultaneous analysis method for 7 carbamate agricultural chemicals such as XMC and the like.

Each agricultural chemical in feeds is extracted with acetone/water, purified by liquid-liquid extraction, coagulation and column treatment and quantified by a gas chromatograph equipped with a flame thermionic detector.

The flow sheet of the analysis method is shown in Figure 6.3.5-1.

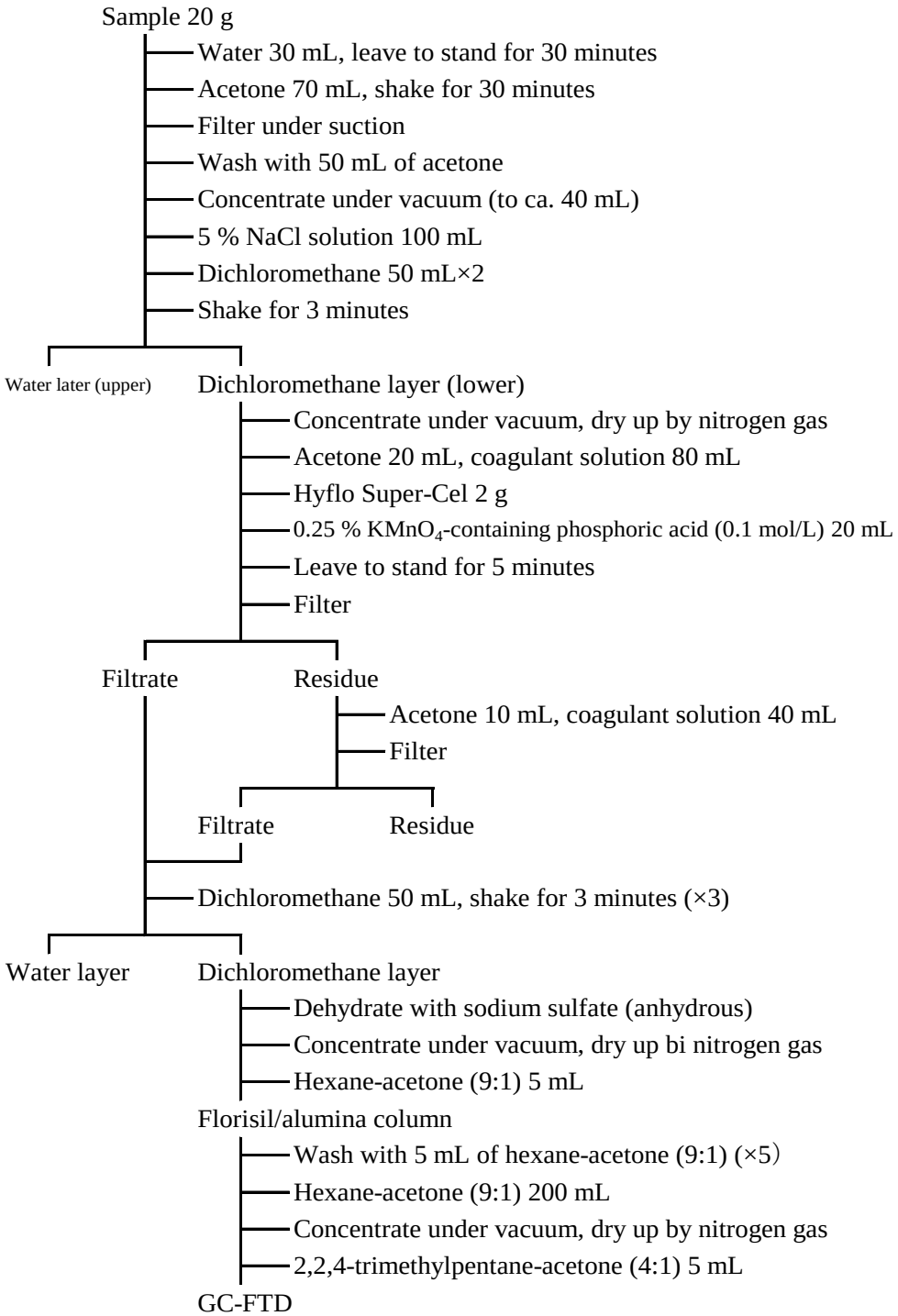


Figure 6.3.5-1. Flow sheet of the simultaneous analysis method for carbamate agricultural chemicals by GC

Reference: Toshiaki Hayakawa, Yukinobu Nakamura: Research Report of Animal Feed, 16, 15 (1991).

«Method validation»

• Spike recovery and repeatability

Compound	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
XMC	formula feed	100~500	3	92.7~100.7	11.2
isoprocarb	formula feed	100~500	3	101.0~105.3	10.5
carbaryl	formula feed	100~500	3	96.3~100.7	9.6
xylylcarb	formula feed	100~500	3	93.7~103.7	12.9
fenobucarb	formula feed	100~500	3	81.3~85.0	5.1
propoxur	formula feed	100~500	3	99.0~107.3	8.5
metolcarb	formula feed	100~500	3	99.0~107.0	6.7

• Collaborative study

Compound	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
XMC	corn	5	250	99.3	8.0	11.7	0.59
isoprocarb	corn	5	250	93.9	6.6	13.2	0.66
carbaryl	corn	5	250	97.0	13.6	13.4	0.68
xylylcarb	corn	5	250	99.1	7.3	10.1	0.51
fenobucarb	corn	5	250	88.7	6.9	13.7	0.68
propoxur	corn	5	250	100.6	6.5	9.2	0.47
metolcarb	corn	5	250	97.3	7.6	13.9	0.70

• Lower limit of quantification: 50 μg/kg each in sample

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers.

[2] Use a Kiriyama funnel of about 9 cm bore. If necessary, the bottom of the funnel is covered with a layer of diatom earth of ca. 1 cm in thickness.

[3] Because of the possibility of bumping, caution is required when concentrating the filtrate.

[4] For feeds containing high levels of pigments or fats, carbamate agricultural chemicals may be adsorbed onto the precipitate. In such cases, it is advisable to increase the amount of acetone to be added. However, when the percentage of acetone exceeds 50 % , purification will not work.

[5] The purpose is to remove pigments and fats.

[6] This solution is added to remove high polarity pigments which cannot be removed by the use of the coagulant alone.

[7] Silver nitrate-coated alumina is used to remove interference caused by thiophosphate.

[8] Both split mode and splitless mode can be used.

[9] DB-17 (Agilent Technologies), for example.

[10] Example of chromatogram is shown in Figure 6.3.5-2.

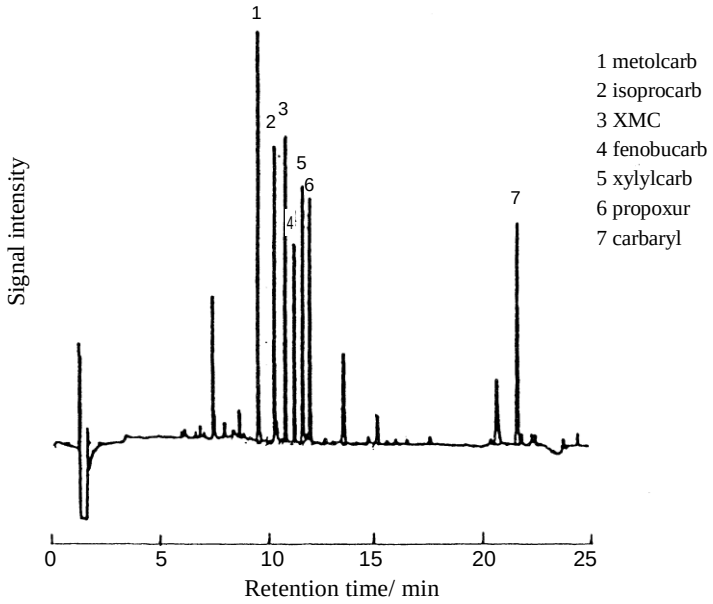


Figure 6.3.5-2. Chromatogram of formula feed spiked with various carbamate agricultural chemicals equivalent to 100 µg/kg

Measurement conditions

Detector: Flame thermionic detector

Column: DB-5 (0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)

Sample injection: Cool-on column injection system

Carrier gas: He (4 mL/min, initial flow rate)

Make up gas: He (26 mL/min)

Hydrogen: 4 mL/min

Air: 100 mL/min

Column oven temperature: Initial temperature 50 °C (hold 1 min) → ramp 20 °C/min → 180 °C (hold 5 min) → ramp 2 °C/min → 190 °C (hold 2 min) → ramp 15 °C/min → 230 °C

Detector temperature: 300 °C

6. Simultaneous analysis method for triazole agricultural chemicals by gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 3, Article 6]

Target Analytes: Triadimenol, triadimefon and propiconazole (3 compounds)

A. Reagent Preparation

1) Triadimenol standard stock solution. Weigh accurately 20 mg of triadimenol [$C_{14}H_{18}ClN_3O_2$][1]. Transfer it to a 100 mL volumetric flask and dissolve by adding 20 mL of acetone. Further, add 2,2,4-trimethylpentane up to the graduation line of the flask to prepare the triadimenol standard stock solution (Each 1 mL of this solution contains 0.2 mg of triadimenol.).

2) Triadimefon standard stock solution. Weigh accurately 20 mg of triadimefon [$C_{14}H_{16}ClN_3O_2$][1]. Transfer it to a 100 mL volumetric flask and dissolve by adding 20 mL of acetone. Further, add 2,2,4-trimethylpentane up to the graduation line of the flask to prepare the triadimenol standard stock solution (Each 1 mL of this solution contains 0.2 mg of triadimefon.).

3) Propiconazole standard stock solution. Weigh accurately 20 mg of propiconazole [$C_{15}H_{17}Cl_2N_3O_2$][1]. Transfer it to a 100 mL volumetric flask and dissolve by adding 20 mL of acetone. Further, add 2,2,4-trimethylpentane up to the graduation line of the flask to prepare the triadimenol standard stock solution (Each 1 mL of this solution contains 0.2 mg of propiconazole.).

4) Mixed standard solution. Before use, mix a certain amount of the respective standard stock solutions of triadimenol, triadimefon and propiconazole, dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare the mixed standard solutions containing 0.05 – 2 μg each of the respective agricultural chemicals per 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 20 mL of acetonitrile – water (3 : 1) and leave to stand for 10 minutes. Further, add it 100 mL of acetonitrile and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or below to almost dryness[2] and add 20 mL of sodium chloride solution to prepare a sample solution to be subjected to column treatment I.

Column treatment I. Load the sample solution on a porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that had contained the sample solution three times with 20 mL each of hexane – ethyl acetate (9 : 1), add the washings to the column in order of precedence and elute agricultural chemicals by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 60 mL of the same solvent to the column and elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 10 mL of cyclohexane – ethyl acetate (4 : 1) accurately, filter the solution through a membrane filter (pore size: 0.5 μm) and use the filtrate as sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph[3]. Dispense each elution fraction to quantify each agricultural chemical into a 200 mL recovery flask, concentrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue with 2 mL of hexane – acetone (29 : 1) and use this solution as a sample solution for column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer (20 mm in inner diameter, 300 m in length, particle size 15 µm) packed column

Guard column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 100 mm in length, particle size 15 µm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 70-100 mL

Column treatment II. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane – acetone (29 : 1)[4].

Load the sample solution on the minicolumn. Wash the recovery flask that had contained the sample solution with 2 mL each of the same solvent twice, add the washings to the column in order of precedence and elute by natural flow until the liquid level reaches the upper end of the column packing material.

Place a 100 mL recovery flask under the minicolumn and add 25 mL of hexane – acetone (17 : 3) to the minicolumn to elute agricultural chemicals. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding exactly 2 mL of hexane – acetone (49 : 1) and use this solution as a sample solution for column treatment III.

Column treatment III. Wash a silica gel minicolumn (690 mg) with 10 mL of hexane – acetone (17 : 3).

Load the sample solution on the minicolumn. Wash the recovery flask that had contained the sample solution with 2 mL each of the same solvent twice, add the washings to the column in order of precedence and elute by natural flow until the liquid level reaches the upper end of the column packing material.

Place a 100 mL recovery flask under the minicolumn and add 20 mL of hexane – acetone (17 : 3) to the minicolumn to elute agricultural chemicals. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution for gas chromatography.

Gas chromatography. Inject 2 µL each of the sample solution and mixed standard solution into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame thermionic detector

Column: Fused silica capillary column (5 % diphenyl/95 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm) [5]

Carrier gas: He (1.5 mL/min)

Make up gas: He (30 mL/min)

Hydrogen: 4 mL/min

Dry air: 140 mL/min

Sample injection : Splitless mode

Injection port temperature: 270 °C

Column oven temperature: Initial temperature 60 °C (hold 2 min) → ramp 30 °C/min → 200 °C → ramp 10 °C/min → 280 °C (hold 40 min)

Detector temperature: 280 °C

Calculation. Obtain the sum of the respective two peak heights of triadimenol and piconazole, as well as the peak height of triadimefon from the resulting chromatograms[7] to prepare a calibration curve and subsequently calculate the amount of the respective pesticides present in the sample.

«Summary of analysis method»

This method is a simultaneous analysis method for 3 triazole agricultural chemicals, triadimenol, triadimefon and propiconazole.

Each agricultural chemical in sample is extracted with acetonitrile/water, purified by the use of a porous diatomite column, GPC, Florisil minicolumn and a silica gel minicolumn and quantified by a gas chromatograph equipped with a flame thermionic detector.

The flow sheet of the analysis method is shown in Figure 6.3.6-1.

Sample 10 g
- Acetonitrile-water (3:1) 20 mL, leave to stand for 10 minutes
- Acetonitrile 100 mL, stir for 30 minutes
- Filter under suction
- Wash with 50 mL of acetonitrile
- Concentrate under vacuum (40 °C or less)
- Saturated sodium chloride solution 20 mL

Chem Elut cartridge (for 20 mL retention)
- Load sample solution, leave to stand for 5 minutes
- Wash with 20 mL of hexane-ethyl acetate (9:1) (×3)
- Elute with 60 mL of hexan-ethyl acetate (9:1)
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- Cyclohexane-acetone (4:1) 10 mL
- Filter (pore size 0.5μm)

GPC (inject 5 mL of sample solution)
- Elution fraction 70-100 mL
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- Hexane-acetone (29:1) 2 mL

Sep-Pak Plus Florisil cartridge (washed by 5 mL of hexane-acetone (29:1) before use)
- Load sample solution
- Wash with 2 mL of hexane-acetone (29:1) (×2)
- Elute with 25 mL of hexane-acetone (17:3)
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- Hexane-acetone (49:1) 2 mL

To be continued

Sep-Pak Plus Silica cartridge (washed by 10 mL of hexane-acetone (49:1) before use)
- Load sample solution
- Wash with 2 mL of hexane-acetone (49:1) (×2)
- Elute with 20 mL of hexane-acetone (17:3)
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- 2,2,4-trimethylpentane-acetone (4:1) 5 mL

GC-FTD

Figure 6.3.6-1. Flow sheet of the simultaneous analysis method for triazole agricultural chemicals

Reference: Fumio Kojima, Akira Furukawa: Research Report of Animal Feed, 26, 29 (2001).

«Method validation»

• Spike recovery and repeatability

Compound	Sample type	Spike concentration (μg/kg)	Replicate	Spike recovery (%)	Repeatability RSD (% or less)
triadimenol	adult hen formula feed	100~500	3	93.8~96.6	5.2
	suckling pig formula feed	100~500	3	88.4~95.6	10.6
	alfalfa	100~500	3	82.7~86.3	4.5
	corn	100~500	3	97.0~104.8	1.1
triadimefon	adult hen formula feed	100~500	3	99.6~102.9	3.7
	suckling pig formula feed	100~500	3	101.4~104.0	4.5
	alfalfa	100~500	3	98.1~101.6	5.4
	corn	100~500	3	100.7~103.5	2.7
propiconazole	adult hen formula feed	100~500	3	99.4~102.8	8.7
	suckling pig formula feed	100~500	3	95.3~101.4	3.3
	alfalfa	100~500	3	96.4~96.5	4.5
	corn	100~500	3	98.7~104.4	3.7

• Collaborative study

Compound	Sample type	No. of labs	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
triadimenol	adult hen formula feed	6	100	98.7	4.0	9.2	0.42
triadimefon	adult hen formula feed	6	100	94.9	5.4	8.1	0.37
propiconazole	adult hen formula feed	6	100	96.4	3.2	8.1	0.37

• Lower limit of quantification: 50 μg/kg each in sample

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Hayashi Pure Chemical and other manufacturers.

[2] Because of possible sudden bubbling, caution is required when concentrating the filtrate after extraction.

Concentrate the filtrate under vacuum until almost no liquid remains.

[3] For gel permeation chromatography, see aloso [6] and [7] of **«Notes and precautions»** in Article 1, Section 2 "Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography" of this Chapter.

[4] Use a new synthetic magnesium silicate minicolumn having enough high activity.

[5] DB-5 (Agilent Technologies), for example.

[6] When the mixed standard solution is injected, the retention time at 280 °C of ca. 10 minutes is sufficient. However, in the case of injecting a sample solution, the retention time at 280 °C should be ca. 40 minutes.

[7] Example of chromatogram is shown in Figure 6.3.6-2.

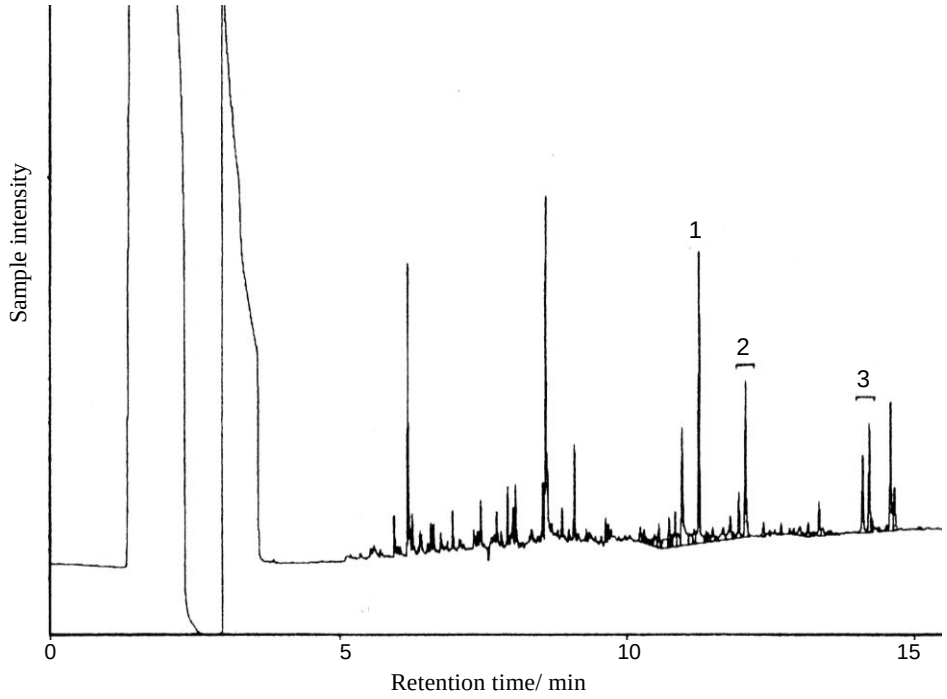


Figure 6.3.6-2. Chromatogram of chicken formula feed spiked with various triazole agricultural chemicals equivalent to 100 µg/kg

(1: triadimefon, 2: triadimenol, 3: propiconazole)

7. Simultaneous analysis method for 2,4-D and 2,4,5-T by gas chromatography[*1] [Analytical Standards of Feeds, Chapter 6, Section 3, Article 7]

Target Analytes: 2,4-D and 2,4,5-T (2 compounds)

A. Reagent Preparation

1) 2,4-D standard stock solution. Weigh accurately 25 mg of 2,4-D [$C_8H_6Cl_2O_3$][1], transfer to a 50 mL brown volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the 2,4-D standard stock solution (Each 1 mL of these solutions contains 0.5 mg of 2,4-D).

2) 2,4,5-T standard stock solution. Weigh accurately 25 mg of 2,4,5-T [$C_8H_5Cl_3O_3$][1] , transfer to a 50 mL brown volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the 2,4,5-T standard stock solution (Each 1 mL of these solutions contains 0.5 mg of 2,4,5-T).

3) Methyl-esterified agricultural chemicals mixed standard solution. Before use, transfer 1 mL each of the 2,4-D and 2,4,5-T standard stock solution to a 50 mL recovery flask, dry up by the flor of nitrogen gas, add 1 mL of methanol and 0.5 mL of trimethylsilyldiazomethane solution[2] and leave to stand for 30 minutes. Dry up this reaction solution by the flow of nitrogen gas. Dissolve the residue by adding hexane, further dilute with hexane accurately to prepare some methyl-esterified agricultural chemicals mixed standard solutions containing 0.002-0.1 μg each of 2,4-D and 2,4,5-T per 1 mL.

4) Magnesium silicate. Dry synthetic magnesium silicate (particle size 149-250 μm (100-60 mesh)) at 130 °C for 16 hour. Let it cool, add water equivalent to 1 % v/w and shake.

B. Quantification

Extraction. Weigh 20.0 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 30 mL of water to moisten and 5 mL of hydrochloric acid (1 mol/L), and leave to stand for 30 minutes. Further, add it 70 mL of acetone and extract by shaking for 30 minutes[3]. Place a 200 mL volumetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Further, add acetone up to the graduation line of the volumetric flask to prepare a sample solution to be subjected to purification.

Purification. Transfer accurately 10 mL of sample solution to a 100 mL recovery funnel, concentrate under reduced pressure to ca. 5 mL, add 5 mL of sodium hydroxide (2 mol/L)[4] and leave to stand for 30 minutes. Transfer it to a 300 mL separating funnel A already containing 50 mL of sodium chloride solution (10 w/v%) and 50 mL of hydrochloric acid (2 mol/L), add 50 mL of diethyl ether – hexane (2 : 1), shake for 5 minutes and leave to stand. Transfer the water layer (lower layer) to a 300 mL separating funnel B, and the diethyl ether – hexane layer (upper layer) to a 200 mL recovery flask, respectively. Add 50 mL of diethyl ether – hexane (2 : 1) to the separating funnel B, shake for 5 minutes, leave to stand and transfer the diethyl ether – hexane layer to the recovery flask. Dehydrate the diethyl ether – hexane layer with a suitable amount of sodium sulfate (anhydrous) and filter into a 500 mL recovery flask with filter paper (No. 2S). Wash the recovery flask and the filter paper with a small amount of diethyl ether – hexane (2 : 1) sequentially, filter the washings through the filter and combine with the

filtrate. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Transfer the residue with 50 mL diethyl ether to a 200 mL separating funnel C, add 25 mL of sodium hydrogen carbonate solution (4 w/v%), shake for 5 minutes and leave to stand. Transfer the water layer (lower layer) to a 200 mL separating funnel D. Add 25 mL of sodium hydrogen carbonate solution (4 w/v%) to the separating funnel C and operate in the similar way. Combine the water layer to that in the separating funnel D. Add 20 mL of hydrochloric acid (1 : 5) solution to the separating funnel D, wait for formation of carbon dioxide gas to subside, add 50 mL of diethyl ether, shake for 5 minutes and leave to stand. Transfer the water layer to a 200 mL separating funnel E and the diethyl ether layer to a 200 mL recovery flask, respectively. Add 50 mL of diethyl ether to the separating funnel E, operate in the similar way and transfer the diethyl ether layer to the 200 mL recovery flask. Dehydrate the diethyl ether layer with a suitable amount of sodium sulfate (anhydrous) and filter into a 200 mL recovery flask with filter paper (No. 2S). Wash the recovery flask above and the filter paper with a small amount of diethyl ether sequentially, filter the washings through the filter paper and combine with the filtrate. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Methyl-esterification[5]. Add 1 mL of methanol and 0.5 mL of trimethylsilyldiazomethane solution to the residue, leave to stand for 30 minutes and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of hexane and use the solution as a sample solution for column treatment.

Column treatment. Suspend 5 g of magnesium silicate and 2 g of sodium sulfate (anhydrous) in hexane respectively, pour the suspensions into a column (10 mm inner diameter) sequentially and elute so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Load the sample solution on the column. Wash the recovery flask that had contained the sample solution with 5 mL of hexane, add the washings to the column and elute by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Further, add 50 mL of hexane – diethyl ether (19 : 1) to the column to elute in the similar way. Place a 300 mL recovery flask under the column, add 100 mL of hexane – diethyl ether (4 : 1) to the column to elute 2,4-D methyl ester and 2,4,5-T methyl ester. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of hexane accurately and use this solution as a sample solution for gas chromatography.

Gas chromatography. Inject 1 µL each of the sample solution and methyl-esterified agricultural chemicals mixed standard solution into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Electron capture detector

Column: Fused silica capillary column (50 % trifluoropropyl methyl/ 50 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)[6]

Carrier gas: He (1 mL/min)

Make up gas: N_2 (60 mL/min)

Sample injection : Splitless mode (60 s)

Injection port temperature:280 °C

Column oven temperature: Initial temperature 80 °C (hold 2 min) → ramp 10 °C/min → 280 °C (hold 5 min)

Detector temperature: 300 °C

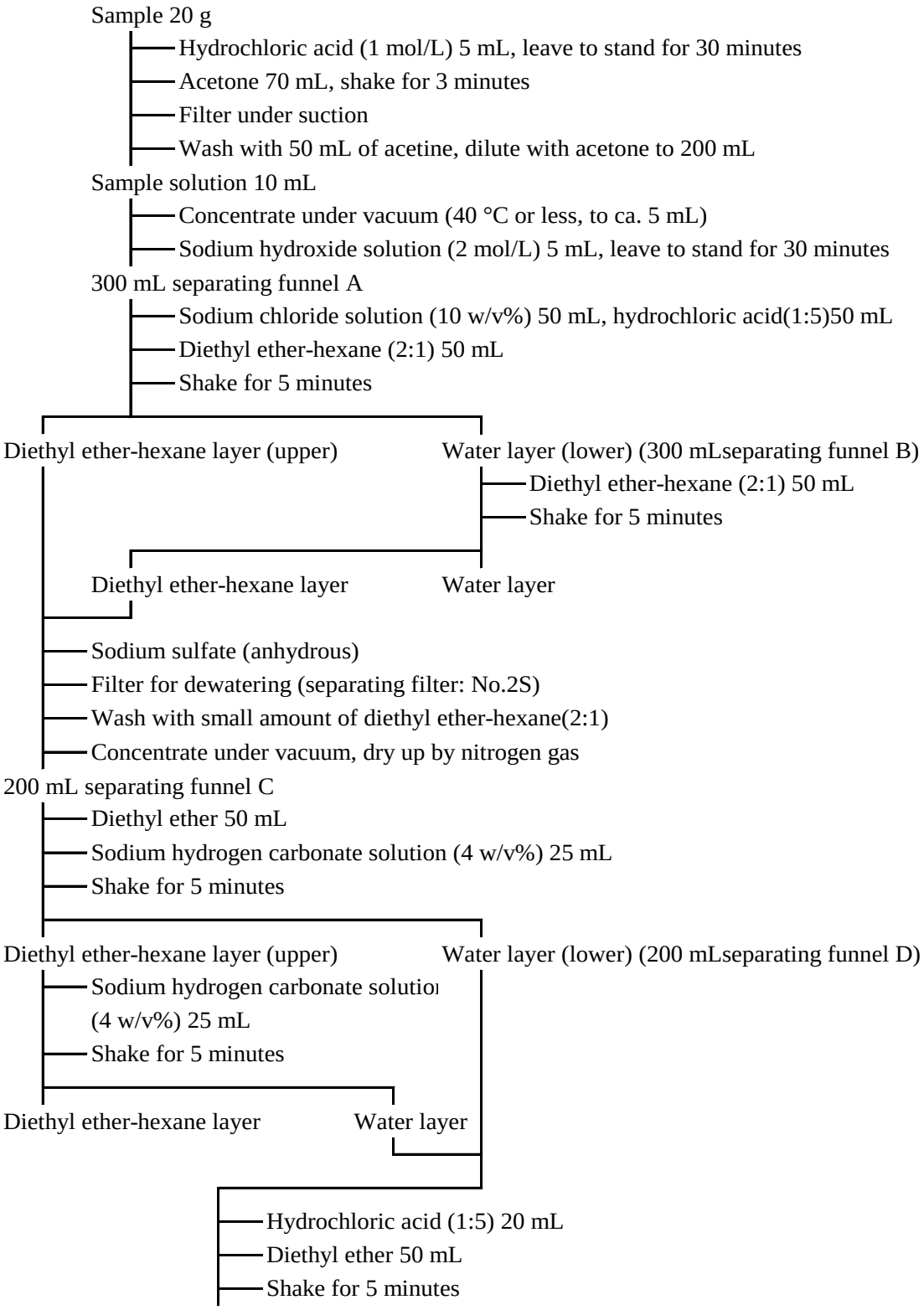
Calculation. Obtain the peak height or peak area from the resulting chromatograms[7] to prepare a calibration curve and subsequently calculate the amount of 2,4-D and 2,4,5-T in the sample.

* 1. Water to be used is that obtained by shaking 1 L of distilled water with 200 mL of hexane.

«Summary of analysis method»

This is an analysis method for 2,4-D and 2,4,5-T in feed by extraction with HCl-acidified acetone, hydrolysis, solvent exchange, methyl esterification, purification using synthetic magnesium silicate and quantification by a gas chromatograph equipped with an electron capture detector.

The flow sheet of the analysis method is shown in Figure 6.3.7-1.



To be continued

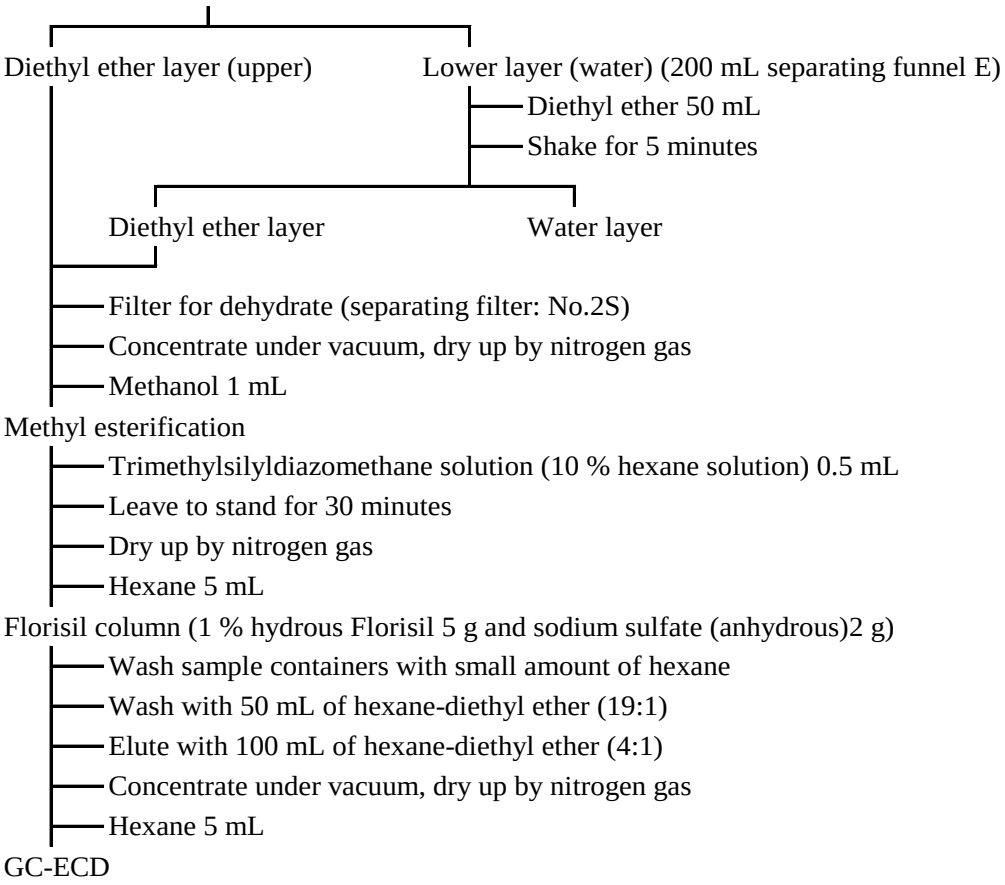


Figure 6.3.7-1. Flow sheet of the simultaneous analysis method for 2,4-D and 2,4,5-T

Reference: Ryuji Koga: Research Report of Animal Feed, 21, 69 (1996).

«Method validation»

• Spike recovery and repeatability

Compounds	Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
2,4-D	finishing pig formula feed	50~500	3	73.7~95.3	15.3
	finishing period broiler formula feed	50~500	3	76.7~89.3	17.2
	Italian ray grass	50~500	3	86.7~105.0	15.0
2,4,5-T	finishing pig formula feed	50~500	3	72.7~82.7	21.4
	finishing period broiler formula feed	50~500	3	72.7~73.7	17.7
	Italian ray grass	50~500	3	67.3~76.3	18.1

• Collaborative study

Compounds	Sample type	No. of labs	Spike concentration (μg/kg)	Recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
2,4-D	growing chick formula feed	6	250	81.4	5.2	9.8	0.48
2,4,5-T	growing chick formula feed	6	250	89.4	5.6	12.5	0.63

«Notes and precautions»

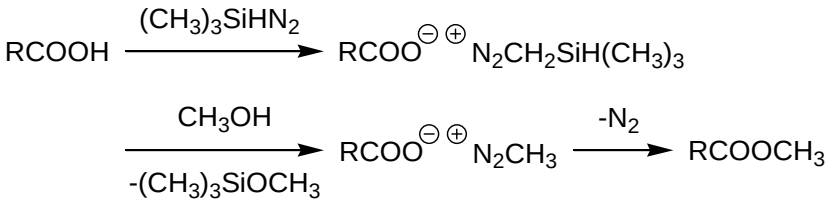
[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and Tokyo Chemical Industry.

[2] 10 % hexane solution and 2.0 mol/L hexane solution are available from Tokyo Chemical Industry and Wako Pure Chemical Industries, respectively.

[3] Simultaneously with 2,4-D, its salts and esters are eluted.

[4] Esters of 2,4-D are hydrolyzed.

[5] 2,4-D and 2,4,5-T are esterificated according to the following equation.



[6] The operating temperature should be over 280 °C. Use Rtx-200 (Restek) or equivalents.

[7] Examples of chromatogram are shown in Figure 6.3.7-2 and 6.3.7-3.

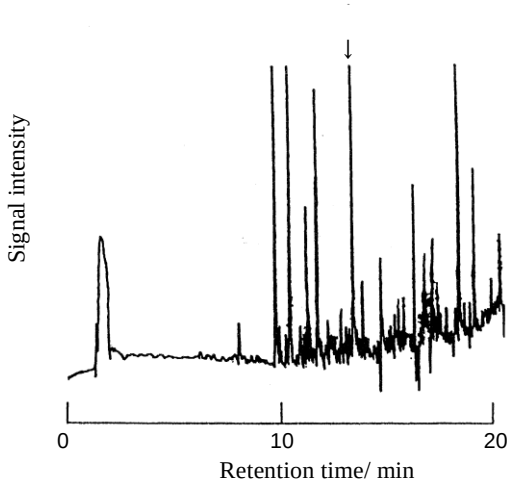


Figure 6.3.7-2. Chromatogram of formula feed spiked with 0.25 mg/kg of 2,4-D
(The arrow indicates the peak of 2,4-D methyl ester)

Measurement conditions

Detector: Electron capture detector (ECD)

Column: Rtx-200 (0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)

Carrier gas: He (1 mL/min, initial flow rate)

Make up gas: N_2 (60 mL/min)

Column oven temperature: Initial temperature 80 °C (hold 2 min) → ramp 10 °C/min → 280 °C (hold 5 min)

Injection port temperature: 280 °C

Detector temperature: 300 °C

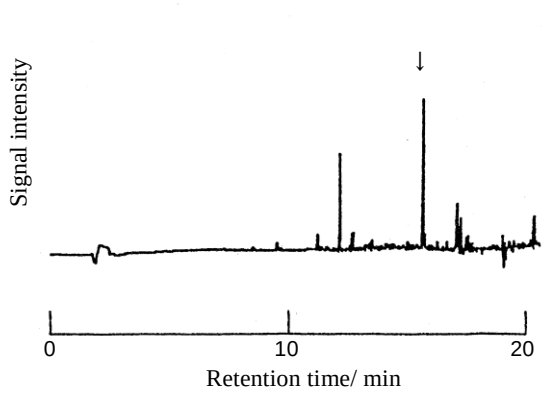


Figure 6.3.7-3. Chromatogram of formula feed spiked with 0.25 mg/kg of 2,4,5-T (The arrow indicates the peak of 2,4,5-T methyl ester)

Measurement conditions

Detector：Electron capture detector (ECD)

Column: DB-1 (0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)

Carrier gas: He (1 mL/min, initial flow rate)

Make up gas: N_2 (60 mL/min)

Column oven temperature: Initial temperature 80 °C (hold 2 min) → ramp 10 °C/min → 280 °C (hold 5 min)

Sample injection: Splitless mode

Injection port temperature: 280 °C

Detector temperature: 300 °C

8. Simultaneous analysis method for EPTC and ethylene dibromide by gas chromatograph-mass spectrometer [Analytical Standards of Feeds, Chapter 6, Section 3, Article 8]

Target Analytes: EPTC and ethylene dibromide (2 compounds)

A. Reagent Preparation

1) EPTC standard stock solution. Weigh accurately 25 mg of EPTC [$C_9H_{19}NOS$], transfer to a 50 mL brown volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flasks to prepare the 2,4-D standard stock solution (Each 1 mL of these solutions contains 0.5 mg of EPTC).

2) Ethylene dibromide standard stock solution. Weigh accurately 25 mg of ethylene dibromide [$C_2H_4Br_2$], transfer to a 50 mL brown volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flasks (Each 1 mL of these solutions contains 0.5 mg of ethylene dibromide).

3) Mixed standard solution. Mix a certain amount of EPTC and ethylene dibromide standard stock solutions and dilute the mixture accurately by adding hexane to prepare the mixed standard solutions containing 0.001 – 0.5 µg each of the respective agricultural chemicals per 1 mL.

B. Quantification

Extraction. Weigh 20.0 g of the sample, transfer it to a 1 L distillation flask for Dean-Stark distillation apparatus and add 400 mL of water. Further, add accurately 20 mL of hexane, then ca. 0.2 mL of silicon oil. Place the distillation flask under a Dean-Stark distillation apparatus*1 [1], heat by a mantle heater[2] under reflux for 60 minutes after boiling and then, let it cool. Discard water in the distilling trap, filter the hexane layer through a separating filter paper[3] and use the filtrate as sample solution for measurement by gas chromatograph-mass spectrometer[4].

Measurement by gas chromatograph-mass spectrometer. Inject 2 µL each of the sample solution and the mixed standard solution to a gas chromatograph-mass spectrometer to obtain selected ion monitoring chromatograms.

Example of measurement conditions

Column: Fused silica capillary column (6 % cyanopropyl-phenyl/ 94 % dimethyl-polysiloxane chemically-bound, 0.32 mm in inner diameter, 30 m in length, film thickness 1.8 µm)[5]

Carrier gas: He (3.6 mL/min, initial flow rate)

Sample injection: Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 50 °C → ramp 10 °C/min → 180 °C → ramp 30 °C/min → 250 °C (hold 10 min)

Interface temperature: 250 °C

Detector: Quadrupole mass spectrometer*2

Ion source temperature: 200 °C

Ionization method: Electron ionization (EI)

Ionizing voltage: 70 eV

Monitor ion: Quantification ion *m/z* 189 (EPTC), 109 (ethylene dibromide), Qualifier ion *m/z* 128 (EPTC), 107 (ethylene dibromide)

Calculation. Obtain the peak area or peak height from the resulting chromatograms[6] to prepare a calibration curve and subsequently calculate the amount of EPTC and ethylene dibromide in the sample.

* 1. To prevent volatilization of hexane, the temperature of cooling water should be 5 °C or lower.
2. Measurement conditions according to GCMS-QP2010 (Shimadzu Corporation).

«Summary of analysis method»

This is a simultaneous analysis method to steam-distill EPTC and ethylene dibromide in feed by a Dean-Stark distillation apparatus, captured by hexane and quantified by a gas chromatograph-mass spectrometer.

The flow sheet of the analysis method is shown in Figure 6.3.8-1.

Sample 20 g
- Water 400 mL
- Hexane 20 mL
- Silicon oil 0.2 mL

Dean-Stark distillation apparatus
- Heat under reflux for 60 minutes
- Filter the hexane layer through a separating filter paper

GC-MS

Figure 6.3.7-1. Flow sheet of the simultaneous analysis method for EPTC and ethylene dibromide

Reference: Mitsunori Yakata, Kazuya Washio: Research Report of Animal Feed, 33, 1 (2008).

«Method validation»

• Spike recovery and repeatability

Compounds	Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
EPTC	chicken formula feed	25~200	3	88.1~91.9	11
	cattle formula feed	10~200	3	88.5~98.3	11
	corn	25~200	3	93.5~95.5	8.0
	rye	25~200	3	93.1~95.5	6.4
ethylene dibromide	chicken formula feed	5~200	3	96.2~98.7	3.5
	cattle formula feed	5~200	3	101.2~101.3	4.1
	corn	5~200	3	99.3~102.7	6.3
	rye	2~200	3	96.7~99.3	3.3

• Collaborative study

Compounds	Sample type	No. of labs	Spike concentration (μg/kg)	Recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
EPTC	corn	8	40	109	6.1	7.7	0.35
	finishing beef cattle formula feed	8	40	113	1.9	6.9	0.31
ethylene dibromide	corn	8	10	106	5.8	14	0.61
	finishing beef cattle formula feed	8	10	106	3.9	11	0.51

• Lower limit of quantification: 10 μg/kg for EPTC 2 μg/kg for ethylene dibromide (*SN* ratio)

• Lower limit of detection: 3 μg/kg for EPTC 0.7 μg/kg for ethylene dibromide (*SN* ratio)

«Notes and precautions»

[1] Dean-Stark distillation apparatus is available from Vidrex Company. For the summary of the Dean-Stark distillation apparatus, see Figure 6.1.63-1 in Article 63, Section 1 "Chloropicrin" of this Chapter.

[2] When the heating temperature of a mantle heater is too high, bumping can occur, even if silicon oil has been added. Temperature control is required throughout the process.

[3] ADVANTEC No. 2S from Toyo Roshi Kaisya, for example.

[4] As ethylene dibromide is highly volatile (Vapor pressure is 1.5 kPa at 20 °C.), quantification should be performed expeditiously.

[5] The capillary column used for development of this method is DB-624 (Agilent Technologies).

[6] Examples of selected ion monitoring chromatogram (SIM) are shown in Figure 6.3.8-2.

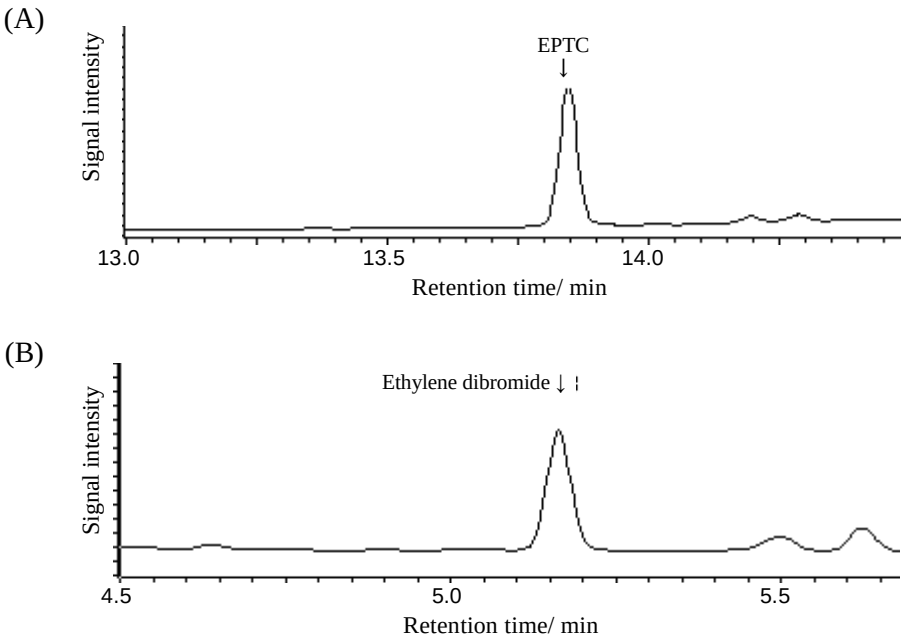


Figure 6.3.8-2. SIM chromatogram of corn spiked with 25 μg/kg of EPTC and 10 μg/kg of ethylene dibromide
(A: EPTC, B: ethylene dibromide)

9. Simultaneous analysis method for azinphos-methyl and profenofos by gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 3, Article 9]

Target Analytes: Azinphos-methyl and profenofos (2 compounds)

A. Reagent Preparation

1) Azinphos-methyl standard stock solution. Weigh accurately 25 mg of azinphos-methyl [$C_{10}H_{12}N_3O_3PS_2$][1], transfer to a 50 mL brown volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the azinphos-methyl standard stock solution (Each 1 mL of these solutions contains 0.5 mg of azinphos-methyl).

2) Profenofos standard stock solution. Weigh accurately 25 mg of profenofos [$C_{11}H_{15}BrClO_3PS$][1], transfer to a 50 mL brown volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the profenofos standard stock solution (Each 1 mL of these solutions contains 0.5 mg of profenofos).

3) Mixed standard solution. Before use, mix a certain amount of azinphos-methyl and profenofos standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare the mixed standard solutions containing 0.02 – 1 μg each of azinphos-methyl and profenofos per 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 10 mL of water to moisten and leave to stand for 30 minutes. Further, add it 100 mL of acetonitrile and extract by shaking for 30 minutes. Place a 300 mL volumetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas. Dissolve the residue by adding 10 mL of cyclohexane – acetone (7 : 3) (for cottonseed oil, 20 mL) accurately, transfer the solution to a 10 mL centrifuge tube and centrifuge at 3,000×*g* for 5 minutes. Filter the supernatant through a membrane filter[2] (pore size: 0.45 μm) and use the filtrate as sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph[3]. Dispense each elution fraction into a 100 mL recovery flask, concentrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue with 2 mL of hexane and use this solution as a sample solution for column treatment.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 300 mm in length, particle size 15 μm)

Guard column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 100 mm in length, particle size 15 μm)

Eluent: Cyclohexane – acetone (7 : 3)

Flow rate: 5 mL/min

Fraction collection: 70-120 mL

Column treatment. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane. Load the sample solution on the column. Wash the recovery flask that had contained the sample solution with 2 mL each of hexane twice, add the washings to the column in order of precedence and elute by natural flow until the liquid level reaches the upper end of the column packing material.

Place a 50 mL recovery flask under the column and add 15 mL of hexane – acetone (17 : 3) to the column to elute azinphos-methyl and profenofos. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding exactly 1 mL of 2,2,4-trimethylpentane – acetone (4 : 1) and use this solution as a sample solution for gas chromatography.

Gas chromatography. Inject 2 µL each of the sample solution and mixed standard solution into a gas chromatograph[*1] to obtain chromatograms.

Example of measurement conditions

Detector: Flame thermionic detector (Filter for phosphorus detection)

Column: Fused silica capillary column (35 % trifluoropropyl methyl/ 65 % dimethyl-polysiloxane chemically-bound, 0.25 mm in inner diameter, 15 m in length, film thickness 0.25 µm)[4]

Carrier gas: He (2.0 mL/min, initial flow rate)

Make up gas: He (30 mL/min)

Hydrogen: 75 mL/min

Dry air: 100 mL/min

Sample injection : Splitless mode (45 s)

Inlet temperature: 250 °C

Column oven temperature: Initial temperature 70 °C (hold 1 min) → ramp 20 °C/min → 250 °C (hold 4 min)

Detector temperature: 250 °C

Calculation. Obtain the peak area from the resulting chromatograms[5] to prepare a calibration curve and subsequently calculate the amount of azinphos-methyl and profenofos in the sample.

* 1. Use a silanized inlet insert without glass wool.

«Summary of analysis method»

This is a simultaneous analysis method to extract azinphos-methyl and profenofos in feed with acetonitrile/water, purify by using a gel permeation chromatograph and a Florisil minicolumn and quantify with the use of a gas chromatograph equipped with a flame thermionic detector.

The flow sheet of the analysis method is shown in Figure 6.3.9-1.

Sample 10.0 g
- Add 10 mL of water, leave to stand for 30 minutes
- Ass 100 mL of acetinitrile, shake for 30 minutes to elute
- Filter under suction (No. 5B)
- Wash with 50 mL of acetonitrile
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- Cyclohexane-acetone (7:3) 10 mL (for cottonseed: 20 mL)
- Centrifuge (3,000×g , 5 minutes)
- Membrane filter (pore size 0.45μm)

GPC (inject 5 mL of sample solution)
- Elution fraction 70-120 mL
- Concentrate under vacuum (40°C or less), dry up by nitrogen gas
- Hexane 2 mL

Sep-Pak Plus Florisil cartridge (washed with 5 mL of hexane)
- Wash with 2 mL of hexane (×2)
- Elute with 15 mL of hexane-acetone (17:3)
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- 2,2,4-trimethylpentane-acetone (4:1) 1 mL

GC-FPD

Figure 6.3.9-1. Flow sheet of the simultaneous analysis method for azinphos-methyl and profenofos

Reference: Ryosuke Yamoto: Research Report of Animal Feed, 33, 13 (2008).

«Method validation»

• Spike recovery and repeatability

Compounds	Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
azinphos-methyl	dairy cattle formula feed	10~3,000	3	76.3~90.9	13
	adult hen formula feed	50~3,000	3	103.6~103.8	3.3
	wheat	50~3,000	3	86.4~111.3	2.0
	cotton seed	20~3,000	3	110.2~116.5	4.6
	ricegrass straw	10~10,000	3	78.3~108.1	12
profenofos	dairy cattle formula feed	10~3,000	3	96.6~111.5	7.7
	adult hen formula feed	50~3,000	3	92.9~109.7	10
	wheat	50~3,000	3	98.5~110.3	2.8
	cotton seed	20~3,000	3	108.9~119.5	4.5
	ricegrass straw	10~10,000	3	88.0~105.3	8.3

• Collaborative study

Compounds	Sample type	No. of labs	Spike concentration (μg/kg)	Recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
azinphos-methyl	alfalfa	8	100	99.3	4.1	12	0.54
	dairy cattle formula feed	8	100	88.0	7.2	9.7	0.44
profenofos	alfalfa	8	100	96.6	6.8	12	0.56
	dairy cattle formula feed	8	100	92.4	7.0	14	0.65

• Lower limit of quantification: feed (except for cotton seed): 10 μg/kg each for azinphos-methyl and profenofos, cotton seed: 20 μg/kg each for azinphos-methyl and profenofos (spike recovery and relative standard deviation)

• Lower limit of detection: feed (except for cotton seed): 2 μg/kg each for azinphos-methyl and profenofos, cotton seed: 3 μg/kg each for azinphos-methyl and profenofos (*SN* ratio)

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, and other manufacturers.

[2] HLC-DISK 25 (Kanto Chemical), for example. Both filters made of PTFE and PVDF can be used.

[3] For gel permeation chromatography (GPC), see also [6] and [7] of **«Notes and precautions»** in Article 1, Section 2 "Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography" of this Chapter.

[4] Rtx-200 (Restek), for example.

[5] Example of chromatogram is shown in Figure 6.3.9-2.

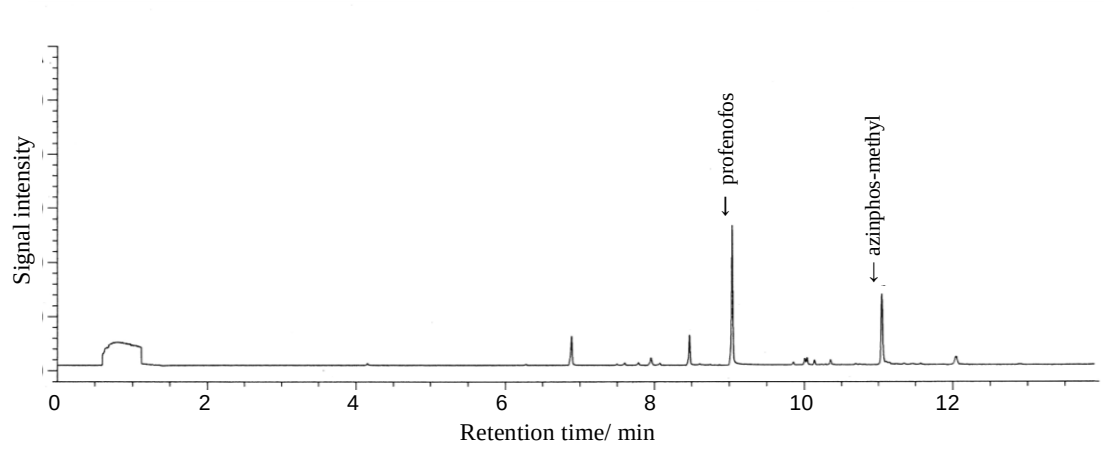


Figure 6.3.9-2. Chromatogram of formula feed spiked with azinphos-methyl and profenofos equivalent to 50 μg/kg each

10. Simultaneous analysis method for atrazine and simazine by gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 3, Article 10]

Target Analytes: Atrazine and simazine (2 compounds)

A. Reagent Preparation

1) Atrazine standard stock solution. Weigh accurately 25 mg of atrazine [$C_8H_{14}ClN_5$][1], transfer to a 50 mL volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the atrazine standard stock solution (Each 1 mL of these solutions contains 0.5 mg of atrazine).

2) Simazine standard stock solution. Weigh accurately 25 mg of simazine [$C_7H_{12}ClN_5$][1], transfer to a 50 mL volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the simazine standard stock solution (Each 1 mL of these solutions contains 0.5 mg of simazine).

3) Mixed standard solution. Before use, mix a certain amount of atrazine and simazine standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare the mixed standard solutions containing 0.01 – 1 μg each of atrazine and simazine per 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 15 mL of water to moiseten and leave to stand for 30 minutes. Further, add 100 mL of acetone and extract by shaking for 60 minutes.

Place a 300 mL volumetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to about 15 mL and use as a smaple solution for column treatment I.

Column treatment I. Load the sample solution on a porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that had contained the sample solution three times with 10 mL each of hexane, add the washings to the column in order of precedence and elute atrazine and simazine by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 120 mL of hexane to the column and elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 10 mL of cyclohexane – acetone (4 : 1) accurately, filter the solution through a membrane filter (pore size: 0.5 μm or less) and use the filtrate as sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph[2]. Dispense each elution fraction into a 100 mL recovery flask, concentrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of hexane – acetone (49 : 1) accurately and use this solution as a

sample solution for column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 300 mm in length, particle size 15 μm)

Guard column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 100 mm in length, particle size 15 μm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 75-110 mL

Column treatment II. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane – acetone.

Load 2.0 mL of the sample solution on the minicolumn and elute by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 8 mL of hexane – acetone (49 : 1) to the minicolumn and elute in the similar way.

Place a 50 mL recovery flask under the minicolumn and add 15 mL of hexane – acetone (9 : 1) to the minicolumn to elute atrazine and simazine. Concentrate the eluate under reduced pressure on a water bath at 40°C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding exactly 2 mL of 2,2,4-trimethylpentane – acetone (4 : 1) and use this solution as a sample solution for gas chromatography.

Gas chromatography. Inject 2 μL each of the sample solution and mixed standard solution into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame thermionic detector

Column: Fused silica capillary column (50 % trifluoropropyl methyl/ 50 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)[3]

Carrier gas: He (1.6 mL/min)

Make up gas: He (30 mL/min)

Hydrogen: 3 mL/min

Dry air: 90 mL/min

Sample injection : Splitless mode (60 s)

Inlet temperature: 250 °C

Column oven temperature: Initial temperature 60 °C (hold 2 min) → ramp 20 °C/min → 170 °C → ramp 2 °C/min → 200 °C → ramp 20°C/min → 280 °C (hold 10 min)

Detector temperature: 280 °C

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[4] to prepare a calibration curve and subsequently calculate the amount of atrazine and simazine in the sample.

«Summary of analysis method»

This is a simultaneous analysis method to extract atrazine and simazine in feed with acetone/water, purify by using a porous diatomite column, GPC and a Florisil minicolumn and quantify with the use of a gas chromatograph equipped with a flame thermionic detector or a nitrogen phosphorus detector.

The flow sheet of the analysis method is shown in Figure 6.3.10-1.

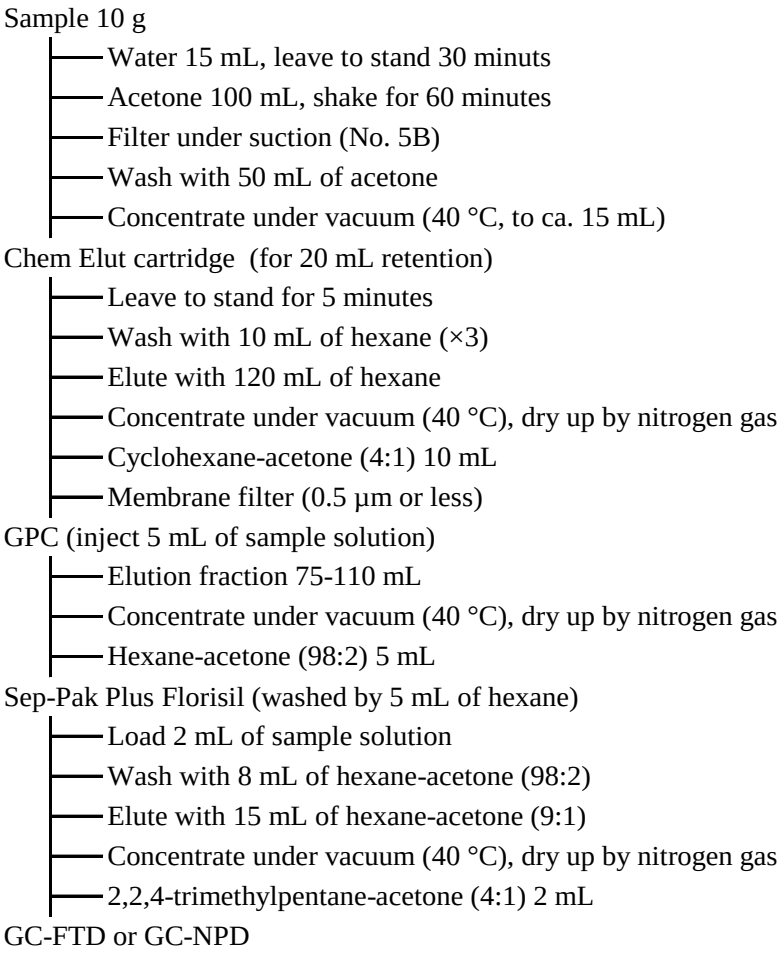


Figure 6.3.10-1. Flow sheet of the simultaneous analysis method for atrazine and simazine

Reference: Reiko Kazama: Research Report of Animal Feed, 28, 30 (2003).

«Method validation»

• Spike recovery and repeatability

Compounds	Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
atrazine	starting chick formula feed	50~250	3	91.4~98.9	8.7
	finishing pig formula feed	50~250	3	96.4~101.7	5.6
	corn	50~250	3	98.9~101.3	2.4
	oats hay	50~250	3	90.5~91.8	8.8
simazine	starting chick formula feed	50~250	3	93.1~101.9	10.0
	finishing pig formula feed	50~250	3	97.4~105.9	6.7
	corn	50~250	3	96.7~97.5	3.0
	oats hay	50~250	3	90.4~92.0	10.6

• Collaborative study

Compounds	Sample type	No. of labs	Spike concentration (μg/kg)	Recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
atrazine	finishing beef cattle formula feed	6	100	95.5	2.9	9.4	0.43
	milo		100	92.7	1.4	10.6	0.48
simazine	finishing beef cattle formula feed	6	100	99.6	3.9	8.1	0.37
	milo		100	101.4	3.4	6.7	0.30

• Lower limit of quantification: 20 μg/kg each in sample

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, and other manufacturers.

[2] For gel permeation chromatography (GPC), see also [6] and [7] of **«Notes and precautions»** in Article 1, Section 2 “Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography” of this Chapter.

[3] Rtx-200 (Restek), for example.

[4] Example of chromatogram is shown in Figure 6.3.10-2.

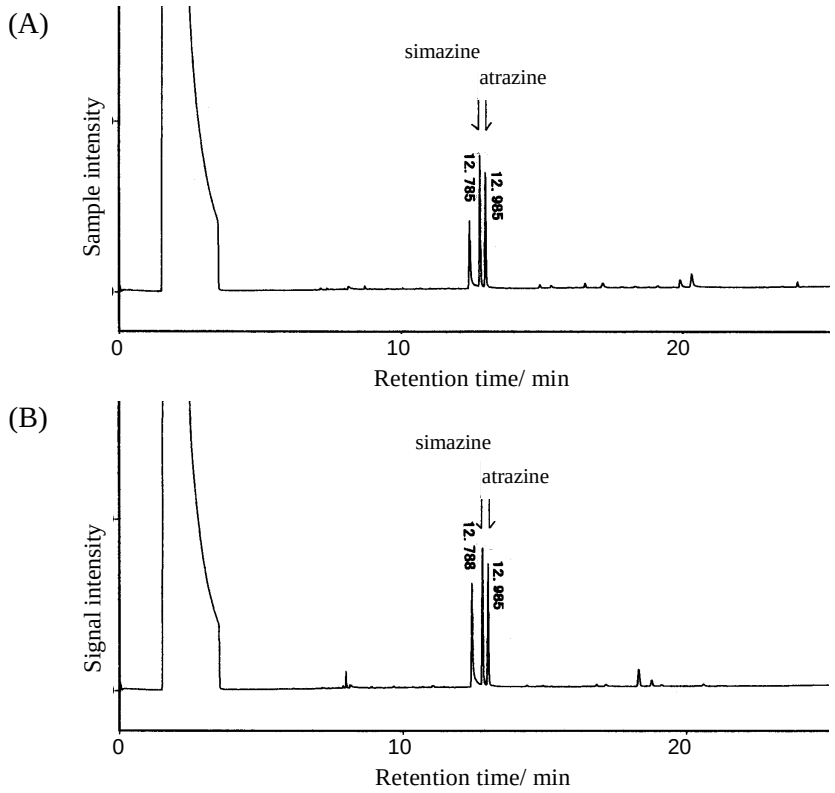


Figure 6.3.10-2. Chromatogram of chicken formula feed (A) and oats hay (B) spiked with atrazine and simazine equivalent to 250 µg/kg each

11. Simultaneous analysis method for ametryn, cyanazine and prometryn by liquid chromatograph-mass spectrometer [Analytical Standards of Feeds, Chapter 6, Section 3, Article 11]

Target Analytes: Ametryn, cyanazine and prometryn (3 compounds)

A. Reagent Preparation

1) Ametryn standard stock solution. Weigh accurately 25 mg of ametryn[$C_9H_{17}N_5S$][1], transfer to a 50 mL volumetric flask and dissolve by adding acetone. Further, add acetone up to the graduation line of the flask to prepare the ametryn standard stock solution (Each 1 mL of these solutions contains 0.5 mg of ametryn).

2) Cyanazine standard stock solution. Weigh accurately 25 mg of cyanazine [$C_9H_{13}ClN_6$][1], transfer to a 50 mL volumetric flask and dissolve by adding acetone. Further, add acetone up to the graduation line of the flask to prepare the cyanazine standard stock solution (Each 1 mL of these solutions contains 0.5 mg of cyanazine).

3) Prometryn standard stock solution. Weigh accurately 25 mg of prometryn [$C_{10}H_{19}N_5S$][1], transfer to a 50 mL volumetric flask and dissolve by adding acetone. Further, add acetone up to the graduation line of the flask to prepare the prometryn standard stock solution (Each 1 mL of these solutions contains 0.5 mg of prometryn).

4) Mixed standard solution. Before use, mix a certain amount of ametryn, cyanazine and prometryn standard stock solutions and dilute the mixture accurately by adding acetonitrile to prepare the agricultural chemicals mixed standard solutions containing 0.5 – 100 ng each of the respective pesticides per 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 20 mL of water (30 mL to grass hay) and leave to stand for 30 minutes. Further, add it 100 mL of acetone and extract by shaking for 30 minutes. Place a 200 mL volumetric flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way. Further, add acetone up to the graduation line of the flask.

Transfer 10 mL of this solution accurately to a 50 mL recovery flask and concentrate under reduced pressure on a water bath at 40 °C or lower to be around 2 mL to prepare a sample solution to be subjected to column treatment I.

Column treatment I. Load the sample solution on a porous diatomite column (for 20 mL retention). Wash the recovery flask that had contained the sample solution with 5 mL of water, add the washings to the column and leave to stand for 5 minutes. Place a 200 mL recovery flask under the column, wash the recovery flask that had contained the sample solution with 10 mL each of ethyl acetate – hexane (17 : 3) three times, add the washings to the column in order of precedence and elute each agricultural chemical by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 50 mL of the same solvent to the column and elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

For analysis of feed other than grass hay, dissolve the residue with 30 mL of hexane and use this solution as a sample solution for liquid-liquid extraction. For analysis of grass hay, dissolve the residue with 5 mL of hexane and use this solution as a sample solution for column treatment II.

Liquid-liquid extraction[2]. Transfer a sample solution to a 100 mL separating funnel, wash the recovery flask that had contained the sample solution with 2 mL each of hexane twice and combine the washings with the sample solution. Further, add 30 mL of acetonitrile saturated with hexane to the separating funnel, shake for 5 minutes and leave to stand. Transfer the acetonitrile layer (lower layer) to a 200 mL recovery flask. Add 30 mL of acetonitrile saturated with hexane to the separating funnel and operate in the similar way. Transfer the acetonitrile layer to the recovery flask.

Concentrate the acetonitrile layer under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas. Dissolve the residue by adding 5 mL of hexane and use this solution as a sample solution for column treatment II.

Column treatment II. Wash a graphite carbon/aminopropylsilylated silica gel layered minicolumn (500 mg/500 mg)[*1] with 5 mL of ethyl acetate and 10 mL of hexane[3].

Load the sample solution on the minicolumn and elute by natural flow so that the liquid level reaches the upper end of the column packing material. Wash the recovery flask that had contained the sample solution with 5 mL each of hexane twice, add the washings to the minicolumn in order of precedence and elute in the similar way.

Place a 50 mL recovery flask under the minicolumn, wash the recovery flask above with 5 mL each of hexane – ethyl acetate (1 : 1) twice, add the washings to the minicolumn in order of precedence and elute each agricultural chemical by natural flow. Further, add 10 mL of hexane – ethyl acetate (1 : 1) to the minicolumn and elute in the similar way.

Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas. Dissolve the residue by adding 3 mL of hexane and use this solution as a sample solution for column treatment III.

Column treatment III. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane[3].

Load the sample solution on the column and elute by natural flow until the liquid level reaches the upper end of the column packing material. Wash the recovery flask that had contained the sample solution with 3 mL each of hexane twice, add the washings to the column in order of precedence and elute in the similar way.

Place a 50 mL recovery flask under the minicolumn, wash the recovery flask with 5 mL each of hexane – acetone (17 : 3) twice, add the washings to the minicolumn in order of precedence and elute each agricultural chemical in the similar way. Further, add 10 mL of hexane – acetone (17 : 3) to the minicolumn and elute in the similar way.

Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas. Dissolve the residue by adding accurately 1 mL of acetonitrile, centrifuge at 3,000×*g* for 5 minutes and use the supernatant as a sample solution to be subjected to measurement by liquid chromatograph-mass spectrometer.

Measurement by liquid chromatograph-mass spectrometer. Inject 4 µL each of the sample solution and

the mixed standard solution to a liquid chromatograph-mass spectrometer to obtain selected ion monitoring chromatograms.

Example of measurement conditions

Column: Octadecylsilylated silica gel column (2.1 mm in inner diameter, 150 mm in length, particle size 5 μm)[*2]

Eluent: 0.01% formic acid solution – acetonitrile (3 : 1) (hold 5 min) → 2 min → (2 : 3) (hold 3 min) → 2 min → (1 : 9) (hold 8 min)[4][5]

Flow rate: 0.2 mL/min

Column oven temperature: 40 °C

Detector: Quadrupole mass spectrometer[*3]

Ionization method: Electrospray ionization (ESI) (positive ion mode)

Fragmentor voltage: 120 V

Nebulizer gas: N_2 (340 kPa)

Drying gas: N_2 (10 L/min, 350 °C)

Capillary voltage: 4,000 V

Monitored ion: *m*/*z* 228 (ametryn), 241 (cyanazine), 242 (prometryn)

Calculation. Obtain the peak height from the resulting selected ion monitoring chromatograms [6] to prepare a calibration curve and subsequently calculate the amount of ametryn, cyanazine and prometryn in the sample.

* 1. ENVI-Carb/LC-NH_2 (Supelco) or equivalents.

2. Inertsil ODS-SP (GL Sciences, the retention time for ametryn, cyanazine and prometryn under these measurement conditions is ca. 15, 14 and 17 minutes, respectively) or equivalents.

3. Example of measurement conditions for Agilent 1100 Series MSD SL (Agilent Technologies).

«Summary of analysis method»

This is a simultaneous analysis method to extract ametryn, cyanazine and prometryn in feed with acetone/water, purify by using a porous diatomite column, liquid-liquid extraction, a graphite carbon/aminopropylsilylated silica gel layered minicolumn and a Florisil minicolumn and quantify with the use of a liquid chromatograph-mass spectrometer.

The flow sheet of the analysis method is shown in Figure 6.3.11-1.

Sample 10 g
- Water 20 mL (for grass hay 30 mL), leave to stand for 30 minutes
- Acetone 100 mL, shake for 30 minutes
- Filter under suction (No. 5B)
- Wash with acetone, dilute to 200 mL

Sample solution 10 mL
- Concentrate under vacuum (40 °C or less, to 2 mL)

Chem Elut cartridge (for 20 mL retention)
- Load sample solution, wash with 5 mL of water, leave to stand for 5 minutes
- Wash and elute with 80 mL of hexane-ethyl acetate (17:3)
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas

For formula feed to be continued to *1, for others to*2.

To be continued

(*1)
- Hexane 30 mL

100 mL separating funnel
- Wash with 4 mL of hexane
- Acetonitrile saturated with hexane 30 mL
- Shake for 5 minutes

Hexane layer (upper) / Acetonitrile layer (lower)

Hexane layer (upper)
- Acetonitrile saturated with hexane 30 m
- Shake for 5 minutes

Hexane layer (discard) / Acetonitrile layer

Acetonitrile layer (lower)
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas

(*2)
- Hexane 5 mL

Envi-Carb/NH_2 cartridge (Washed with 5 mL of ethyl acetate and 10 mL of hexane)
- Load sample solution
- Wash with 10 mL of hexane
- Elute with 20 mL of hexane-ethyl acetate (1:1)
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- Hexane 3 mL

Sep-Pak Plus Florisil cartridge (washed with 5 mL of hexane)
- Load sample solution
- Wash with 6 mL of hexane
- Elute with 20 mL of hexane-acetone (17:3)
- Concentrate under vacuum (40 °C or less), dry up by nitrogen gas
- Acetonitrile 1 mL

LC-MS

Figure 6.3.11-1. Flow sheet of the simultaneous analysis method for ametryn, cyanazine and prometryn

Reference: Tomoharu Nozaki, Toshiaki Yamata: Rsearch Report of Animal Feed, 33, 26 (2008).

«Method validation»

• Spike recovery and repeatability

Compounds	Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
ametryn	starting broiler chick formula feed	2~100	3	89.5~95.7	9.4
	Sudan grass	2~100	3	74.7~85.7	5.4
cyanazine	starting broiler chick formula feed	2~100	3	81.1~93.5	4.7
	Sudan grass	2~100	3	77.2~80.6	5.9
prometryn	starting broiler chick formula feed	2~100	3	85.8~94.5	6.6
	Sudan grass	2~100	3	73.8~87.2	4.0

• Collaborative study

Compounds	Sample type	No. of labs	Spike concentration (μg/kg)	Recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
ametryn	starting broiler chick formula feed	9	10.0	98.4	4.3	6.2	0.28
	Sudan grass	9	10.0	92.2	4.2	15	0.66
cyanazine	starting broiler chick formula feed	9	10.0	98.9	6.6	9.4	0.43
	Sudan grass	9	10.0	94.4	4.1	17	0.76
prometryn	starting broiler chick formula feed	9	10.0	93.6	2.7	6.1	0.28
	Sudan grass	9	10.0	89.6	3.2	11	0.52

• Lower limit of quantification: 2 μg/kg each in sample for ametryn, cyanazine and prometryn (*SN* ratio)

• Lower limit of detection: 0.7 μg/kg each in sample for ametryn, cyanazine and prometryn (*SN* ratio)

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, and other manufacturers.

[2] In this method, the step of liquid-liquid extraction is skipped only for grass hay. However, as the purpose of liquid-liquid extraction is to remove lipid-soluble substances, this step may be skipped for feed definitely containing low-level of lipid-soluble substances, too.

[3] As the step of washing minicolumn before sample loading is not affected by the flow rate, some measures such as adding pressure can shorten the operate time.

[4] Peaks may not be detected depending on models of liquid chromatograph or columns used. In such cases, it is required to change measurement conditions, gradient, for example. An example of gradient program used by another laboratory is as follows: Example of gradient 0.01 % formic acid solution – acetonitrile (9 : 1) (hold 17 min) → 3 min → (2 : 3) (hold 5 min) → 2 min → (1 : 9) (hold 8 min)

[5] If peaks cannot be detected well because of interference etc., gradient program should be modified.

[6] Example of selected ion monitoring chromatogram (SIM) is shown in Figure 6.3.11-2.

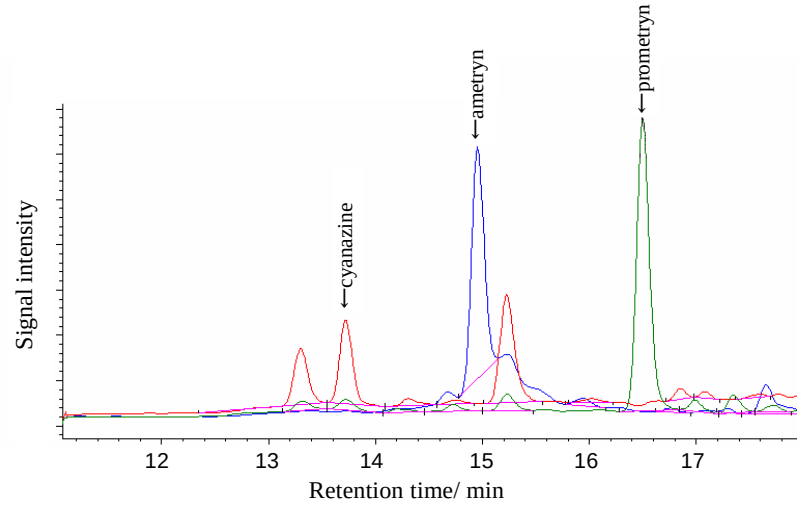


Figure 6.3.11-2. SIM chromatogram of broiler formula feed spiked with 10 µg/kg each of ametryn, cyanazine and prometryn

12. Simultaneous analysis method for chlorpyrifos-methyl and pirimiphos-methyl by gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 3, Article 12]

Target Analytes: Chlorpyrifos-methyl and pirimiphos-methyl (2 compounds)

A. Reagent Preparation

1) Chlorpyrifos-methyl standard stock solution. Weigh accurately 20 mg of chlorpyrifos-methyl [$C_7H_7Cl_3NO_3PS$][1], transfer to a 100 mL brown volumetric flask and dissolve by adding 20 mL of acetone. Further, add 2,2,4-trimethylpentane up to the graduation line of the flask to prepare the chlorpyrifos-methyl standard stock solution (Each 1 mL of these solutions contains 0.2 mg of chlorpyrifos-methyl).

2) Pirimiphos-methyl standard stock solution. Weigh accurately 20 mg of pirimiphos-methyl [$C_{11}H_{20}N_3O_3PS$][1], transfer to a 100 mL volumetric flask and dissolve by adding 20 mL of acetone. Further, add 2,2,4-trimethylpentane up to the graduation line of the flask to prepare the pirimiphos-methyl standard stock solution (Each 1 mL of these solutions contains 0.2 mg of pirimiphos-methyl).

3) Mixed standard solution. Before use, mix a certain amount of chlorpyrifos-methyl and pirimiphos-methyl standard stock solutions and dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare the mixed standard solutions containing 0.1 – 4 µg each of chlorpyrifos-methyl and pirimiphos-methyl per 1 mL.

4) Silica gel. Store a silica gel (particle size 63-200 µm (230-70 mesh))[*1] in a desiccator after opening.

5) Coagulant solution. Dissolve 1 g of ammonium chloride in 1 L of water and add 2 mL of phosphoric acid to prepare a coagulant solution.

6) Diatom earth. Wash diatom earth[*2] with warm water and methanol, and air-dry.

B. Quantification

Extraction. Weigh 10.0 - 20.0 g of the sample[2], transfer it to a 500 mL separating funnel, add 60 mL of water to moisten and leave to stand for 30 minutes. Further, add it 140 mL of acetone and extract by shaking for 30 minutes. Place a tall beaker under a Büchner funnel[3] and filter the extract through a filter paper (No. 5B) by suction. Then, wash the separating funnel above and the residue with 100 mL of acetone – water (7 : 3) sequentially, and filter the washings by suction in the similar way to use the filtrate as a sample solution for purification.

Purification. Transfer sample solution to a 1 L separating funnel already containing 400 mL of sodium chloride solution (5 w/v%) and 100 mL of dichloromethane, shake vigorously for 3 minutes and leave to stand. Transfer the dichloromethane layer (lower layer) to an Erlenmeyer flask. Add 100 mL of dichloromethane to the residual solution, shake gently and leave to stand. Combine the dichloromethane layer with the content of the Erlenmeyer flask. Dehydrate the dichloromethane layer with a suitable amount of sodium sulfate (anhydrous) and filter into a 500 mL recovery flask through a glass fiber filter paper[*3] [4]. Wash the Erlenmeyer flask and the glass fiber filter paper with a small amount of dichloromethane sequentially, filter the washings through the glass fiber filter paper and combine with the filtrate. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 30 mL of acetone[5], add 0.5 g of diatom earth and 40 mL of the

coagulant solution, swirl slightly and leave to stand for 5 minutes[6]. Filter the supernatant through a glass fiber filter paper*3 [4] into the separating funnel A. Add 30 mL of acetone to the recovery flask , add 40 mL of coagulant solution, swirl slughtly and operate in the similar way. Further, wash the recovery flask and the glass fiber filter paper with a small amount of coagulant solution – acetone (4 : 3) sequentially, filter the washings through the glass fiber filter paper and combine with the content of the separating funnel A. Add 50 mL of hexane to the separating funnel A, sake vigorously for 3 minutes and leave to stand. Transfer the water layer (lower layer) to a 300 mL separating funnel B, and the hexane layer (upper layer) to a Erlenmeyer flask. Add 50 mL of hexane to the separating funnel B, operate in the similar way and combine the hexane layer with the content of the Erlenmeyer flask. Dehydrate the hexane layer with a suitable amount of sodium sulfate (anhydrous) and filter into a 300 mL recovery flask through a glass fiber filter paper*3 [4]. Wash the recovery flask and the glass fiber filter paper with a small amount of hexane sequentially, filter the washings through the glass fiber filter paper and combine with the filtrate. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue with 5 mL of hexane – diethyl ether (9 : 1) and use this solution as a sample solution for column treatment.

Column treatment. Suspend 5 g sodium sulfate (anhydrous), 5 g of silica gel and 5 g of sodium sulfate (anhydrous) respectively in hexane – diethyl ether (9 : 1), pour the suspensions into a column (15 mm inner diameter) sequentially and elute so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Place a 200 mL recovery flask under the column and load the sample solution on the column. Wash the recovery flask that had contained the sample solution with 5 mL of hexane – diethyl ether (9 : 1) four times, add the washings to the column in order of precedence and elute chlorpyrifos-methyl and pirimiphos-methyl by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Further, add 75 mL of hexane – diethyl ether (9 : 1) to the column to elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue with 5 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution for gas chromatography.

Gas chromatography. Inject 1 µL each of the sample solution and respective mixed standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame thermionic detector[7]

Column: Fused silica capillary column (100 % dimethylpolysiloxane coating, 0.32 mm in inner diameter, 30 m in length, film thickness 0.25 µm)[8]

Carrier gas: He (4 mL/min)

Make up gas: He (26 mL/min)

Hydrogen: 4 mL/min

Dry air: 100 mL/min

Sample injection: Cool on column[9]

Injection port temperature: 280 °C

Column oven temperature: 50 °C (hold 1 min) → ramp 20 °C/min → 180 °C (hold 1 min) → ramp 3 °C/min → 200 °C

Detector temperature: 240 °C

Calculation. Obtain the peak area from the resulting chromatograms[10] to prepare a calibration curve and subsequently calculate the amount of chlorpyrifos-methyl and pirimiphos-methyl in the sample.

* 1. Silica Gel 60 (Merck) or equivalents.

2. Hyflo Supercel (Celite Corporation) or equivalents.

3. GA-100 (Toyo Roshi Kaisya) or equivalents.

«Summary of analysis method»

This method is a simultaneous analysis method for chlorpyrifos-methyl and pirimiphos-methyl. Each agricultural chemical in feeds is extracted with acetone/water, purified by liquid-liquid extraction, coagulation and a silica gel column, and quantified by a gas chromatograph equipped with a flame thermionic detector.

The flow sheet of the analysis method is shown in Figure 6.3.12-1.

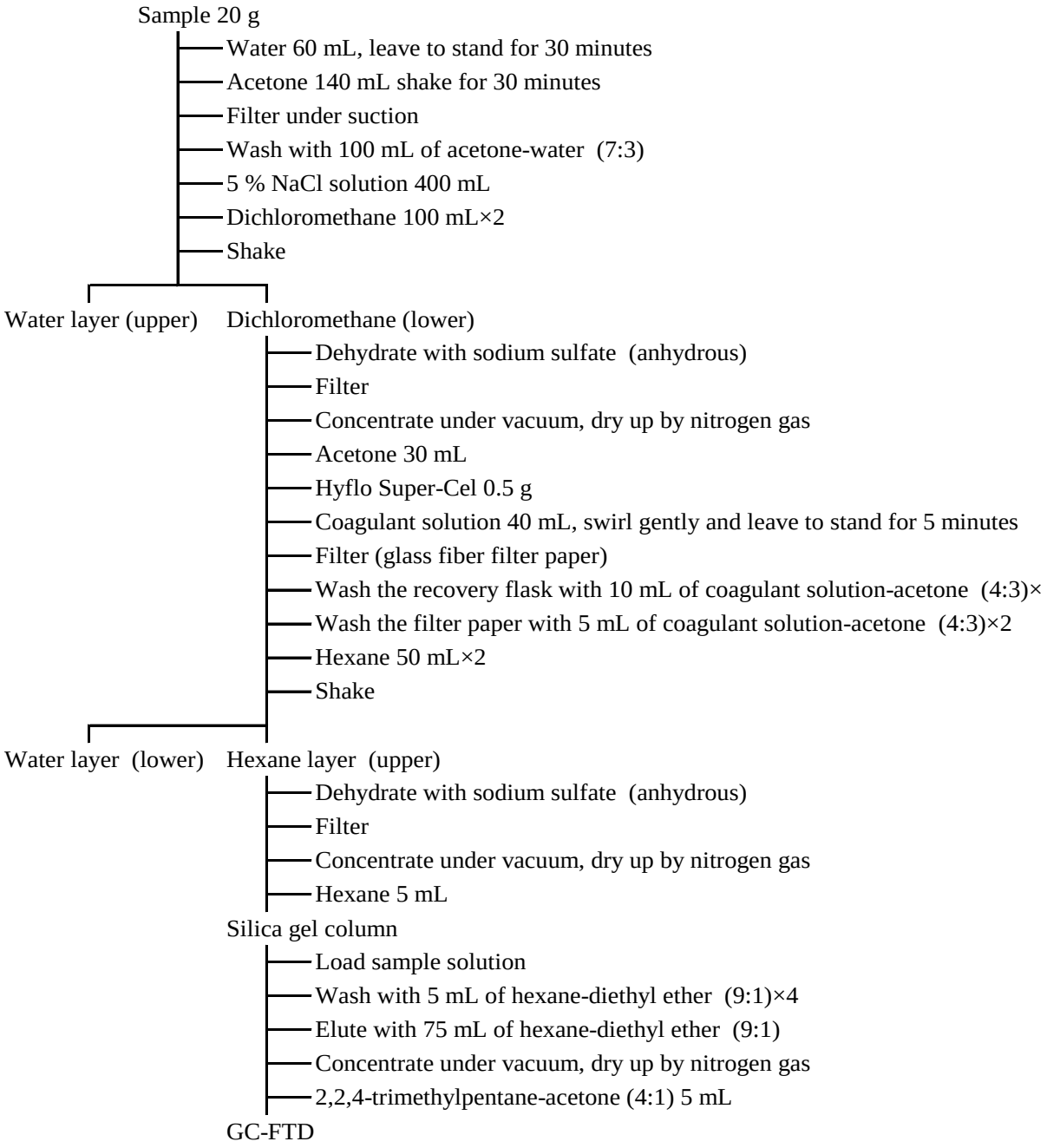


Figure 6.3.12-1. Flow sheet of the simultaneous analysis method for chlorpyrifos-methyl and pirimiphos-methyl

Reference: Toshiaki Hayakawa, Miyako Yoshimoto, Yukinobu Nakamura: Research Report of Animal Feed, 17, 12 (1992).

«Method validation»

• Spike recovery and repeatability

Compounds	Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
chlorpyrifos-methyl	broiler finishing chick formula feed	250~500	3	92.1~94.4	3.7
	Finishing pig formula feed	250~500	3	88.1~94.1	6.6
	finishing beef cattle formula feed	250~500	3	87.1~100.9	4.0
pirimiphos-methyl	broiler finishing chick formula feed	250~500	3	97.0~99.6	2.3
	Finishing pig formula feed	250~500	3	102.7~106.8	5.8
	finishing beef cattle formula feed	250~500	3	100.0~104.6	3.7

• Collaborative study

Compounds	Sample type	No. of labs	Spike concentration (μg/kg)	Recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
chlorpyrifos-methyl	broiler finishing chick formula feed	6	250	89.8	3.5	4.2	0.21
pirimiphos-methyl	broiler finishing chick formula feed	6	250	101.6	4.4	7.9	0.40

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers.

[2] For samples containing high levels of pigments, grass hay, for example, 10 g of the sample is used.

[3] Use a Kiriyama funnel of about 9 cm bore. If necessary, the bottom of the funnel is covered with a layer of diatom earth of ca. 1 cm in thickness.

[4] To prevent the adsorption of pirimiphos-methyl on the filter paper, it is advisable to use a glass fiber filter paper.

[5] The residue is dissolved by supersonic treatment before the subsequent steps.

[6] Shake several times to precipitate the coagulation and leave to stand.

[7] FPD can also be used.

[8] DB-1 (Agilent Technologies) , for example.

[9] All of split mode, splitless mode and cool on can be used.

[10] Example of chromatogram is shown in Figure 6.3.12-2.

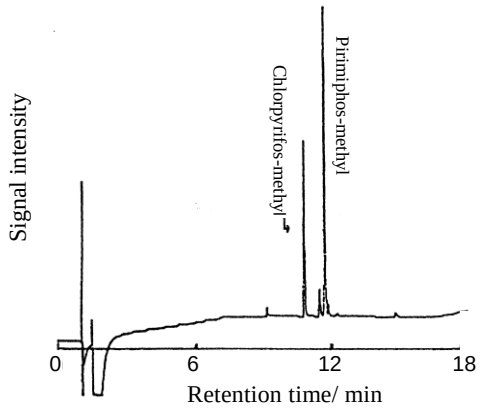


Figure 6.3.12-2. Chromatogram of formula feed spiked with chlorpyrifos-methyl and pirimiphos-methyl equivalent to 250 µg/kg

Measurement Conditions

Detector: Flame thermionic detector (FTD)

Column: DB-1 (0.32 mm in inner diameter, 30 m in length, film thickness 0.25 µm)

Carrier gas: He (4 mL/min, initial flow rate)

Make up gas: He (26 mL/min)

Hydrogen: 4 mL/min

Dry air: 100 mL/min

Sample injection: Cool-on column

Column oven temperature: 50 °C (hold 1 min) → ramp 20 °C/min → 180 °C (hold 1 min) → ramp 3 °C/min → 200 °C

Detector temperature: 240 °C

13. Simultaneous analysis method for fenbutatin oxide and cyhexatin by gas chromatography

[Analytical Standards of Feeds, Chapter 6, Section 3, Article 13]

Target Analytes: Fenbutatin oxide and cyhexatin (2 compounds)

A. Reagent Preparation

1) Mixed standard stock solution. Weigh accurately 20 mg each of fenbutatin oxide [$C_{60}H_{78}OSn_2$][1] and cyhexatin [$C_{18}H_{34}OSn$][1]. Transfer them to a 100 mL volumetric flask and dissolve by adding ethyl acetate – acetic acid (99 : 1). Further, add the same solvent up to the graduation line of the flask to prepare the mixed standard stock solution (Each 1 mL of this solution contains 0.2 mg each of fenbutatin oxide and cyhexatin).

2) Magnesium silicate. Dry synthetic magnesium silicate (particle size 149-250 μm (100-60 mesh)) for 16 hours at 130 °C.

B. Quantification

Extraction. Weigh 10.0 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 15 mL of water to moisten and leave to stand for 30 minutes. Further, add it 80 mL of acetone – acetic acid (99:1) and extract by shaking for 30 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B), by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to be around 15 mL and use as a sample solution for column treatment I.

Column treatment I. Load the sample solution on a porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 200 mL recovery flask under the column, wash the recovery flask that had contained the sample solution three times with 10 mL each of hexane, add the washings to the column in order of precedence and elute fenbutatin oxide and cyhexatin by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 40 mL of hexane to the column and elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of hexane accurately and use the solution as sample solution for ethylation.

Ethylation. Transfer accurately 1 mL of sample solution [2] to a 50 mL test tube, add 1 mL of ethylmagnesium bromide solution and leave to stand for 20 minutes to ethylate fenbutatin oxide and cyhexatin. Add it 10 mL of sulfuric acid[*1] (0.5 mol/L) by little and little[3] to decompose excess ethylmagnesium bromide, add 10 mL of water and 5 mL of hexane, shake and leave to stand. Transfer the hexane layer (upper layer) by using Pasteur pipette to a 50 mL Erlenmeyer flask. Add 5 mL of hexane to the test tube, operate in the similar way and combine the obtained hexane layer with the content of the Erlenmeyer flask. Dehydrate the hexane layer with a suitable amount of sodium sulfate (anhydrous), filter it through a filter paper (No. 2S) to a 100 mL recovery flask. Then, wash the Erlenmeyer flask and the filter paper with a small amount of hexane sequentially, and filter the washings through the filter paper and combine the filtrate. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue with 5mL of hexane and use the solution as a sample solution for column treatment II.

Column treatment II. Suspend 10 g of magnesium silicate in hexane, pour the suspension into a column (15 mm inner diameter) and elute so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Place a 200 mL recovery flask under the column and load the sample solution on the column. Wash the recovery flask that had contained the sample solution with 5 mL each of hexane three times, add the washings to the column sequentially and elute ethylated fenbutatin oxide and ethylated cyhexatin by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material.

Further, add 70 mL of hexane – diethyl ether (99 : 1) to the column to elute ethylated fenbutatin oxide and ethylated cyhexatin, concentrate the eluate on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 2 mL of hexane accurately and use this solution as a sample solution for gas chromatography.

Ethylation of standard solution. Transfer accurately 1 mL of the mixed standard stock solution to a 50 mL test tube and operate in the similar way described in "Ethylation". Concentrate the filtrate on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas. Dissolve the residue by adding accurately 10 mL of hexane, dilute a certain amount of this solution with hexane to prepare some mixed standard solutions containing 0.01 – 2 µg each of fenbutatin oxide and cyhexatin.

Gas chromatography. Inject 2 µL each of the sample solution and mixed standard solutions into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame photometric detector (filter for tin detection)

Column: Capillary column (50 % trifluoropropyl methyl/ 50 % dimethyl-polysiloxane coating, 0.32 mm in inner diameter, 30 m in length, film thickness 0.5 µm)[4]

Carrier gas: He (2 mL/min)

Make up gas: N_2 (30 mL/min)

Hydrogen: 80 mL/min

Dry air: 100 mL/min

Sample injection : Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 150 °C (hold 1 min) → ramp 10 °C/min → 280 °C (hold 5 min)

Detector temperature: 280 °C

Calculation. Obtain the peak area or peak height from the resulting chromatograms[5] to prepare a calibration curve and subsequently calculate the amount of fenbutatin oxide and cyhexatin in the sample.

* 1. Reagents for determination of toxic metals, or equivalents.

«Summary of analysis method»

This method is a simultaneous analysis method for cyhexatin and fenbutatin oxide. Each agricultural chemical in feeds is extracted with acetic acid in aqueous acetone, purified by a porous diatomite column, ethylated, purified by a magnesium silicate column and quantified by a gas chromatograph equipped with a flame photometric detector (filter for tin detection).

The flow sheet of the analysis method is shown in Figure 6.3.13-1.

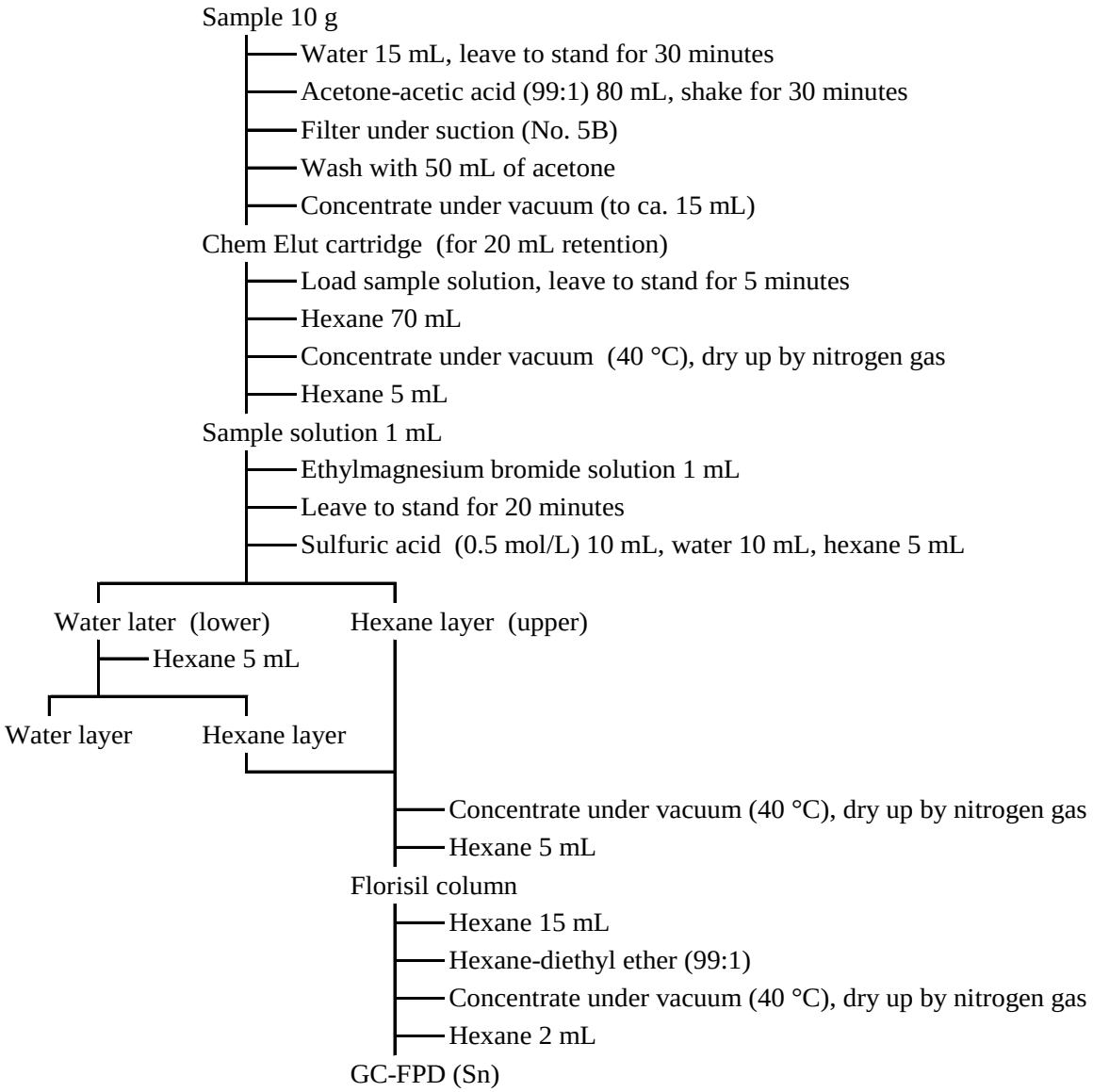


Figure 6.3.13-1. Flow sheet of the simultaneous analysis method for fenbutatin oxide and cyhexatin in feed

Reference: Akira Furukawa, Yutaka Kunugi: Research Report of Animal Feed, 24, 37 (1999).

«Method validation»

• Spike recovery and repeatability

Compounds	Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
fenbutatin oxide	finishing period broiler formula feed	100~1,000	3	84.8~93.6	14.2
	piglet formula feed	100~1,000	3	80.0~89.0	17.6
	alfalfa hay	100~1,000	3	84.4~96.0	8.6
cyhexatin	finishing period broiler formula feed	100~1,000	3	84.6~90.6	5.2
	piglet formula feed	100~1,000	3	87.1~92.7	8.3
	alfalfa hay	100~1,000	3	85.7~89.6	8.8

• Collaborative study

Compounds	Sample type	No. of labs	Spike concentration (μg/kg)	Recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
fenbutatin oxide	adult hen formula feed	7	500	95.3	5.2	9.5	0.53
cyhexatin	adult hen formula feed	7	500	78.7	5.6	17.1	0.93

• Lower limit of quantification: 20 μg/kg each in sample

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries and Kanto Chemical.

[2] In cases where precipitates are formed, they should be separated by centrifugation for prevention of analysis error.

[3] Because of possibility of bumping, add sulfuric acid at first drop by drop carefully.

[4] Rtx-200 (Restek), for example.

[5] Example of chromatogram is shown in Figure 6.3.13-2.

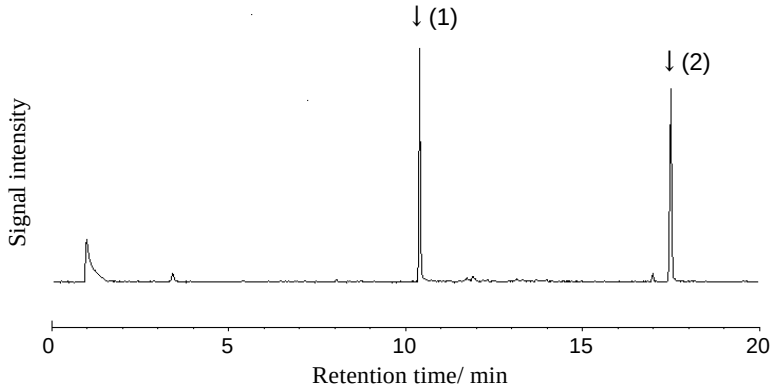


Figure 6.3.13-2. Chromatogram of formula feed spiked with fenbutatin oxide and cyhexatin equivalent to 500 μg/kg
((1) ethylated cyhexatin, (2) ethylated fenbutatin oxide)

Measurement Conditions

Detector: Flame photometric detector (filter for tin detection)

Column: Rtx-200 (0.32 mm in inner diameter, 30 m in length, film thickness 0.5 μm)

Carrier gas: He (2 mL/min, initial flow rate)

Make up gas: N_2 (30 mL/min)

Hydrogen: 80 mL/min

Dry air: 100 mL/min

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 150 °C (hold 1 min) → ramp 10 °C/min → 280 °C (hold 5 min)

Detector temperature: 280 °C

14. Simultaneous analysis method for cyanazine and myclobutanil by gas chromatograph-mass spectrometer [Analytical Standards of Feeds, Chapter 6, Section 3, Article 14]

Target Analytes: Cyanazine and myclobutanil (2 compounds)

A. Reagent Preparation

1) Cyanazine standard stock solution. Weigh accurately 50 mg of cyanazine [$C_9H_{13}ClN_6$]. Transfer it to a 100 mL brown volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the cyanadine standard stock solution (Each 1 mL of this solution contains 0.5 mg of cyanadine).

2) Myclobutanil standard stock solution. Weigh accurately 50 mg of myclobutanil [$C_{15}H_{17}ClN_4$]. Transfer it to a 100 mL brown volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the myclobutanil standard stock solution (Each 1 mL of this solution contains 0.5 mg of myclobutanil).

3) Mixed standard solution. Before use, mix a certain amount of the standard stock solutions of cyanazine and myclobutanil, dilute the mixture accurately by adding a diluting solvent to prepare the mixed standard solutions containing 0.02 – 1.0 μg each of cyanazine and myclobutanil per 1 mL.

4) Diluting solvent. 0.1 v/v% polyethylene glycol[1]-containing acetone

B. Quantification

Extraction. Weigh 10.0 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 15 mL of water[2] and leave to stand for 30 minutes. Further, add it 100 mL of acetone and extract by shaking for 60 minutes. Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to ca. 15 mL[3] and add 5 g of sodium chloride to prepare a sample solution to be subjected to column treatment I.

Column treatment I. Load the sample solution on a porous diatomite column (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that had contained the sample solution three times with 10 mL each of hexane – ethyl acetate (4 : 1), add the washings to the column in order of precedence and elute cyanazine and myclobutanil by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 70 mL of hexane – ethyl acetate (4 : 1) to the column and elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 20 mL of cyclohexane – acetone (4 : 1) accurately, filter the solution through a membrane filter (pore size: 0.5 μm or less) and use the filtrate as sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph[4]. Dispense each elution fraction to quantify cyanazine and myclobutanil into a 100 mL recovery flask, concentrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue with 5 mL of hexane – acetone (19 : 1) and use this solution as a sample solution for column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 300 m in length, particle size 15 µm)

Guard column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 100 mm in length, particle size 15 µm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 80-120 mL

Column treatment II. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane – acetone (19 : 1)[5].

Load the sample solution on the minicolumn and elute by natural flow until the liquid level reaches the upper end of the column packing material. Wash the recovery flask that had contained the sample solution with 5 mL each of hexane – acetone (19 : 1) twice, add the washings to the column in order of precedence and elute in the similar way.

Place a 50 mL recovery flask under the minicolumn and add 20 mL of hexane – acetone (7 : 3) to the minicolumn to elute cyanazine and myclobutanil. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 2 mL of acetone accurately and use this solution as a sample solution for gas chromatography.

Gas chromatography. Inject 2 µL each of the sample solution and the mixed standard solution into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Flame thermionic detector[6]

Column: Fused silica capillary column (5 % diphenyl/ 95 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)

Carrier gas: He (2.5 mL/min)

Make up gas: He (30 mL/min)

Hydrogen: 3 mL/min

Dry air: 90 mL/min

Sample injection : Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 70 °C (hold 2 min) → ramp 30 °C/min → 230 °C → ramp 2.5 °C/min → 245 °C → ramp 20 °C/min → 280 °C (hold 10 min)

Detector temperature: 280 °C

Calculation. Obtain the peak area from the resulting chromatograms[7] to prepare a calibration curve and subsequently calculate the amount of cyanazine and myclobutanil in the sample.

«Summary of analysis method»

This method is a simultaneous analysis method for cyanazine and myclobutanil. Each agricultural chemical in sample is extracted with acetone/water, purified by the use of a porous diatomite column and GPC, Florisil minicolumn and quantified by a gas chromatograph equipped with a flame thermionic detector.

The flow sheet of the analysis method is shown in Figure 6.3.14-1.

Sample 10 g
- Water 15 mL, leave to stand for 30 minutes
- Acetone 100 mL, shake for 60 minutes
- Filter under suction (No. 5B)
- Wash with 50 mL of acetone
- Concentrate under vacuum (40 ℃, to ca. 15 mL)
- Sodium chloride 5 g

Chem Elut cartridge (for 20 mL retention)
- Load sample solution, leave to stand for 5 minutes
- Wash with 10 mL of hexane-ethyl acetate (4:1) (×3)
- Elute with 70 mL of hexane-ethyl acetate (4:1)
- Concentrate under vacuum (40 ℃ or less), dry up by nitrogen gas
- Cyclohexane-acetone (4:1) 20 mL
- Membrane filter (pore size 0.45 μm)

GPC (inject 5 mL of sample solution)
- Elution fraction 80-120 mL
- Concentrate under vacuum (40 ℃ or less), dry up by nitrogen gas
- Hexane-acetone (19:1) 5 mL

Sep-Pak Plus Florisil cartridge (washed by 5 mL of hexane-acetone (19:1) before use)
- Load sample solution
- Wash with 5 mL of hexane-acetone (19:1) (×2)
- Elute with 20 mL of hexane-acetone (7:3)
- Concentrate under vacuum (40 ℃ or less), dry up by nitrogen gas
- Acetone 2 mL

GC-FTD

Figure 6.3.14-1. Flow sheet of the simultaneous analysis method for cyanazine and myclobutanil

Reference: Yuji Fukumoto, Keisuke Aoyama, Hideki Taniguchi, Masashi Watahara: Research Report of Animal Feed, 29, 21 (2004).

«Method validation»

• Spike recovery and repeatability

Compounds	Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
cyanazine	chicken formula feed	50~500	3	98.8~108.5	9.6
	cattle formula feed	50~500	3	114.4~121.6	5.4
	corn	50~500	3	96.1~104.0	4.9
	barley	50~500	3	104.7~106.3	3.6
myclobutanil	chicken formula feed	50~500	3	88.7~92.8	4.5
	swine formula feed	50~500	3	99.4~100.3	7.2
	corn	50~500	3	85.0~91.0	4.8
	barley	50~500	3	89.7~92.6	4.7

• Collaborative study

Compounds	Sample type	No. of labs	Spike concentration (μg/kg)	Recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
cyanazine	starting broiler chick formula feed	7	200	93.4	5.0	13.9	0.68
	corn	7	200	94.1	6.9	7.9	0.38
myclobutanil	starting broiler chick formula feed	7	200	89.1	5.3	10.0	0.48
	corn	7	200	91.1	5.2	14.9	0.72

• Lower limit of quantification: 10 μg/kg for cyanazine, 20 μg/kg for myclobutanil (spike concentration and relative standard deviation)

«Notes and precautions»

[1] The sensitivity of a flame thermionic detector to cyanazine and myclobutanil in GC analysis increases in the presence of traces of organic substances.

Because the sample solution contains traces of organic substances derived from the sample, it is required to add polyethylene glycol to the mixed standard solution as well in order to increase the sensitivity.

[2] In column treatment I, adding more water by mistake may lead to clogging of the porous diatomite column.

[3] Because of possible bumping, caution is required.

In column treatment I, loading an inadequately-concentrated filtrate on the porous diatomite column may lead to clogging of the column.

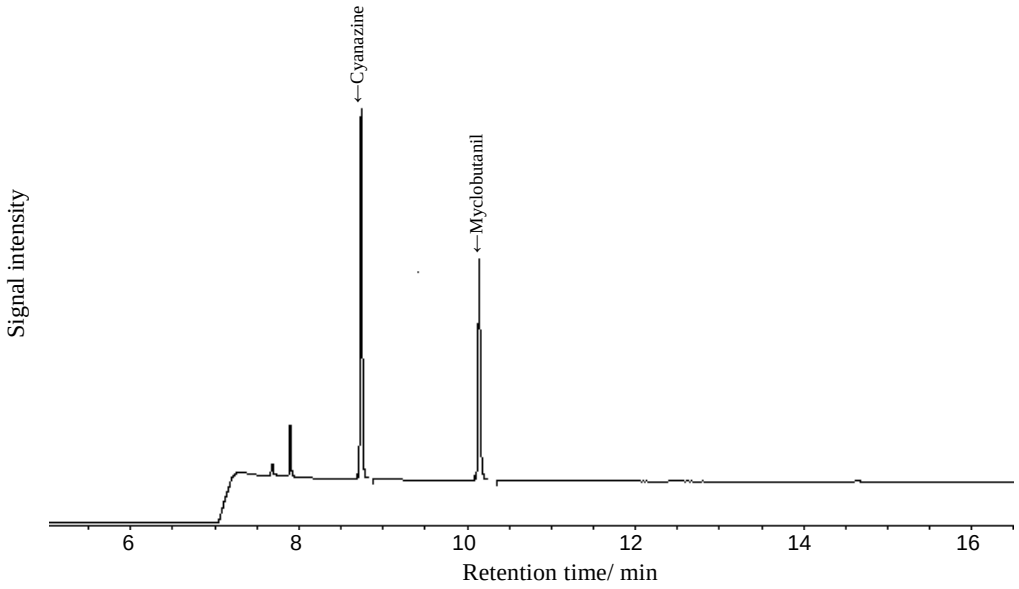
[4] For gel permeation chromatography (GPC), see also [6] and [7] of **«Notes and precautions»** in Article 1, Section 2 "Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography" of this Chapter.

[5] Prewash of a synthetic magnesium silicate minicolumn is imperative.

[6] As a flame thermionic detector deteriorates due to moisture in the air, remove it from the GC and store in a desiccator (for 2003 models).

[7] Example of chromatograms obtained with the use of the mixed standard solution and sample solution is shown in Figure 6.3.14-2.

(A)



(B)

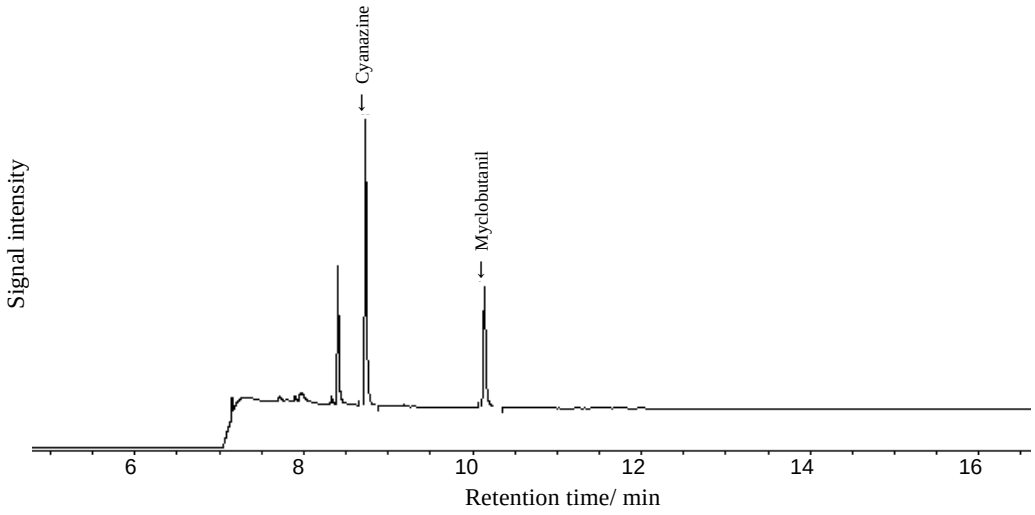


Figure 6.3.14-2. Chromatogram of mixed standard solution and sample solution

(A) Mixed standard solution (equivalent to 2 ng)

(B) Starting broiler chick formula feed (equivalent to 0.5 mg/kg)

15. Simultaneous analysis method for dicofol and trifluralin by gas chromatography [Analytical Standards of Feeds, Chapter 6, Section 3, Article 15]

Target Analytes: Dicofol and trifluralin (2 compounds)

A. Reagent Preparation

1) Dicofol standard stock solution. Weigh accurately 50 mg of dicofol [$C_{14}H_9Cl_5O$]. Transfer it to a 100 mL brown volumetric flask and dissolve by adding 20 mL of acetone. Further, add 2,2,4-trimethylpentane up to the graduation line of the flask to prepare the dicofol standard stock solution (Each 1 mL of this solution contains 0.5 mg of dicofol).

2) Trifluralin standard stock solution. Weigh accurately 50 mg of trifluralin [$C_{13}H_{16}F_3N_3O_4$]. Transfer it to a 100 mL brown volumetric flask and dissolve by adding 20 mL of acetone. Further, add 2,2,4-trimethylpentane up to the graduation line of the flask to prepare the trifluralin standard stock solution (Each 1 mL of this solution contains 0.5 mg of trifluralin).

3) Mixed standard solution. Before use, mix a certain amount of the respective standard stock solutions of dicofol and trifluralin, dilute the mixture accurately by adding 2,2,4-trimethylpentane – acetone (4 : 1) to prepare the mixed standard solutions containing 0.01 – 1.0 µg each of dicofol and trifluralin per 1 mL.

B. Quantification

Extraction. Weigh 10.0 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 15 mL of water and leave to stand for 30 minutes. Further, add it 80 mL of acetone and extract by shaking for 60 minutes. Place a 500 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetone sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to ca. 15 mL[1] and use the concentrate as a sample solution for purification.

Purification. Transfer sample solution to a 500 mL separating funnel A already containing 200 mL of sodium chloride solution (5 w/v%) and 100 mL of hexane, shake for 5 minutes and leave to stand. Transfer the water layer (lower layer) to a 500 mL separating funnel B, and the hexane layer (upper layer) to a 300 mL Erlenmeyer flask. Add 50 mL of hexane to a separating funnel B, shake for 5 minutes and leave to stand. Combine the obtained hexane layer with the content of the Erlenmeyer flask. Dehydrate the hexane layer with a suitable amount of sodium sulfate (anhydrous) and filter through a filter paper (No. 5B) into a 300 mL recovery flask. Wash the Erlenmeyer flask and the filter paper with a small amount of hexane sequentially, filter the washings through the filter paper and combine with the filtrate. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and dry up by the flow of nitrogen gas. Dissolve the residue by adding 10 mL of cyclohexane – acetone (4 : 1) accurately, filter the solution through a membrane filter (pore size: 0.5 µm or less) and use the filtrate as sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph[2]. Dispense each elution fraction into a 100 mL recovery flask, concentrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue with 5 mL of hexane and use this solution as a sample solution for column treatment.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 300 mm in length, particle size 15 μm)

Guard column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 100 mm in length, particle size 15 μm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 60-110 mL

Column treatment. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane.

Place a 100 mL recovery flask under the minicolumn and transfer accurately 2 mL of the sample solution to the minicolumn. Elute by natural flow until the liquid level reaches the upper end of the column packing material. Then, add 30 mL of hexane – diethyl ether (99 : 1) to the column to elute dicofol and trifluralin. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding 2 mL of 2,2,4-trimethylpentane – acetone (4 : 1) accurately and use this solution as a sample solution for gas chromatography.

Gas chromatography. Inject 2 μL each of the sample solution and mixed standard solution into a gas chromatograph to obtain chromatograms[3].

Example of measurement conditions

Detector: Electron capture detector

Column: Fused silica capillary column (50 % diphenyl/ 50 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)

Carrier gas: He (2.5 mL/min)

Make up gas: N_2 (60 mL/min)

Sample injection : Splitless mode (60 s)

Injection port temperature:200 °C

Detector temperature: 300 °C

Column oven temperature: Initial temperature 70 °C (hold 2 min) → ramp 20 °C/min → 280 °C (hold 10 min)

Calculation. Obtain respective the peak height or peak area from the resulting chromatograms[4] to prepare a calibration curve and subsequently calculate the amount of dicofol and trifluralin in the sample.

«Summary of analysis method»

This method is a simultaneous analysis method for dicofol and trifluralin. Each agricultural chemical in feeds is extracted with acetone/water, purified by liquid-liquid extraction, GPC and Florisil minicolumn, and quantified by a gas chromatograph equipped with an electron capture detector.

The flow sheet of the analysis method is shown in Figure 6.3.15-1.

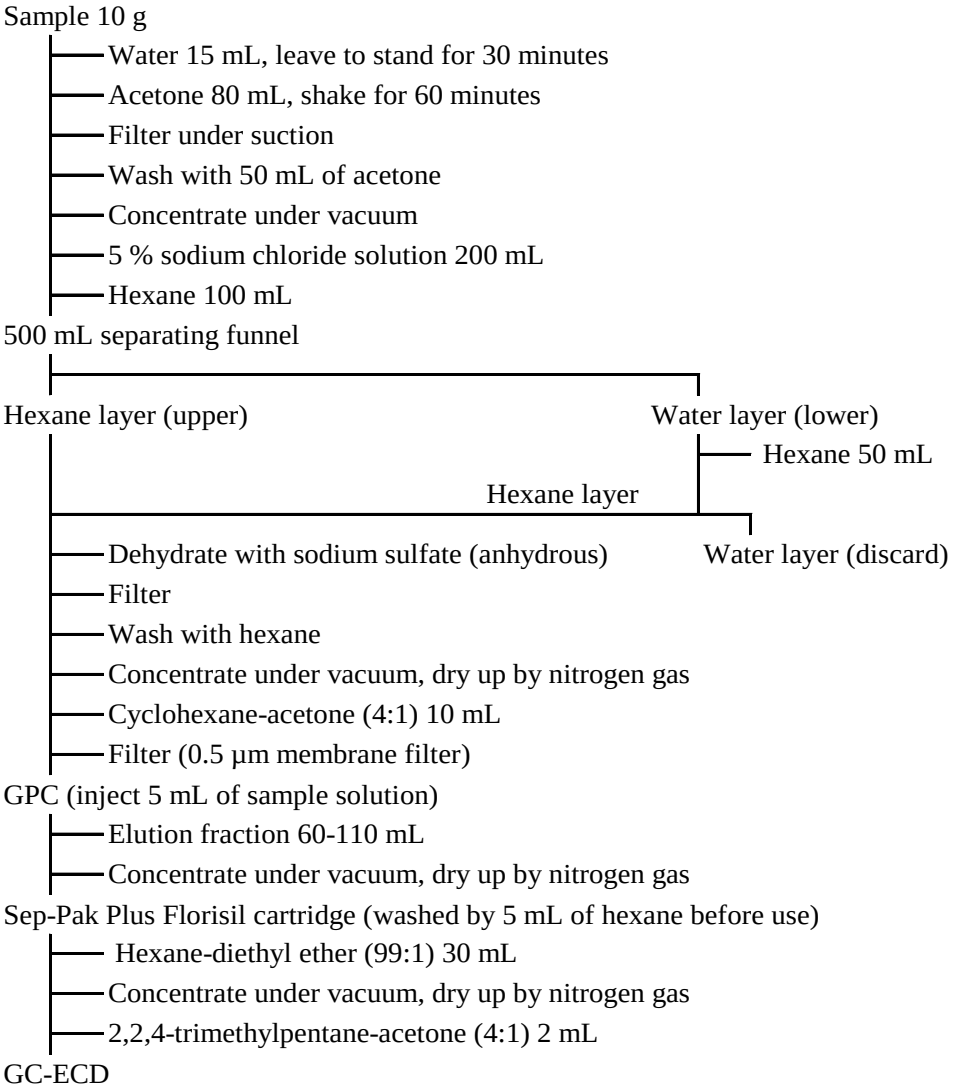


Figure 6.3.15-1. Flow sheet of the simultaneous analysis method for dicofol and trifluralin

Reference: Manabu Matsuzaki, Miyuki Matsuzaki: Research Report of Animal Feed, 29, 32 (2004).

«Method validation»

• Spike recovery and repeatability

Compounds	Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
dicofol	chicken formula feed	100~500	3	88.5~94.8	10.9
	swine formula feed	100~500	3	89.8~98.9	3.8
	alfalfa	100~500	3	89.0~94.3	8.2
	timothy	100~500	3	85.7~90.4	10.8
trifluralin	chicken formula feed	100~500	3	90.7~95.4	10.0
	swine formula feed	100~500	3	91.9~106.5	5.2
	alfalfa	100~500	3	91.6~101.0	8.8
	timothy	100~500	3	87.6~90.1	8.7

• Collaborative study

Compounds	Sample type	No. of labs	Spike concentration (μg/kg)	Recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
dicofol	finishing pig formula feed	5	200	88.2	5.6	14.2	0.68
	milo	5	200	89.9	6.5	17.5	0.84
trifluralin	finishing pig formula feed	5	200	98.6	5.5	9.0	0.44
	milo	5	200	99.7	5.9	11.8	0.58

• Lower limit of quantification: 10 μg/kg each for dicofol and trifluralin (spike recovery and relative standard deviation)

«Notes and precautions»

[1] In the extraction step, caution is required because of possible bumping during concentration under vacuum.

[2] For gel permeation chromatography (GPC), see also [6] and [7] of **«Notes and precautions»** in Article 1, Section 2 "Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography" of this Chapter.

[3] Dicofol is decomposed to 4,4′-dichlorobenzophenone, when injected into a gas chromatograph as a solution in 2,2,4-trimethylpentane – acetone (4 : 1). Therefore, in cases where it is measured with the use of a mass spectrometer, both peaks of dicofol-derived ion (*m*/*z* 251) and 4,4′-dichlorobenzophenone-derived ion (*m*/*z* 250) may be obtained.

[4] Example of chromatograms of dicofol and trifluralin is shown in Figure 6.3.15-2.

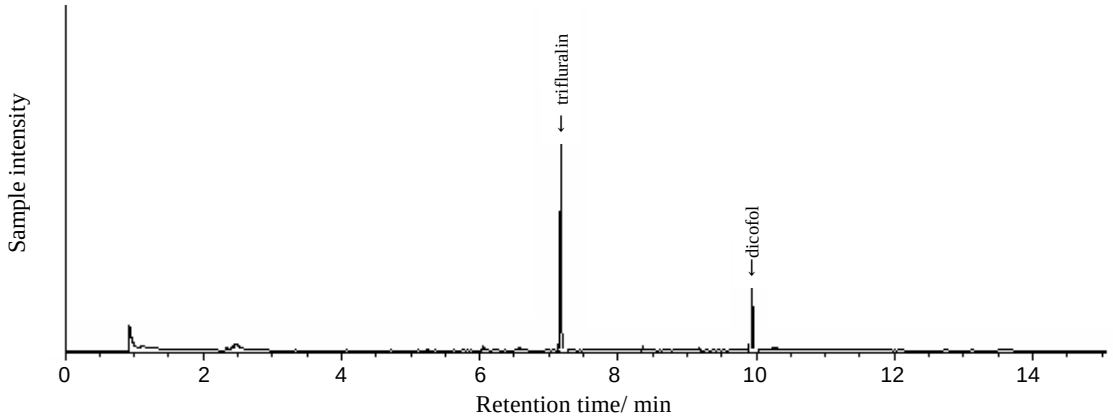


Figure 6.3.15-2. Chromatogram of adult hen formula feed spiked with dicofol and trifluralin equivalent to 50 µg/kg each

16. Simultaneous analysis method for tebuconazole and fenarimol by gas chromatography

[Analytical Standards of Feeds, Chapter 6, Section 3, Article 16]

Target Analytes: Tebuconazole and fenarimol (2 compounds)

A. Reagent Preparation

1) Tebuconazole standard stock solution. Weigh accurately 25 mg of tebuconazole [$C_{16}H_{22}ClN_3O$][1], transfer to a 50 mL brown volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the tebuconazole standard stock solution (Each 1 mL of these solutions contains 0.5 mg of tebuconazole).

2) Fenarimol standard stock solution. Weigh accurately 25 mg of fenarimol [$C_{17}H_{12}Cl_2N_2O$][1], transfer to a 50 mL brown volumetric flask and dissolve by adding acetone. Further, add the same solvent up to the graduation line of the flask to prepare the fenarimol standard stock solution (Each 1 mL of these solutions contains 0.5 mg of fenarimol).

3) Mixed standard solution. Before use, mix a certain amount of tebuconazole and fenarimol standard stock solutions and dilute the mixture accurately by a diluting solvent to prepare the mixed standard solutions containing 0.01 – 1.0 µg each of tebuconazole and fenarimol per 1 mL.

4) Diluting solvent. Add 50 µL of polyethylene glycol (average molecular weight 400) to 100 mL of 2,2,4-trimethylpentane – acetone (4 : 1) to prepare a diluting solvent.

B. Quantification

Extraction. Weigh 10.0 g of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 20 mL of acetonitrile – water (3 : 1) and leave to stand for 10 minutes. Further, add it 100 mL of acetonitrile and extract by shaking for 30 minutes.

Place a 300 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 50 mL of acetonitrile sequentially, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 40 °C or lower to almost dryness[2], add 20 mL of water and use this solution as a smaple solution for column treatment I.

Column treatment I Load the sample solution on a porous diatomite column[3] (for 20 mL retention) and leave to stand for 5 minutes. Place a 300 mL recovery flask under the column, wash the recovery flask that had contained the sample solution three times with 20 mL each of hexane, add the washings to the column in order of precedence and elute tebuconazole and fenarimol by natural flow until the liquid level reaches the upper end of the column packing material. Further, add 60 mL of hexane to the column and elute in the similar way. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas. Dissolve the residue by adding 10 mL of cyclohexane – acetone (4 : 1) accurately, filter the the solution through a membrane filter (pore size: 0.5 µm or less)[4] and use the filtrate as sample solution for gel permeation chromatography.

Gel permeation chromatography. Inject 5.0 mL of the sample solution into the gel permeation chromatograph[5]. Dispense each elution fraction into a 100 mL recovery flask, concentrate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue with 2 mL of hexane and use this solution as a sample solution for column treatment II.

Example of operating conditions

Column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 300 mm in length, particle size 15 μm)

Guard column: Styrene-divinylbenzene copolymer column (20 mm in inner diameter, 100 mm in length, particle size 15 μm)

Eluent: Cyclohexane – acetone (4 : 1)

Flow rate: 5 mL/min

Fraction collection: 70-125 mL

Column treatment II. Wash a synthetic magnesium silicate minicolumn (910 mg) with 5 mL of hexane – acetone (29 : 1).

Load the sample solution on the minicolumn and elute by natural flow until the liquid level reaches the upper end of the column packing material. Wash the recovery flask that had contained the sample solution with 2 mL each of hexane twice, add the washings to the column in order of precedence and elute in the similar way. Further, add 10 mL of hexane – acetone (19 : 1) to the minicolumn and elute in the similar way. Place a 50 mL recovery flask under the minicolumn and add 20 mL of hexane – acetone (7 : 3) to the minicolumn to elute tebuconazole and fenarimol. Concentrate the eluate under reduced pressure on a water bath at 40 °C or lower to almost dryness and further dry up by the flow of nitrogen gas.

Dissolve the residue by adding accurately 2 mL of the diluting solvent and use this solution as a sample solution for measurement by gas chromatograph-mass spectrometer.

Measurement by gas chromatograph-mass spectrometer. Inject 2 μL each of the sample solution and the mixed standard solution to a gas chromatograph-mass spectrometer to obtain selected ion monitoring chromatograms.

Example of measurement conditions

Column: Fused silica capillary column (5 % diphenyl/ 95 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 μm)[6]

Carrier gas: He (1.0 mL/min, initial flow rate)

Sample injection: Splitless mode (60 s)

Injection port temperature: 250 °C

Column oven temperature: Initial temperature 70 °C (hold 2 min) → ramp 20 °C/min → 280 °C (hold 10 min)

Detector: Quadrupole mass spectrometer[*1]

Interface temperature: 280 °C

Ion source temperature: 200 °C

Ionization method: Electron ionization (EI)

Ionizing voltage: 70 eV

Monitor ion: *m*/*z* 250 (tebuconazole), 330 (fenarimol)

Calculation. Obtain the peak area from the resulting selected ion monitoring chromatograms[7] to prepare a calibration curve and subsequently calculate the amount of tebuconazole[*2] and fenarimol in the sample.

* 1. Measurement conditions according to GCMS-QP2010 (Shimadzu Corporation).

2. If the amount of tebuconazole in feed exceeds 5 mg/kg, the test[8] described in Article 107-3, Section 1 of this Chapter should be performed, as the recovery may decrease with the use of this method.

«Summary of analysis method»

This method is a simultaneous analysis method for tebuconazole and fenarimol. Each agricultural chemical in feeds is extracted with acetonitrile/water, purified by a porous diatomite column, GPC and Florisil minicolumn, and quantified by a gas chromatograph-mass spectrometer.

The flow sheet of the analysis method is shown in Figure 6.3.16-1.

Sample 10 g
- Acetonitrile-water (3:1) 20 mL, leave to stand for 10 minutes
- Acetonitrile 100 mL, shake for 30 minutes
- Filter under suction (No. 5B)
- Wash with 50 mL of acetonitrile
- Concentrate under vacuum (40 °C)
- Water 20 mL

Chem Elut cartridge (for 20 mL retention)
- Load sample solution, leave to stand for 5 minutes
- Elute with 120 mL of hexane
- Concentrate under vacuum (40 °C), dry up by nitrogen gas
- Cyclohexane-acetone (4:1) 10 mL
- Membrane filter (pore size 0.5μm)

GPC (inject 5 mL of sample solution)
- Elution fraction 70-125 mL
- Concentrate under vacuum (40 °C), dry up by nitrogen gas
- Hexane 2 mL

Sep-Pak Plus Florisil cartridge (washed with 5 mL of hexane)
- Wash with 2 mL of hexane (×2)
- Wash with 10 mL of hexane-acetone (19:1)
- Elute with 20 mL of hexane-acetone (7:3)
- Concentrate under vacuum (40 °C), dry up by nitrogen gas
- Containing 0.05 v/v% polyethylene glycol 2,2,4-trimethylpentane-acetone (4:1) 2 mL

GC-MS

Figure 6.3.16-1. Flow sheet of the simultaneous analysis method for tebuconazole and fenarimol

Reference: Keisuke Aoyama, Tomoe Inoue: Research Report of Animal Feed, 30, 20 (2005).

«Method validation»

• Spike recovery and repeatability

Compounds	Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
tebuconazole	finishing period broiler formula feed	50~500	3	86.6~91.9	9.8
	growing cattle formula feed	50~500	3	99.5~101.0	11.1
	corn	50~500	3	90.1~91.9	10.8
	ryegrass	50~500	3	85.4~87.1	4.7
fenarimol	finishing period broiler formula feed	50~500	3	95.3~98.6	9.0
	growing cattle formula feed	50~500	3	100.1~102.2	5.1
	corn	50~500	3	96.9~101.2	4.6
	ryegrass	50~500	3	97.6~97.9	3.6

• Collaborative study

Compounds	Sample type	No. of labs	Spike concentration (μg/kg)	Recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
tebuconazole	finishing period broiler formula feed	5	200	89.1	2.1	9.3	0.45
	barley	5	200	91.0	6.3	11.6	0.56
fenarimol	finishing period broiler formula feed	5	200	92.7	5.4	6.0	0.29
	barley	5	200	90.9	7.3	10.2	0.49

• Lower limit of quantification: 5 μg/kg for tebuconazole, 10 μg/kg for fenarimol (spike recovery and relative standard deviation)

• Upper limit of quantification: 5 mg/kg for tebuconazole

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries and other manufacturers.

[2] To prevent bumping, warm up filtrates and eluates on a water bath by holding a recovery flask on the level where its bottom touches the hot water.

[3] In a porous diatomite cartridge, agricultural chemicals in water solution are transferred into hexane by solvent exchange.

[4] DISMIC-25HP (ADVANTEC MFS) or equivalents.

[5] For gel permeation chromatography (GPC), see also [6] and [7] of **«Notes and precautions»** in Article 1, Section 2 "Systematic analysis methods for organochlorine and acid-amide agricultural chemicals by gas chromatography" of this Chapter.

[6] HP-5MS (Agilent Technologies), for example.

[7] Example of chromatogram is shown in Figure 6.3.16-2.

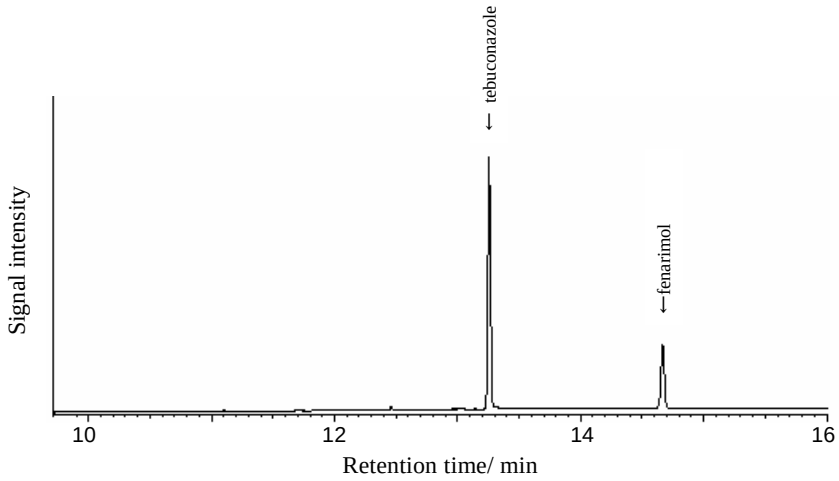


Figure 6.3.16-2. Chromatogram of agricultural chemicals mixed standard solution (2 ng each)

[8] See Article 107.3 in Section 1 “Tebuconazole” of this Chapter (p.853).

17. Simultaneous analysis method for fenvalerate and permethrin by gas chromatography

[Analytical Standards of Feeds, Chapter 6, Section 3, Article 17]

Target Analytes: Fenvalerate and permethrin (2 compounds)

A. Reagent Preparation

1) Fenvalerate standard stock solution. Weigh accurately 10 mg of fenvalerate [$C_{25}H_{22}ClNO_3$][1], transfer to a 100 mL brown volumetric flask and dissolve by adding hexane. Further, add the same solvent up to the graduation line of the flask to prepare the fenvalerate standard stock solution (Each 1 mL of these solutions contains 0.1 mg of fenvalerate).

2) Permethrin standard stock solution. Weigh accurately 10 mg of permethrin [$C_{21}H_{20}Cl_2O_3$][1], transfer to a 100 mL brown volumetric flask and dissolve by adding hexane. Further, add the same solvent up to the graduation line of the flask to prepare the permethrin standard stock solution (Each 1 mL of these solutions contains 0.1 mg of permethrin).

3) Mixed standard solution. Before use, mix a certain amount of fenvalerate and permethrin standard stock solutions and dilute the mixture accurately by adding hexane to prepare the mixed standard solutions containing 0.05 – 1 μg each of fenvalerate and permethrin per 1 mL.

4) Magnesium silicate. Dry synthetic magnesium silicate (particle size 149-250 μm (100-60 mesh)) at 130 °C for 5 hours.

B. Quantification

Extraction. Weigh 5-10 g[2] of the sample, transfer it to a stoppered 200 mL Erlenmeyer flask, add 100 mL of acetonitrile – water (7 : 3) and extract by shaking for 30 minutes. Place a 500 mL recovery flask under a Büchner funnel and filter the extract through a filter paper (No. 5B) by suction. Then, wash the Erlenmeyer flask and the residue with 10 mL of acetonitrile – water (7 : 3) sequentially twice, and filter the washings by suction in the similar way. Concentrate the filtrate under reduced pressure on a water bath at 50 °C or lower to ca. 30 mL[3] and use as a sample solution for purification.

Purification. Transfer sample solution to a 300 mL separating funnel already containing 100 mL of sodium chloride solution (10 w/v%) and 30 mL of dichloromethane. Wash the recovery flask that had contained the sample solution with 10 mL each of dichloromethane twice, combine the washings with the content of the separating funnel, shake for 5 minutes and leave to stand. Transfer the dichloromethane layer (lower layer) to an Erlenmeyer flask. Add 50 mL of dichloromethane to the separating funnel, operate in the similar way and transfer the dichloromethane layer to the Erlenmeyer flask. Dehydrate the dichloromethane layer with a suitable amount of sodium sulfate (anhydrous) and filter into a 300 mL recovery flask through a filter paper (No. 2S). Wash the recovery flask and the filter paper with a small amount of dichloromethane, filter the washings through the filter paper and combine the filtrate with the filtrate. Concentrate the filtrate under reduced pressure on a water bath at 50 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue with 10 mL of hexane and use this solution as a sample solution for column treatment I.

Column treatment I. Suspend 10 g of magnesium silicate and 3 g of sodium sulfate (anhydrous) in hexane respectively, pour the suspensions into a column (15 mm inner diameter) sequentially and elute

so that the liquid level reaches to the height of 3 mm from the upper end of the column packing material to prepare a column.

Load the sample solution on the column and elute by natural flow until the liquid level reaches to the height of 3 mm from the upper end of the column packing material. Further, add 90 mL of hexane to the column and elute in the similar way. Place a 300 mL recovery flask under the column, add 100 mL of hexane – diethyl ether (7 : 3) to the column to elute fenvalerate and permethrin. Concentrate the eluate under reduced pressure on a water bath at 50 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of acetonitrile, add 5 mL of water[4] and use this solution as a sample solution for column treatment II.

Column treatment II. Wash an octadecylsilylated silica gel minicolumn (360 mg) with 10 mL of water.

Filter the sample solution through a membrane filter (pore size: 0.5 µm or less)[5] into the minicolumn. Wash the recovery flask that had contained the sample solution and the membrane filter with a small amount of acetonitrile – water (1 : 1), add the washings through the membrane filter to the minicolumn and elute by positive pressure*1. Add 10 mL of acetonitrile – water (1 : 1) to the minicolumn and elute in the similar way to wash the minicolumn. Place a 100 mL recovery flask under the minicolumn and elute fenvalerate and permethrin under positive pressure*1. Concentrate the eluate under reduced pressure on a water bath at 50 °C or lower to almost dryness and dry up by the flow of nitrogen gas.

Dissolve the residue by adding 5 mL of hexane accurately and use this solution as a sample solution for gas chromatography.

Gas chromatography. Inject 1 µL each of the sample solution and the mixed standard solution into a gas chromatograph to obtain chromatograms.

Example of measurement conditions

Detector: Electron capture detector

Column: Capillary column (14 % cyanopropyl-phenyl/ 86 % dimethyl-polysiloxane coating, 0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)[6]

Carrier gas: He (1.5 mL/min)

Make up gas: N_2 (60 mL/min)

Sample injection: Splitless mode (60 s)

Injection port temperature: 260 °C

Column oven temperature: Initial temperature 80 °C (hold 1 min) → ramp 30 °C/min → 250 °C (hold 5 min) → ramp 1 °C/min → 280 °C[7]

Detector temperature: 300 °C

Calculation. Obtain the respective sum[9] of two peak area or peak height from the resulting chromatograms[8] to prepare a calibration curve and subsequently calculate the amount of fenvalerate and permethrin in the sample.

* 1. The flow rate should be 1-2 mL/min.

«Summary of analysis method»

This method is a simultaneous analysis method for fenvalerate and permethrin. Each agricultural chemical in feeds is extracted with acetonitrile/water, purified by liquid-liquid partition, a magnesium silicate column and a C_{18} minicolumn, and quantified by a gas chromatograph equipped with an electron capture detector.

The flow sheet of the analysis method is shown in Figure 6.3.17-1.

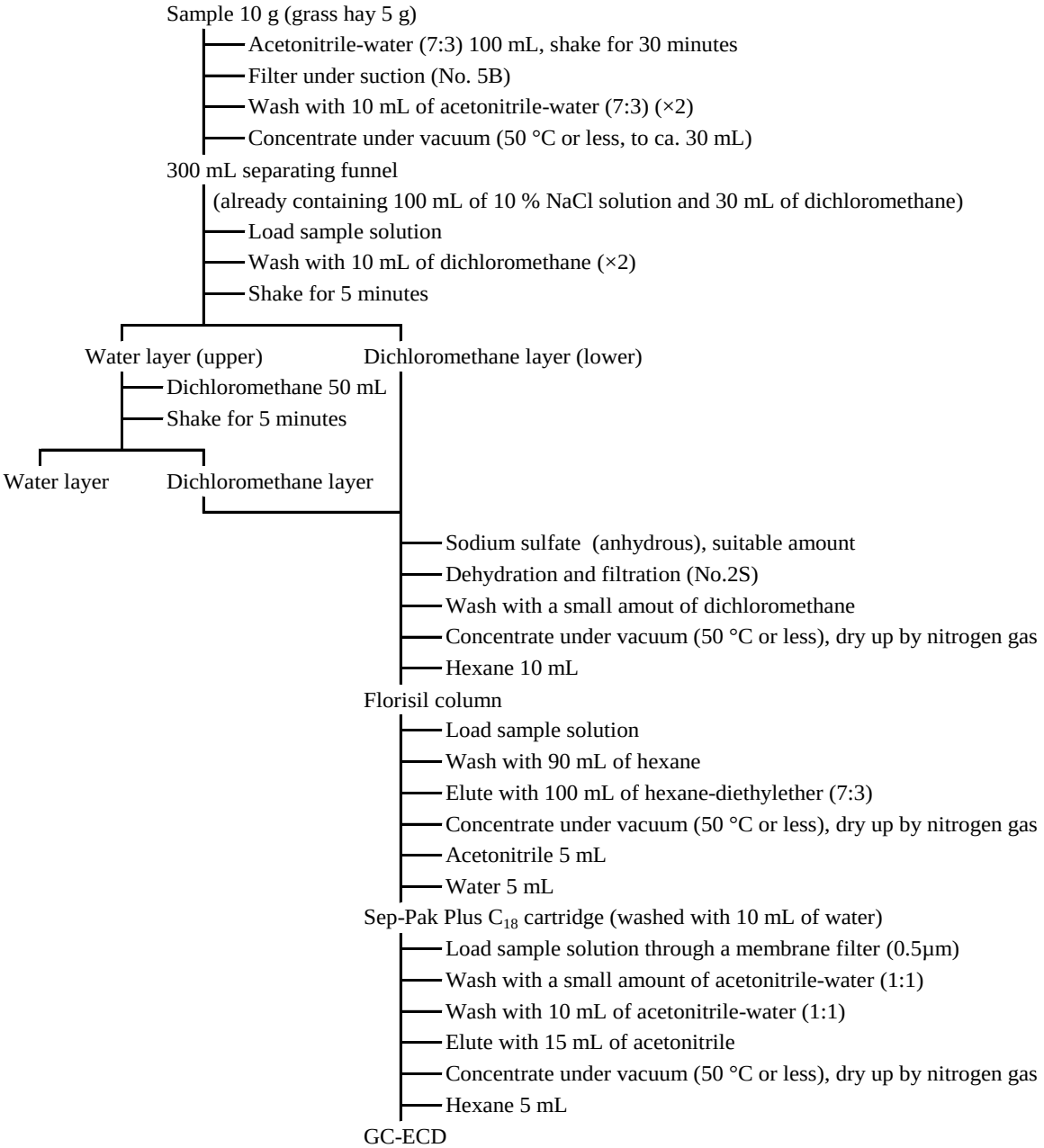


Figure 6.3.17-1. Flow sheet of the simultaneous analysis method for fenvalerate and permethrin in feed

Reference: Norio Saito: Research Report of Animal Feed, 20, 39 (1995).

«Method validation»

• Spike recovery and repeatability

Compounds	Sample type	Spike concentration (μg/kg)	Replicate	Recovery (%)	Repeatability RSD (% or less)
fenvalerate	corn	50~500	3	88.4~105.5	12.5
	alfalfa	50~500	3	91.2~118.9	7.9
	adult hen formula feed	50~500	3	91.7~105.9	9.6
permethrin	corn	50~500	3	86.2~100.9	10.2
	alfalfa	50~500	3	95.3~113.9	6.0
	adult hen formula feed	50~500	3	89.1~100.1	16.6

• Collaborative study

Compounds	Sample type	No. of labs	Spike concentration (μg/kg)	Recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
fenvalerate	finishing period broiler formula feed	6	250	95.3	8.1	11.1	0.56
permethrin	finishing period broiler formula feed	6	250	97.0	6.8	8.3	0.42

• Lower limit of quantification: 50 μg/kg each in sample

«Notes and precautions»

[1] The standards are available from Wako Pure Chemical Industries, Kanto Chemical, Hayashi Pure Chemical, GL Sciences and other manufacturers.

[2] The suitable amount of feed for analysis is 10 g for formula feed and 5 g for grass hay.

[3] Because of extremely high possibility of bumping, caution is required during concentration under vacuum. Adding boiling chips to the filtrate is modestly beneficial for preventing bumping. In cases where boiling chips are used, it is advisable to filter the filtrate through a piece of tissue wiper for laboratory (Kim Wipe from Jujo Kimberly (current Nippon Paper Crecia)) or to use a bump trap (PAT C-1 from EYELA).

[4] The residue obtained from grass hay may dissolve in 10 mL of acetonitrile – water (1 : 1) well, but that from feed does not dissolve well in this solvent. Therefore, in this method, fenvalerate and permethrin are dissolved by adding acetonitrile first, followed by adding the same amount of water.

[5] Adding water to fenvalerate and permethrin dissolved in acetonitrile may produce colorless precipitation, which may cause clogging of the minicolumn. Therefore, the solution needs to be filtered through a membrane filter for both aqueous and solvent solutions, made of hydrophilic PTFE, for example.

[6] DB-1701 (Agilent Technologies), for example.

[7] In cases where other peaks appeared after elution of fenvalerate, the sample solution should be retained at 280 °C for 15 minutes.

[8] Example of chromatogram is shown in Figure 6.3.17-2.

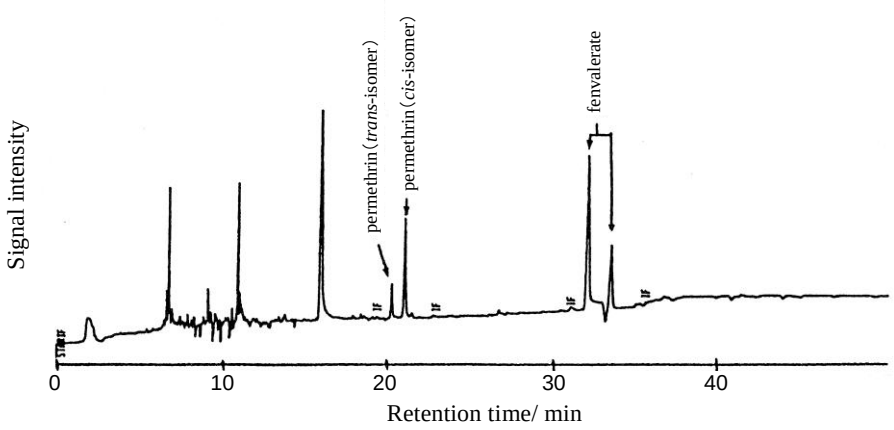


Figure 6.3.17-2. Chromatogram of formula feed spiked with fenvalerate and permethrin equivalent to 0.25 µg/kg each

Measurement conditions

Detector: Electron capture detector (ECD)

Column: DB-1701 (0.25 mm in inner diameter, 30 m in length, film thickness 0.25 µm)

Carrier gas: He (1.5 mL/min、initial flow rate)

Make up gas: N_2 (60 mL/min)

Injection port temperature: 260 °C

Column oven temperature: Initial temperature 80 °C (hold 1 min) → ramp 30 °C/min → 250 °C (hold 5 min) → ramp 1 °C/min → 280 °C (hold 15 min)

Detector temperature: 300 °C

[9] For fenvalerate, two peaks appear in one chromatogram obtained by gas chromatography under the conditions for this method. For permethrin, which has theoretically four isomers, two peaks of *cis*- and *trans*-isomer appear in one chromatogram.