Testing Methods for Fertilizers (2014)

Incorporated Administrative Agency Food and Agricultural Materials Inspection Center

Introduction

"The Official Methods of Analysis of Fertilizers" stipulated by the Ministry of Agriculture, Forestry and Fisheries is the only analysis method to assess main components and harmful components in fertilizers in Japan and contributes to maintaining quality and securing safety of fertilizers. However no new revision has been issued since "The Official Methods of Analysis of Fertilizers 1992" was issued. Some quarters such as fertilizer manufacturers and inspection instruction agencies have requested a revised edition of "The Official Methods of Analysis of Fertilizers" since new kinds of fertilizers and its new components were added into the public standard, and analysis instruments and technologies have progressed during the period.

Incorporated Administrative Agency, Food and Agricultural Materials Inspection Center (FAMIC) has revised the Official Methods of Analysis of Fertilizers by introducing the analysis conditions and the analysis methods, etc. which meet the latest situation. Additionally, FAMIC tried to study how to introduce the analysis methods or the new analysis instruments to cope with new effective components or harmful components and new fertilizers which are not documented in the Official Methods of Analysis of Fertilizers, and established new testing methods. At the same time, FAMIC conducted a validity test according to the requirements of ISO/IEC 17025 and opened the results and new testing methods which were discussed and approved by "the Technical Committee for Fertilizers etc." including outside experts on FAMIC's web site in 2008 as "The Testing Methods for Fertilizers 2008". Since then, the contents have been annually added and updated. In this year, two testing methods such as nitrous acid (High Performance Liquid Chromatography), whose validity were checked at a single laboratory in 2013, were merged as reference methods. In addition, four testing methods such as melamine (High Performance Liquid Chromatography) were formally merged from reference methods and the "Testing Methods for Fertilizes 2014" in which 57 items (components, etc.) and 94 testing methods were incorporated was opened on FAMIC's web site.

In addition, the procedure to validate characteristics of testing methods is appended as an Appendix this year. The Appendix explains the procedure for performance evaluation of testing methods which will be listed in the Testing Methods of Fertilizers, and furthermore evaluation criteria prepared based on the validation test results of new testing methods hitherto conducted and the performance investigation results of existing methods, etc. is shown in the Appendix.

Although the "Testing Methods for Fertilizes" cannot substitute "the Official Methods of Analysis of Fertilizers" which is stipulated as an official method in an official notice of the Ministry of Agriculture, Forestry and Fisheries, reagents and instruments used are stipulated in JIS standards, the validity of the testing method is checked by referencing IUPAC protocols and it is listed as an analysis method whose validity is checked in "The Management Handbook of Heavy Metal in Sludge Fertilizers" published by the Ministry of Agriculture, Forestry and Fisheries in August 2010. Therefore FAMIC would like people engaged in the quality control and analysis of fertilizes to use this as a practical document.

December, 2014

Incorporated Administrative Agency
Food and Agricultural Materials Inspection Center
General Director
Makoto Kimura

The members of The Technical Committee for the Testing Methods for Fertilizers
(As of March, 2014)

(Without honorifics)

Mayumi AIZAKI	Saitama Prefectural Agriculture and Forestry Comprehensive Research Center
Masashi UWASAWA	Japan Fertilizer and Feed Inspection Association
Akira KAWASAKI	Incorporated Administrative Agency: National Institute for Agro-Environmental Sciences
Narihiro NAITO	Incorporated Administrative Agency: National Agriculture and Food Research Organization
Munetomo NAKAMURA	Japan Food Research Laboratories
Akira NOGUCHI	Incorporated Educational Institution: Nihon University
Kazuyuki YAZIMA	Corporate Juridical Person: Niigata Prefectural Institute of Public Health and Environmental Sciences
Akemi YASUI	Incorporated Administrative Agency: National Agriculture and Food Research Organization

Contents

1. General Rules

1.1 Common Items

(1) Applicable range

The Testing Methods for Fertilizers stipulate the official method of analysis of fertilizers and fertilizer materials. The type of samples in the tests is shown in the summary of respective test items.

(2) General matters and terms in common

a) **General notices:** General matters common to analysis are according to JIS K 0050.

b) **Definition:** The definitions of major terms used in the Testing Methods for Fertilizers are according to JIS K 0211, JIS K 0214, JIS K 0215, JIS Z 8101-1, JIS Z 8101-2 or JIS Z 8101-3.

c) **Main components or major components:** The main components or major components in fertilizers in Table 1 are stipulated as components to be calculated by a public notice of the Ministry of Agriculture, Forestry and Fisheries.

Table 1 Components to be calculated as main component or major component in fertilizers

Main component or major component	Component to be calculated
Phosphate	Phosphorus pentoxide (P_2O_5)
Potassium	Potassium oxide (K_2O)
Silicate	Silicon dioxide (SiO_2)
Magnesium	Magnesium oxide (MgO)
Manganese	Manganese oxide (MnO)
Boron	Boron oxide (B_2O_3)
Sulfur content	Sulfur trioxide (SO_3)
Lime	Calcium oxide (CaO)

d) **Organic matters:** Organic fertilizers, sludge fertilizers, compost, etc. Organic compounds such as urea are excluded.

e) **Laboratory sample:** A sample transferred to a laboratory. Laboratory sample as specified in JIS K 0211.

f) **Test sample:** A sample obtained from a laboratory sample after pretreatment such as grinding. Test sample as specified in JIS K 0211.

g) **Analytical sample:** A sample measured from a laboratory sample or a test sample and to be used in one test.

h) **Sample:** A sample in the Test methods indicates a laboratory sample, a test sample or an analytical sample.

i) **Dilution of solution:** "Transfer accurately a predetermined amount (to a vessel)" means the procedure to measure any volume of solution with a measuring instrument specified in JIS K 0050 (into a vessel).
Also, "dilute accurately a predetermined amount (with solvent or solution)" means the procedure to measure any volume of solution with a measuring instrument specified in JIS K 0050 into a volumetric flask of arbitrary volume and fill up to the marked line(with solvent or solution).(1)

j) **Preparation of a calibration curve:** "Transfer A mL - B mL of the standard solution to volumetric flasks step-by-step." means the procedure to transfer a volume of 4 - 6 steps (2) in the range from A mL to B mL of the standard solution to respective volumetric

flasks step-by-step.
Prepare a calibration curve every time a test is conducted. Also, when the same test item is measured under the same conditions for multiple samples continuously, measure the standard solution at regular intervals to check the indicated value.

k) Notes, comments, figures, tables and formulae: Serial numbers for each test item should be recorded in notes, comments, figures, tables and formulae.

l) Rounding numbers: Methods of rounding the numbers are according to JIS Z 8401.

m) Referential matters related to the validity of testing methods: Information related to the validity of respective test methods such as quantification range (minimum and maximum limits of quantification), mean recovery, repeatability and reproducibility is described in a Comment, etc. Note that the numerical values such as Minimum Limit of Quantification, etc. are not standards to be targeted but examples.

n) Absorptiometric analysis: General rules for absorptiometric analysis are according to JIS K 0115.

o) Atomic absorption spectrometry: Atomic absorption spectrometry includes flame atomic absorption spectrometry, electrically heated atomic absorption spectrometry (hereinafter referred to as "electrically heated atomic absorption spectrometry") and other atomic absorption spectrometry. General matters common to these are according to JIS K 0121.

p) Gas chromatography: General matters common to gas chromatography are according to JIS K 0114.

q) Gas chromatography/Mass spectrometry: General matters common to gas chromatography/Mass spectrometry are according to JIS K 0123.

r) Electrical conductivity measuring method: General matters common to electrical conductivity measuring methods are according to JIS K 0130.

s) Test sieving: General matters common to test sieving are according to JIS Z 8815.

t) High Performance Liquid Chromatography: General matters common to High Performance Liquid Chromatography are according to JIS K 0124.

u) High Performance Liquid Chromatography/Mass Spectrometry: General matters common to High Performance Liquid Chromatography mass spectrometry are according to JIS K 0136.

v) ICP Atomic Emission Spectrometry: General matters common to ICP Atomic Emission Spectrometry are according to JIS K 0116.

w) Ion Chromatography: General matters common to Ion Chromatography are according to JIS K 0127.

Note **(1)** When the dilution factor is large, accuracy should be secured by procedures such as repeating the dilution procedure.

(2) Set according to the specification and operation method of the measurement instrument used. There is no need to include the minimum and the maximum values.

(3) Water

a) Water: Water used in the Testing Methods for Fertilizers herein is water of A2 specified in JIS K 0557 or water that is confirmed not to affect a quantitation value. However, when otherwise specified in respective test items, use the specified water.

(4) Reagents

a) Reagents: When the reagent is JIS-specified, use one of highest quality among those marked with the JIS symbol; when none of the reagent is marked with the JIS symbol, use one of quality that will not cause a problem in the test. Use reference materials for volumetric analysis specified in JIS K 8005 for the standardization of titration solutions.

- **b) Concentration of reagent solution:** Unless otherwise specified, the mass concentration is expressed as g/L or mg/L, while the molar concentration is expressed as mol/L or mmol/L. The concentration of the standard solution is expressed as the mass in 1 mL (mg/mL, µg/mL or ng/mL) except for the ion-selective electrode method.
- **c) Concentration in parenthesis shown after the name of reagent solution:** It indicates that the solution is about that concentration except the standard solution. For example, sodium hydroxide solution (0.1 mol/L) means that it is about 0.1 mol/L sodium hydroxide solution. Also, the concentration shown in front of the name of solution means that it is the accurate concentration. However, the concentration is generally expressed as a round figure; calculate the factor separately.
- **d) Description of mixture solution:** Mixture solutions are described as shown in **1) - 4)**.
  - **1) Reagent + reagent**: Describe as reagent name 1 - reagent name 2 (V_1 + V_2). In this case, V_1 volume of reagent name 1 is mixed with V_2 volume of reagent name 2.
    Example: acetonitrile-water (1+1), hexane-ethyl acetate (2+1), methanol-buffer solution (3+1)
  - **2) Reagent + water**: Describe as reagent name 1 (V_1+V_2). In the case of reagents described in Table 1 in JIS K 0050, it means V_1 volume of reagent name 1 is diluted by mixing with V_2 volume of water.
    Example: hydrochloric acid (1+1), sulfuric acid (1+2), ammonia solution (1+3)
  - **3) Solution + reagent**: Describe as solution name a (concentration) - reagent name b [V_1+V_2]. In this case, it means V_1 volume of solution name a of a certain concentration is mixed with V_2 volume of reagent name b.
    Example: sodium hydroxide solution (4 g/L) - methanol [1+4]
  - **4) Diluted reagent + reagent**: Describe as reagents name a (V_1+V_2) - reagent name b [V_3+V_4]. In this case, V_3 volume of the solution in which V_1 volume of reagent name a described in Table 1 in JIS K 0050 diluted by mixing with V_2 volume of water, is mixed with V_4 volume of reagent name b.
    Example: hydrochloric acid (1+100) -methanol [2+3]
- **e) Water for reagent preparation:** Use water in **(3) a)**. However, when otherwise specified in respective test items, use the specified water.
- **f) Reagent name:** Unless otherwise specified, conform to the names by the chemical nomenclature established by the Chemical Society of Japan [in accordance with the International Union of Pure and Applicable Chemistry (IUPAC) nomenclature of inorganic chemistry and nomenclature of organic chemistry] and the names of JIS reagents.
- **g) Handling of reagents and liquid waste, etc.:** Handle with care and in compliance with relevant laws and regulations.

(5) Apparatus

- **a) Glass apparatus:** Unless otherwise specified, use glass apparatus specified in JIS R 3503 and JIS R 3505. Also, when a heating procedure is involved, use borosilicate glass-1 specified in JIS R 3503.
- **b) Desiccants for desiccators:** Unless otherwise specified, use silica gel.
- **c) Porcelain crucibles and porcelain basins:** Use ones specified in JIS R 1301 and JIS R 1302.
- **d) Platinum crucibles and platinum dishes:** Use ones specified in JIS H 6201 and JIS H 6202.
- **e) Filter paper:** Use that specified in JIS P 3801. However, the type of filter paper is specified in respective test items.
- **f) Absorbance measurement (absorptiometric analysis) absorbance cells:** Unless otherwise specified, use ones of 10 mm in optical path length.

1.2 Validity check of testing methods

The Testing Methods for Fertilizers have been discussed and approved by the Technical Committee for Fertilizers etc. The Testing Methods for Fertilizers will be revised such as by adding, modifying, or deleting test methods with the approval of the Technical Committee for Fertilizers etc. due to the needs such as from progress in analysis techniques and changing social situation.

Additionally, although validated test methods can be used instead of the test methods herein, a final judgment should be determined with a stipulated method when a result is doubtful.

Comment The procedure of the validity check of testing methods is shown in the Appendix of this Testing Methods for Fertilizers. This procedure was made based on 5.4.5 Validation of methods in JIS Q 17025 “General requirements for the competence of testing and calibration laboratories” or 2.4 Tests for validation requirements in “Guidelines for the design and implementation of surveillance and monitoring and for the evaluation and publication of the results” which was issued by the Ministry of Agriculture, Forestry and Fisheries. Validated test methods are methods which are conducted according to this procedure and confirmed conforming to the standards such as required accuracy (trueness and precision), quantification range (maximum and minimum limits of quantitation) and so on.

2. Handling of samples

2.1 Storage of samples

(1) Summary

Put a sample in a container suitable for its characteristics and form and seal it tightly, and then store the sample at room or cold temperature. Care should be taken not to freeze it when it is stored at cold temperature.

(2) Apparatus and instruments: Apparatus and instruments are as shown below:

- **a) Refrigerator:** A refrigerator that can be adjusted to 1 ºC - 5 ºC.
- **b) Storage container for a sample:** A storage container for a sample should be clean, strong and completely sealed airtight. In particular, in case it contains sludge for raw materials, the container should be made of non-degradable, non-absorbable material. Additionally, it should be airtight, water-proof, vapor-proof and non-corrosive.

(3) Procedure: Conduct storage as shown below.

- **a)** Store a relatively stable sample in a tightly sealed container to avoid direct sunlight.
- **b)** Store a sample in a desiccator, etc. by tightly sealing it if test results are affected by moisture absorption.
- **c)** Store a sample in a tightly sealed container in a dark place at 1 ºC - 5 ºC if it is easily deteriorated by moisture.

2.2 Preparation of test samples

(1) Summary

- **a)** Prepare a test sample by pre-drying, reducing, and grinding laboratory samples as necessary.
- **b)** Conduct pre-drying if a laboratory sample is moist and hard to grind.
- **c)** A laboratory sample made from such fertilizers as a fluid fertilizer or a particle-fertilizer, etc. that is sufficiently homogeneous can be used as a test sample.
- **d)** If contamination by apparatus affects a test result, procedures such as pre-drying, reduction and grinding are prohibited.
- **e)** Note that part of a test sample should not scatter, nor should surrounding fine particles or other alien substances be mixed with the test sample being prepared

References

1) JIS M 8100: Particulate materials - General rules for methods of sampling (1992)
2) JIS K 0060: Sampling method of industrial wastes (1992)

2.2.1 Pre-drying

(1) Summary

Conduct pre-drying if a laboratory sample is moist and hard to grind. Measure the rate of volatile matter content and convert the analyzed value of respective tests to the content of a laboratory sample (actual article).

(2) Apparatus and instruments: Apparatus and instruments are shown below.

a) Drying apparatus: A drying apparatus that can be adjusted to the pre-drying temperature at ± 2 ºC.

b) Sample drying dish: Measure the mass to the order of 0.1g in advance. Additionally, use materials of a quality that do not affect the measurement of test components.

(3) Procedure: Conduct pre-drying as shown below.

a) Transfer 250 g - 1 kg of a laboratory sample to a sample drying dish, spread uniformly and measure the mass to the order of 0.1g.

b) Place a sample drying dish containing a laboratory sample in a drying apparatus and dry (1).

c) Remove a sample drying dish from a drying apparatus and leave at rest at room temperature until it is balanced with atmospheric temperature (2).

d) After leaving at rest, measure the mass of **c)** to the order of 0.1 g.

e) Calculate loss on drying in the pre-drying by the following formula (1). If necessary, calculate a conversion factor (actual article) by the following formula (2).

Loss on drying (% (mass fraction)) = $((A - B)/A) \times 100$ ………. (1)

Conversion factor (actual article) = B/A ………… (2)

A: Mass (g) of the sampled laboratory sample

B: Mass (g) of the laboratory sample after drying

Note (1) Examples of drying temperature and drying time: About 70 hours at 40 ºC, no less than 5 hours at 65 ºC.

(2) An example of time to leave at rest: About 20 minutes

Reference

1) Mariko AIZAWA, Yuji SHIRAI, Yasushi SUGIMURA, Yuichi TAKAHASHI, Jun OKI, Yukio FUKUCHI and Norio HIKICHI: Evaluation of Pre-drying Procedure to Prepare Test Samples from Sludge Fertilizer, Research Report of Fertilizer Vol.1, 2008, (122 - 128)

(4) Flow sheet for pre-drying: The flow sheet for the pre-drying of a moist laboratory sample is shown below.

250 g – 1 kg of Laboratory sample	Transfer to a sample drying dish and spread uniformly. Measure the mass to the order of 0.1 g
Drying	Example: About 70 hours at 40 ºC, no less than 5 hours at 65 ºC
Standing to cool	Room temperature
Measurement	Measure the mass to the order of 0.1 g

Figure The flow sheet for pre-drying

2.2.2 Reduction (Separation)

(1) Summary

In order to distinguish a test sample from a sample for granularity test and physical characteristics test, etc., reduce (separate) a laboratory sample with an increment reduction method, a riffle sampler method or a conical quartering method.

(2) Apparatus

- **a) Scoop for increment reduction:** A scoop for increment reduction specified in the attached chart 1 of JIS M 8100.
- **b) Riffle sampler:** A riffle sampler specified in the attached chart 3 of JIS M 8100.

(3) Procedure: Conduct reduction (separation) as shown below.

- **a) Increment reduction method:** Conduct as indicated in 6.5.2 of JIS M 8100.
- **b) Riffle sampler method:** Conduct as indicated in 6.5.3 of JIS M 8100.
- **c) Conical quartering method:** Conduct as indicated in 6.5.4 of JIS M 8100.

2.2.3 Grinding

(1) Summary

In order to prepare a homogeneous test sample, grind a laboratory sample with an adequate grinder until it completely passes through the designated granularity.

(2) Apparatus and instruments: Apparatus and instruments are as shown below.

- **a) Grinder:** Use a grinder (2) of a type and competence suitable for the granularity and the physical characteristics (1) of a laboratory sample
- **b) Primary grinder:** A grinder (3) that can primarily grind a large lump.
- **c) Cutter machine:** A cutter that can cut long stems, etc.
- **d) Sieve:** A sieve for the test specified in JIS Z 8801-1 or JIS Z 8801-2 or equivalents.

Note **(1)** The physical characteristics of a laboratory sample are defined by their solidity, toughness, specific gravity and adhesiveness.

(2) A centrifuging type grinder, a cutting mill, a vibration mill type grinder, etc.

(3) A blender with an attachable blade, etc.

(3) Procedure: Conduct grinding as shown below.

(3.1) Fertilizers except ones specified in (3.2): Conduct as specified in 6.4 of JIS M 8100 and as shown below.

- **a)** Break or cut a laboratory sample with a primary grinder or a cutting machine as necessary.
- **b)** Grind with a grinding machine until it completely passes through a sieve of 500 µm - 1 mm aperture.
- **c)** Mix ground samples to make the test sample.

(3.2) Fused phosphate fertilizer, calcined phosphate fertilizer, silica fertilizer, calcareous fertilizer, magnesia fertilizer, manganese fertilizers, etc.: Conduct as specified in 6.4 of JIS M 8100 and as shown below.

- **a)** Grind a laboratory sample with a vibration mill type grinder.
- **b)** Transfer the ground laboratory sample to a sieve of 212 µm apertures.
- **c)** Incline the sieve about 20 degrees, supporting it with one hand or a bent arm, and tap the sieve frame with the other hand at the rate of about 120 times per minute. During the procedure, place the sieve in a horizontal position at the rate of 4 times per minute, rotate it 90 degrees and tap the sieve frame firmly one or two times.
- **d)** When fine powder attaches to the back side of a sieve screen, remove it gently from the back side to make minus sieve.
- **e)** Regarding the plus sieve of a sample, make them pass through by repeating the procedure in **a) - d)**.
- **f)** Combine and mix the sample passed to make the test sample.

Comment 1 The procedures in **b) - d)** are the procedures in 6.1.3 (1.4) of JIS Z 8815.

3. General tests

3.1 Moisture

3.1.a Loss on drying method with drying apparatus

(1) Summary

Use drying apparatus under conditions suitable for the kind of fertilizers to be measured to heat analytical samples to measure loss on drying. This test method corresponds to loss on heating in the Official Methods of Analysis of Fertilizers (1992).

(2) Apparatus and instruments: Use apparatus and instruments as shown below:

- **a) Drying apparatus:** Drying apparatus that can be adjusted to the test temperature ± 2 ºC.
- **b) Ground-in stoppered weighing bottles [(1)]:** Low-form weighing bottles, 50 mm × 30 mm, specified in JIS R 3503. Dry by heating in advance in a drying apparatus at 75 ºC - 130 ºC, stand to cool in a desiccator, and measure the mass to the order of 1 mg.

Note (1) Aluminum weighing dishes described in the Handbook of the Feed Analysis Standards -2009- can also be used.

(3) Measurement: Conduct measurement as shown below.

- **a)** Transfer 2 g-5 g of an analytical sample to a ground-in stoppered weighing bottle, spread so that the thickness is no more than 10 mm, and measure the mass to the order of 1 mg.
- **b)** Place the ground-in stoppered weighing bottle containing the analytical sample in a drying apparatus at 100 ºC ± 2 ºC, and heat for 5 hours.[(2)]
- **c)** After heating, fit the stopper into the ground-in stoppered weighing bottle, and immediately transfer to a desiccator to let it stand to cool.
- **d)** After cooling, remove the ground-in stoppered weighing bottle from the desiccator, and measure the mass to the order of 1 mg.
- **e)** Calculate loss on drying in the analytical sample by the following formula (1) as moisture. If necessary, calculate the conversion factor (dry matter) by the following formula (2):

Loss on drying (% (mass fraction)) = $((A - B)/A) \times 100$ ……… (1)

Conversion factor (dry matter) = A/B……………………………….. (2)

A: Mass (g) of the sampled analytical sample
B: Mass (g) of the analytical sample after drying

Note (2) Heat simultaneously the slightly moved or removed stopper of the ground-in stoppered weighing bottle.

Comment 1 When pre-drying is conducted, calculate the moisture of the laboratory sample (actual article) by the following formula:

Total moisture (% (mass fraction)) in the laboratory sample (actual article) = $A + B \times ((100 - A)/100)$

A: Loss on drying (% (mass fraction)) of the laboratory sample (actual article) by the pre-drying procedure

B: Loss on drying (% (mass fraction)) in the analytical sample by moisture measurement

Comment 2 Use drying conditions in Table 1 for fertilizers of the types shown below:

Table 1 Drying conditions

Type of fertilizers	Sampling amount of analytical samples	Drying temperature	Drying time
Superphosphate, concentrated superphosphate fertilizer, or fertilizers containing these	About 5 g	100 ºC ± 2 ºC	3 hours
Ammonium sulfate, sodium nitrate, and potassium salts	2 g - 5 g	130 ºC ± 2 ºC	Until a constant weight is achieved.
Urea and urea-containing fertilizers	About 5 g	75 ºC ± 2 ºC	4 hours

Comment 3 For samples containing volatile matters, subtract the volatile matter content by the following a) and b) from loss on drying to obtain moisture.

a) Fertilizers containing guano or diammonium hydrogen phosphate, etc.: Determine total nitrogen in the test sample, and in the analytical sample after the drying procedure; convert the difference between the quantitation values into ammonia (NH_3) to make the volatile matter content.

b) Potassium hydrogen carbonate: Determine carbon dioxide in the test sample, and in the analytical sample after the drying procedure; the difference between the quantitation values is the volatile matter content.

Comment 4 Table 2 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 2　Results and analysis results from a simultaneous analysis with the same samples for moisture hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2006	High analysis compound fertilizer	147	1.70	0.30	17.6
2007	Organic compound fertilizer	146	4.99	0.35	7.0
2008	High analysis compound fertilizer	145	2.87	0.24	8.5
2009	Ordinary compound fertilizer	145	3.53	0.15	4.2
2010	High analysis compound fertilizer	143	1.58	0.41	26.0
2011	High analysis compound fertilizer	137	2.03	0.09	12.0
2013	High analysis compound fertilizer	136	2.93	0.84	28.7

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
RSD_{rob} = (NIQR/M) × 100

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.20 - 23, Yokendo, Tokyo (1988)

2) Society for the Study of Feed Analysis Standards：Methods of Analysis of Feeds and Feed Additives-2009-I, p.37 - 39, Incorporated Administrative Agency Food and Agricultural Materials Inspection Center, Saitama (2009)

(4) Flow sheet for moisture: The flow sheet for moisture in fertilizers is shown below:

2 g -5 g analytical sample	Transfer to a ground-in stoppered weighing bottle, spread so that the thickness is no more than 10 mm. Measure the mass to the order of 1 mg.
Heating	100 °C ± 2 °C, 5 hours
Standing to cool	Desiccator
Measurement	Measure the mass to the order of 1 mg.

Figure Example of the flow sheet for moisture in fertilizers
by loss on drying with drying apparatus.

3.1.b Loss on drying method by moisture analyzers

(1) Summary

The test method is applicable to sludge fertilizers, compost, and organic fertilizers, etc. Moisture in an analytical sample is measured by a moisture analyzer in the heat drying method.

(2) Instruments: Instruments are as shown below:

a) **Moisture analyzer:** A moisture analyzer consisted of a heat source to heat an analytical sample (halogen lamp, infrared heater, ceramic heater, etc.) and a built-in balance (1) with calibration function.

Note (1) There is a method to calibrate with calibration weights or a method to calibrate automatically with built-in weights.

(3) Measurement: Conduct measurement as shown below. However, conduct in advance a comparative test with 3.1.a Loss on heating with drying apparatus using sludge fertilizers, compost, and organic fertilizers, etc., to confirm that there is no difference in the quantitation value of moisture.

a) Transfer about 5 g of an analytical sample to a weighing dish, spread so that the thickness is no more than 10 mm, and measure the mass to the order of 1 mg.

b) Heat at 100 ºC (2), until a constant weight is achieved.

c) After the end of heating (2), measure the mass to the order of 1 mg.

d) Calculate loss on drying in the analytical sample by the following formula (1) as moisture. If necessary, calculate the conversion factor (actual article) by the following formula (2):

$$\text{Loss on drying (\% (mass fraction))} = ((A - B) / A) \times 100 \qquad (1)$$

$$\text{Conversion factor (dry matter)} = A/B \qquad (2)$$

A: Mass (g) of the sampled analytical sample

B: Mass (g) of the analytical sample after drying

Note (2) The setup of the drying program and the determination parameter for the end of heating (constant weight) is according to the specification and the operation method of the moisture analyzer used.

Comment 1 When pre-drying is conducted, calculate the moisture of the laboratory sample (actual article) by the following formula:

$$\text{Total moisture (\% (mass fraction)) in the laboratory sample (actual article)} = A + B \times ((100 - A)/100)$$

A: Loss on drying (% (mass fraction)) of the laboratory sample (actual article) by the pre-drying procedure

B: Loss on drying (% (mass fraction)) in the analytical sample by moisture measurement

Comment 2 Table 1 shows results from replicate testing conducted with organic fertilizers, compost and sludge fertilizers.

Additionally, Table 2 shows results and analysis results from a collaborative study for test method validation.

Table 1 Replicate testing on moisture in sludge fertilizers, etc., with a moisture analyzer.

Name of fertilizer	Mean[1)] (%)[2)]	Standard deviation (%)[2)]	Relative standard deviation (%)
Sewage sludge fertilizer	24.40	0.29	1.2
Human waste sludge fertilizer A	5.50	0.03	0.6
Human waste sludge fertilizer B	13.63	0.09	0.7
Industrial sludge fertilizer A	13.39	0.11	0.8
Industrial sludge fertilizer B	90.61	0.14	0.2
Composted sludge fertilizer A	33.92	0.20	0.6
Composted sludge fertilizer B	63.14	0.21	0.3
Fish meal	6.27	0.01	0.2
Steamed leather meal	8.03	0.11	1.4
Rape seed meal and powdered rape seed meal	8.85	0.03	0.3
Rice bran meal and powdered rice bran meal	9.05	0.01	0.1
Byproduct organic fertilizer of vegetable origin	6.26	0.07	1.0
Organic fertilizer mixture	6.47	0.02	0.3
Compost	10.19	0.04	0.4

1) Mean of triplicates.
2) Mass fraction

Table 2 Results and analysis results from a collaborative study for the validation of the moisture test method.

Sample name	Number of laboratories[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	s_R[6] (%)[3]	RSD_R[7] (%)
Sewage sludge fertilizer	9	21.93	0.32	1.4	0.47	2.1
Human waste sludge fertilizer	8	13.36	0.14	1.1	0.37	2.8
Industrial sludge fertilizer	9	34.28	0.21	0.6	0.50	1.5
Calcined sludge fertilizer	9	38.75	0.59	1.5	0.59	1.5
Composted sludge fertilizer	9	27.1	0.26	0.9	0.60	2.2

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Takeshi UCHIYAMA and Chiyo SAKASEGAWA: Validation of a Heating Method Using a Moisture Analyzer for Moisture Content in Sludge Fertilizer. Research Report of Fertilizer Vol.1, 2008, (1- 5)
2) Takeshi UCHIYAMA and Yuji SHIRAI: Determination of Moisture Content in Sludge Fertilizer by a Heating Method Using Moisture Analyzer: A Collaborative Study. Research Report of Fertilizer Vol.1, 2008, (6 - 11)
3) Satono AKIMOTO and Sakiko TAKAHASHI: Validation of a Heating Method Using a Moisture Analyzer for Moisture Content in Sludge Fertilizer. Research Report of Fertilizer Vol.2, 2009, (1 - 5)

(4) Flow sheet for moisture: The flow sheet for moisture in sludge fertilizers, compost, and organic fertilizers, etc. is shown below:

About 5 g analytical sample	Transfer to a weighing dish, spread so that the thickness is no more than 10 mm. Measure the mass to the order of 1 mg.
Heating	100 ºC
End of drying	Constant weight
Measurement	Measure the mass to the order of 1 mg

Figure Flow sheet for moisture in sludge fertilizers, compost, and organic fertilizers, etc. by the heat drying method with a moisture analyzer.

3.2 Ash content

3.2.a Ignition residue method

(1) Summary

The method is applicable to organic fertilizers and fertilizers containing organic matters.
The method ignites an analytical sample with an electric furnace and measures residue on ignition.

(2) Apparatus and instruments: Apparatus and Instruments are shown below.

- **a) Electric furnace:** An electric furnace that can be adjusted to the temperature 550 ºC ± 5 ºC.
- **b) Crucible:** After heating a porcelain crucible for chemical analysis specified in JIS R 1301 with an electric furnace at 550 ºC ± 5 ºC, stand to cool in a desiccator in advance and measure the mass to the order of 1 mg.

(3) Measurement: Conduct measurement as shown below.

- **a)** Transfer about 2 g of an analytical sample into a crucible, and measure the mass to the order of 1 mg.
- **b)** Place it into an electric furnace, heat gently until carbonized (1).
- **c)** Heat at 550 ºC ± 5 ºC for no less than 4 hours.
- **d)** After heating, move the crucible into a desiccator and let it stand to cool.
- **e)** After standing to cool, remove the crucible from the desiccator and measure the mass to the order of 1 mg.
- **f)** Calculate the residue on ignition in the analytical sample by the following formula to make ash content.

Residue on ignition (% (mass fraction)) = $(B/A) \times 100$

A: Mass (g) of the sampled analytical sample

B: Mass (g) of the ignited analytical sample

Note (1) Example of carbonizing procedure: Heat at about 250 ºC until smoke no longer form.

(4) Flow sheet for ash content: The flow sheet for ash content in fertilizers is shown below.

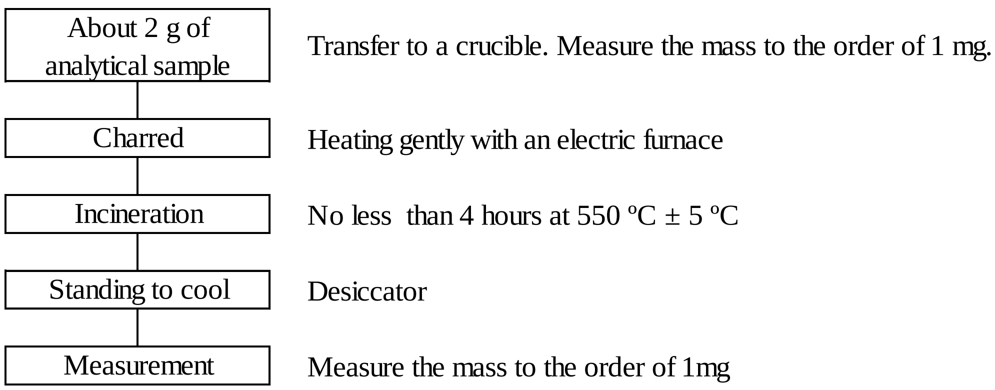

Figure Flow sheet for ash content in fertilizers.

3.3 pH

3.3.a Glass electrode method

(1) Summary

Measure with a pH meter using a glass electrode

(2) Reagent: Reagents are as shown below.

a) **Oxalate pH standard solution:** Oxalate pH standard solution class 2 traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.

b) **Phthalate pH standard solution:** Phthalate pH standard solution class 2 traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.

c) **Neutral phosphate pH standard solution:** Neutral phosphate pH standard solution class 2 traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.

d) **Borate pH standard solution:** Borate pH standard solution class 2 traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.

e) **Carbonate pH standard solution:** Carbonate pH standard solution class 2 traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.

Comment 1 Respective pH standard solutions stored for long time should not be used since the pH value may change during storage period. In particular, note that borate pH standard solution and carbonate pH standard solution easily absorb carbon dioxide in the air, so that the pH values deteriorate. The pH standard solution that was used once or left exposed to the air should not be used.

(3) Instruments: Instruments are shown below.

a) **pH meter:** Use type II specified in JIS Z 8802.

Comment 2 Conduct the calibration of a pH meter as indicated in JIS Z 8802. Actual calibration operation is according to the operation procedure of the pH meter used for measurement.
When the pH of a sample solution is no more than 7, use neutral phosphate pH standard solution and oxalate pH standard solution, or phthalate pH standard solution. When it exceeds 7, use neutral phosphate pH standard solution and borate pH standard solution, or carbonate pH standard solution.

(4) Test procedures

(4.1) Preparation of sample solution: Conduct preparation of sample solution as shown below.

(4.1.1) Fertilizers except inorganic fertilizers

a) Transfer a predetermined amount of an analytical sample [(1)] into a ground-in stopper flask and add water 5 - 10 times the volume.

b) Mix with a magnetic stirrer, filter with Type 3 filter paper to make the sample solution.

Note (1) In the case of a moist laboratory sample, it is recommended to use a sample that is not pre-dried.

(4.1.2) Inorganic fertilizers

a) Transfer a predetermined amount of an analytical sample [1] into a ground-in stopper flask and add water 100 times the volume.

b) Mix with a magnetic stirrer, filter with Type 3 filter paper to make the sample solution.

Comment 3 The procedure in **(4.1.1)** is the same as **3.4.a (4.1)**. Additionally, the sample solution prepared in **4.2.4.a (4.1)** can be used instead of the sample solution prepared by **(4.1.2)**.

(4.2) Measurement: Conduct the measurement as indicated in JIS Z 8802 and as shown below. Actual calibration operation is according to the operation procedure of the pH meter used for measurement.

a) Wash the read station of a calibrated pH meter repeatedly no less than 3 times with water and wipe out with clean and soft paper, etc.

b) Transfer a sample solution into a beaker [2], dip the read station in the solution and measure the pH value.

Note (2) It is necessary to transfer sufficient volume of sample solution to keep a measurement value stable.

Comment 4 If a pH meter has a temperature correction dial or a digital switching, measure the pH value after adjusting the graduation of the pH meter with the temperature of a sample.

Reference

1) JIS Z 8002: Methods for determination of pH of aqueous solutions (2011)

(5) Flow sheet for pH value: The flow sheet for pH value in fertilizers is shown below.

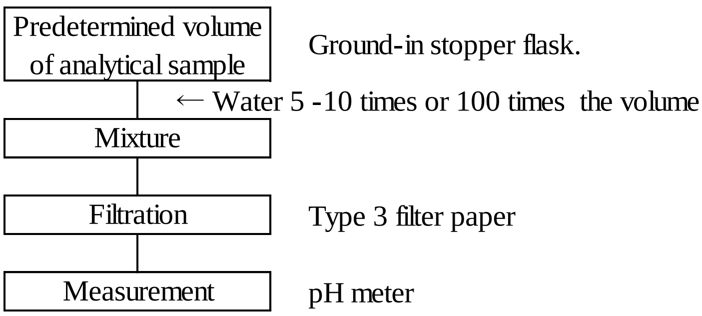

Figure Flow sheet for pH in fertilizers.

3.4 Electrical conductivity

3.4.a Measurement method with an electrical conductivity meter

(1) Summary
Measure the electrical conductivity of organic fertilizers such as compost or sludge fertilizers with an electrical conductivity meter.

(2) Reagents: Reagents are shown below.

a) Potassium chloride: Grind potassium chloride used for measurement of electrical conductivity specified in JIS K 0130 with an agate mortar to powder and heat for 4 hours at 500 ºC ± 5 ºC, and then stand to cool in a desiccator.

b) Potassium chloride standard solution (1)**:** Measure predetermined volume (2) of potassium chloride of **a)** on a weighing dish, dissolve in a small amount of water, transfer it into a 1,000-mL volumetric flask , and add up to the marked line with water.

Note **(1)** Store potassium chloride standard solution in a polyethylene or borosilicate glass bottle and seal the bottle.

(2) The volume that is recommended for an instrument or a cell used.

Comment 1 Potassium chloride standard solution used once or left in the air should not be used.

(3) Instruments: Instruments are shown below.

a) Electrical conductivity meter: An electrical conductivity meter specified in JIS K 0130

Comment 2 Check the indicated value as shown in 6.2 in JIS K 0130 as necessary. Actual procedure to check is according to the operation procedure of the electrical conductivity meter used for measurement.

(4) Test procedure

(4.1) Preparation of sample solution: Conduct the preparation of sample solution as shown below.

a) Transfer the predetermined volume of an analytical sample (3) into a ground-in stopper flask and add water 10 times the equivalent volume of dry matter (4).

b) Mix with a magnetic stirrer, filter with Type 3 filter paper to make the sample solution.

Note **(3)** In the case of a moist laboratory sample, it is recommended to use a sample that is not pre-dried.

(4) If the sample solution becomes hard to measure because it is gelled by the influence of flocculants in sludge fertilizer, etc., increase the volume of water added. However this fact should be expressed in the test result.

Comment 3 The procedure in **(4.1)** is the same as **3.3.a (4.1.1)**.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0132 and as shown below. Actual measurement operation is according to the operation procedure of the electrical conductivity meter used for measurement.

a) Wash the read station of an electrical conductivity meter repeatedly no less than 3 times with water.

b) Transfer a sample solution into a beaker [5], dip the read station and measure electrical conductivity.

Note (5) It is necessary to transfer sufficient volume of sample solution to keep the measurement value stable.

Reference

1) JIS K 0130: General rules for electrical conductivity measuring method (2008)

(5) Flow sheet for electrical conductivity: The flow sheet for electrical conductivity is showed below.

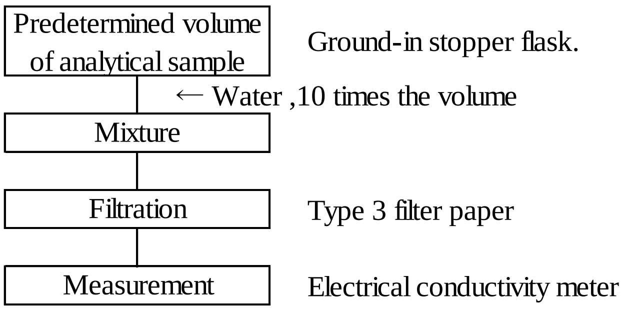

Figure Flow sheet for electrical conductivity in fertilizers.

3.5 Granularity

3.5.a Dry-type sieving testing method

(1) Summary

Measure the particle diameter distribution of particulates and powdery fertilizers with a dry-type sieving analysis.

(2) Apparatus: Apparatus are shown below.

- **a) Sieve:** A sieve for testing specified in JIS Z 8815
- **b) Clogging removal brush:** A brush which is adequately hard for the apertures and does not damage the sieving screen.
- **c) Weighing dish:** A container that can contain about 250 g of a sample. Measure the mass to the order of 0.1g in advance.

(3) Dry-type sieving analysis procedure: Conduct sieving analysis corresponding to the aperture size of a sieve used as indicated in JIS Z 8815 and as shown below.

(3.1) More than 1mm and no more than 4mm

- **a)** Stack a large aperture sieve on an acceptor so that the large sieve is on top.
- **b)** Measure the total mass (2) of a laboratory sample or a separated test sample (1) to the order of 0.1g and transfer it to the sieve at the top section.
- **c)** After putting a stopper on it, hold the stacked sieves with both hands, and vibrate (3) them back and forth at about 70 mm amplitude along a unidirectional and horizontal plane at about 60 times per minute.
- **d)** Transfer respective plus and minus sieves to a weighing dish (4).

Note **(1)** Conduct the separation of a sample as indicated in **2.2.2(3).**

(2) The minimum volume of a separated test sample is 250 g.

(3) Conduct more circular motion at the rate of about 3 revolutions per minute as necessary.

(4) Turning over the back side of a sieve, remove the clogged particles from the sieve screen with a clogging removal brush and combine them with the plus sieve.

(3.2) Less than and equal to 1mm

- **a)** Stack a large aperture sieve on an acceptor so that the large sieve is on top.
- **b)** Measure the total mass (2) of a laboratory sample, a separated test sample (1) or minus sieve of **(3.1) c)** to the order of 0.1g and transfer it to the sieve at the top section.
- **c)** After putting a stopper on it, incline the stacked sieves about 20 degrees, supporting with one hand or a bent arm, and tap the sieve frame with the other hand at the rate of about 120 times per a minute.
- **d)** During the procedure in **c)**, place the sieve in a horizontal position at the rate of 4 times per minute, rotate it 90 degrees and tap the sieve frame hard one or two times.
- **e)** Transfer respective plus and minus sieves (5) to a weighing dish (4).

Note **(5)** When fine powder attaches to the back side of a sieve screen, remove them gently from the back side with a clogging removal brush and combine them with the minus sieve

(4) Measurement of granularity distribution: Calculate the granularity distribution in an analytical sample as shown below.

- **a)** Measure respective mass of plus and minus sieves to the order of 0.1g.

b) Calculate "plus sieve percentage" and "integrated minus sieve percentage" with the following formula and round the results to the first decimal place.

c) Confirm that the sum of the mass of the plus sieve and the mass of the minus sieve with the smallest aperture is in the range of ± 2 % of the mass of sample measured in **(3.1)b)** or **(3.2)b)**.

Mass percentage of plus sieve or minus sieve (%) (R) = $(A/T) \times 100$

A: Mass of plus sieve or minus sieve (g)
T: Sum of the mass of plus and minus sieve (g)

References

1) JIS Z 8815: Test sieving - General requirements (1994).
2) JIS K 0069: Test methods for sieving of chemical products (1992).

(5) **Flow sheet of granularity:** The flow sheet of granularity of particulate or powdery fertilizers is shown below.

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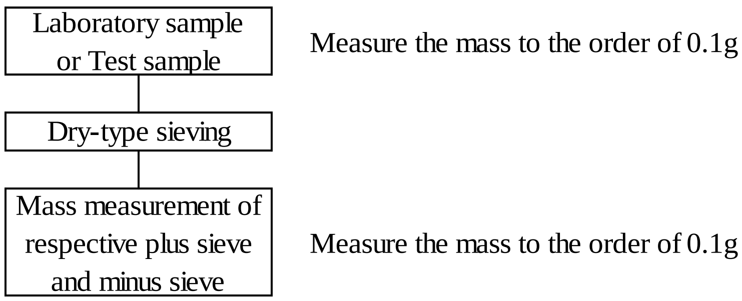


Figure Flow sheet for granularity of particulate or powdery fertilizers

3.6 Oil content

3.6.a Diethyl ether extraction method

(1) Summary

The method is applicable to organic fertilizers.

Extract an analytical sample with diethyl ether using a Soxhlet extractor to measure the extract obtained.

The oil content contains not only fat but also fat-soluble pigments (carotenoid, chlorophyll, etc.), wax, and free fatty acids, etc..

(2) Reagents: Reagents are as shown below.

- **a) Diethyl ether:** A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

- **a) Drying apparatus:** Drying apparatus that can be adjusted to the test temperature ± 2 ºC
- **b) Soxhlet extractor:** An inter-changeable Soxhlet extractor, cooling apparatus and weighing bottles (Example: JIS R 3503, attached figure 71)
- **c) Water bath:** A water bath that can be adjusted to about 60 ºC
- **d) Weighing bottle:** A flat bottle flask connectable to a Soxhlet extractor. After heating with a drying apparatus at 100 ºC - 105 ºC in advance, stand to cool in a desiccator and measure the mass to the order of 1mg.
- **e) Cylindrical filter paper:** A cylindrical filter paper made of cellulose. Example: 22 mm external diameter, 20 mm internal diameter, 90 mm total length (1).

(4) Measurement: Conduct measurement as shown below

- **a)** Weigh 2 g - 5 g of an analytical sample to the order of 1mg, and transfer it into a cylindrical filter paper.
- **b)** Place absorbent cotton on the upper end of an analytical sample (2), as if gently pushing it, and heat it at 100 ºC – 105 ºC for 2 hours.
- **c)** As soon as heating is complete, move the cylindrical filter paper to a desiccator and stand to cool.
- **d)** After standing to cool, transfer it into a Soxhlet extractor and connect it to a cooling apparatus.
- **e)** Transfer adequate volume of diethyl ether (3) into a weighing bottle, connect it to the Soxhlet extractor and heat (4) it for 8 hours to extract.
- **f)** Recover the diethyl ether (5).
- **g)** Disconnect the Soxhlet extractor from the weighing bottle and vaporize the diethyl ether (6).
- **h)** Heat the weighing bottle (7) at 100 ºC - 105 ºC for 3 hours.
- **i)** As soon as heating is complete, move the weighing bottle to the desiccator and stand to cool.
- **j)** After standing to cool, remove the weighing bottle from the desiccator and measure the mass to the order of 1mg.
- **k)** Calculate oil content with the following formula.

$$\text{Oil content (\% (mass fraction))} = (B/A) \times 100$$

A: Mass of a sampled analytical sample (g)

B: Mass of extract of diethyl ether (g)

Note (1) Select a scale according to the volume of a Soxhlet extractor.

(2) The purpose is to prevent overflow at the upper end of an analytical sample.
(3) The amount of diethyl ether depends on the volume of a weighing bottle.
(4) Adjust the temperature for diethyl ether to circulate 16 - 20 times per hour. (Target temperature is about 60 ºC)
(5) Remove the cylindrical filter paper from the Soxhlet extractor. In the case of a cock attached Soxhlet extractor, open the cock and recover it.
(6) It is dangerous if diethyl ether resides in a weighing bottle when the bottle is transferred to a drying apparatus.
(7) Wipe the outside of a weighing bottle since there is a risk of garbage or stain sticking to it.

References

1) Japan Oil Chemist ‘Society: Standard Method for the Analysis of Fats, Oils and Related Materials (2003), 1.5 Oil content p.1 - 2, Incorporated Foundation Japan Oil Chemist ‘Society, Tokyo (2009)

2) Feed Analysis Standard Task Force: Feed Analysis Method/Handbook -2009 - I, p.37 - 39, Incorporated Administrative Agency, Food and Agriculture Materials Inspection Center, Saitama (2009)

(5) Flow sheet of oil content: Flow sheet of oil content in organic fertilizers is shown below.

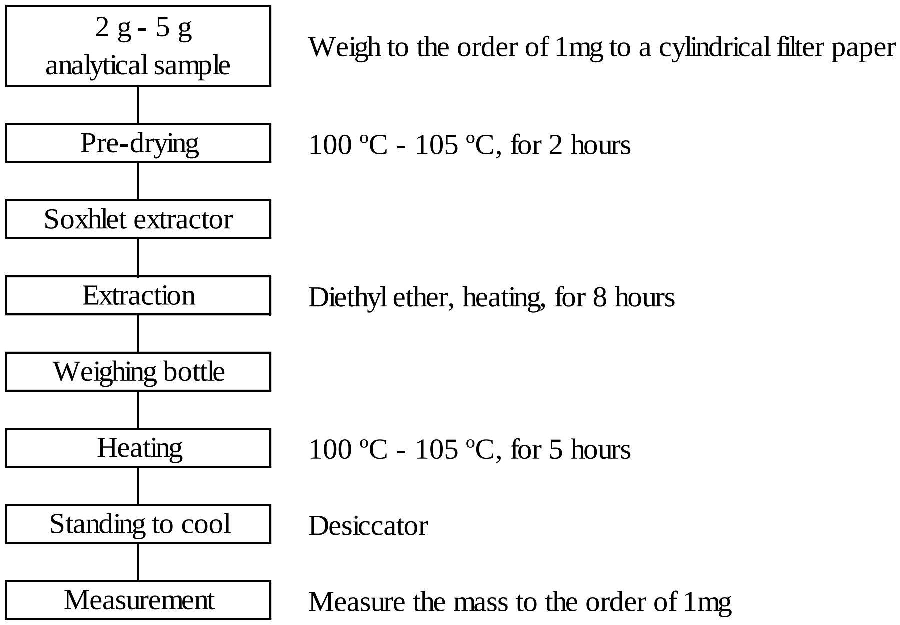


Figure Flow sheet for oil content in organic fertilizers.

4. Main components, guaranteed components, etc.

4.1 Nitrogen

4.1.1 Total nitrogen

4.1.1.a Kjeldahl method

(1) Summary

This test method is applicable to fertilizers containing no nitrate nitrogen.

Add sulfuric acid, potassium sulfate and copper (II) sulfate pentahydrate to an analytical sample, pretreat by Kjeldahl method to change total nitrogen (T-N) to ammonium ion, and add sodium hydroxide solution to subject to steam distillation. Measure separated ammonium ion by neutralization titration, to obtain total nitrogen (T-N) in the analytical sample. This test method corresponds to the sulfuric acid method in the Official Methods of Analysis of Fertilizers (1992).

(2) Reagents: Reagents are as shown below:

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution(1):** Transfer about 30 mL of water to a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Transfer 5.5 mL -11 mL of the supernatant to a ground-in stoppered storage container, and add 1,000 mL of water containing no carbonic acid.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then transfer about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line.(1) Transfer a predetermined amount of the solution to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 mg/100 mL) as an indicator, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)
$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C_1)$

W_1: Mass (g) of sulfamic acid sampled
A: Purity (% (mass fraction)) of sulfamic acid
V_1: Volume (mL) of sulfamic acid solution transferred
V_2: Constant volume (250 mL) of sulfamic acid solution
V_3: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sulfuric acid:** A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) **0.25 mol/L sulfuric acid(1) (2):** Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1,000 mL.

Standardization: Transfer a predetermined amount (3) of 0.25 mol/L sulfuric acid to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red-methylene blue mixture solution, and titrate with 0.1 mol/L -0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green.(4) Calculate the volume of 0.1 mol/L - 0.2

mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
$= V_4/V_5$ …… (1)

Factor of 0.25 mol/L sulfuric acid (f_2)
$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2)$ …… (2)

V_4: Volume (mL) of 0.1 mol/L -0.2 mol/L sodium hydroxide solution needed for titration
V_5: Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
C_2: Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) Boric acid solution (40 g/L): Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1,000 mL.
e) Catalyst (5): Mix potassium sulfate specified in JIS K 8962 and copper (II) sulfate pentahydrate (6) specified in JIS K 8983 in the ratio of 9 to 1.
f) Sodium hydroxide solution (200 g/L - 500 g/L) (1): Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.
g) Bromothymol blue solution (0.1 g/100 mL): Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, and add water to make 100 mL.
h) Methyl red solution (0.1 g/100 mL): Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
i) Methylene blue solution (0.1 g/100 mL): Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.
j) Methyl red - methylene blue mixture solution: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).
k) Bromocresol green solution (0.5 g/100 mL): Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.
l) Methyl red - bromocresol green mixture solution: To methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).
(3) 5 mL -10 mL
(4) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.
(5) A tablet is commercially available.
(6) Crush into powder as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are as shown below.
a) Steam distillation apparatus
b) Digestion flask: Kjeldahl flask
c) Distillation flask: A Kjeldahl flask or round bottom flask that can be connected to a steam distillation apparatus.

(4) Test procedures

(4.1) Kjeldahl method: Conduct digestion as shown below.

a) Weigh 0.5 g - 5 g of an analytical sample to the order of 1 mg, and put it in a 300-mL - 500-mL digestion flask.

b) Add 5 g - 10 g of catalyst, and further add 20 mL - 40 mL of sulfuric acid, shake to mix and heat gently.

c) After bubbles cease to form, heat until white smoke of sulfuric acid evolves.

d) Ignite until organic matters are completely digested [7].

e) After standing to cool, add a small amount of water, mix well by shaking, transfer to a 250-mL - 500-mL volumetric flask with water[8], and further mix by shaking.

f) After standing to cool, add water up to the marked line to make the digestion solution.

Note **(7)** When the solution has finished changing color, heat further for no less than 2 hours.

(8) When the entire sample solution volume is used in measurement, it is not necessary to transfer it to a volumetric flask.

Comment 1 The procedure in **(4.1)** is the same as that in **(4.1) a) - f)** in **4.2.1.a**.

Comment 2 In the case of fish meal containing amino acids that are not easily digested, use 0.5 g -1 g analytical sample, 10 g catalyst and 30 mL - 40 mL sulfuric acid.

Comment 3 In the case of nitrolime, moisten by adding a small amount of water before the procedure in **(4.1) b)**. Care should be taken because bubbles are produced by the addition of sulfuric acid.

(4.2) Distillation: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Transfer a predetermined amount[9] of 0.25 mol/L sulfuric acid to an acceptor,[10] add a few drops of methyl red - methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, transfer a predetermined amount[9] of boric acid solution (40 g/L) to an acceptor,[10] add a few drops of methyl red - bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Transfer a predetermined amount of the digestion solution to a 300-mL distillation flask, add a proper amount of sodium hydroxide solution (200 g/L - 500 g/L) [11], and immediately connect this distillation flask to the steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate has reached 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note **(9)** 5 mL - 20 mL

(10) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or boric acid solution (40 g/L).

(11) An amount sufficient to make the solution strong alkalinity. A blue color will appear.

(4.3) Measurement: Conduct measurement as shown below.

(4.3.1) When 0.25 mol/L sulfuric acid is used in **(4.2)**:

a) Titrate the distillate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green [(4)].

b) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (14.007/W_2) \times (100/1{,}000)$
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (1.4007/W_2)$

B: Volume of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
V_6: Volume (mL) of 0.25 mol/L sulfuric acid transferred to the acceptor in **(4.2) a)**
V_7: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f_1: Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
V_8: Predetermined volume (mL) of the digestion solution in **(4.1) e)**
V_9: Transferred amount (mL) of the digestion solution subjected to distillation in **(4.2) b)**
W_2: Mass (g) of the analytical sample

(4.3.2) When boric acid solution (40 g/L) is used in **(4.2)**:

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red [(12)].

b) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample
$= V_{10} \times C_2 \times 2 \times f_2 \times (V_{11}/V_{12}) \times (14.007/W_2) \times (100/1{,}000)$
$= V_{10} \times C_2 \times f_2 \times (V_{11}/V_{12}) \times (2.8014/W_2)$

V_{10}: Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
C_2: Set concentration (mol/L) of 0.25 mol/L sulfuric acid
f_2: Factor of 0.25 mol/L sulfuric acid
V_{11}: Predetermined volume (mL) of the digestion solution in **(4.1) e)**
V_{12}: Transferred amount (mL) of the digestion solution subjected to distillation in **(4.2) b)**
W_2: Mass (g) of the analytical sample

Note **(12)** The endpoint is reached when the color changes from green to light red.

Comment 4 The titration procedures in **(2) a) Standardization**, **(2) c) Standardization** and **(4.3)** can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Comment 5 The nitrogen content in the analytical sample can be measured by using an automatic nitrogen analyzer (Kjeldahl method) instead of the test procedure in **(4)**. The setup of the program and the parameter of the analyzer as well as vessels etc. are according to the specification and the operation method of the automatic nitrogen analyzer used. However, conduct in advance a comparative test with the test procedure in **(4)** using fertilizers containing no nitrate nitrogen, to confirm that there is no difference in the quantitation value of total nitrogen.

Comment 6 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from a simultaneous analysis with the same samples for total nitrogen hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2006	High analysis compound fertilizer	158	14.60	0.13	0.9
2007	Organic compound fertilizer	145	8.74	0.07	0.8
2010	High analysis compound fertilizer	132	14.11	0.11	0.8

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:

$$RSD_{rob} = (NIQR/M) \times 100$$

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.27 - 31, Yokendo, Tokyo (1988)

2) Society for the Study of Feed Analysis Standards: Methods of Analysis of Feeds and Feed Additives-2009-I, p.28 - 33, Incorporated Administrative Agency Food and Agricultural Materials Inspection Center, Saitama (2009)

3) Takashi KUBOTA, Tomoko OSHIDA, Kozue YANAI, Yuzuru INOUE, Seiji MATSUI, Takaharu MATSUMOTO, Eiichi ISHIKURO and Akemi YASUI: Improvement of the Conditions for the Determination of Total Nitrogen in Fish Meal in Kjeldahl Method and Its Comparison with Dumas Method, Bunsekikagaku, 60, 67 - 74 (2011)

(5) Flow sheet for total nitrogen: The flow sheet for total nitrogen in fertilizers is shown below.

0.5 g - 5 g analytical sample	Weigh to the order of 1 mg into a 300-mL digestion flask.
← 5 g - 10 g catalyst	
←20 mL - 40 mL sulfuric acid	
Heating	Gently
Heating	After foam no longer evolves, ignite until organic matters are completely digested.
Standing to cool	
← small amount of water	
Transfer	250-mL - 500-mL volumetric flask, water
Standing to cool	Room temperature
← Water (up to the marked line)	
Aliquot (predetermined volume)	300-mL distillation flask
← Sodium hydroxide solution (200 g/L - 500 g/L)	
Steam distillation apparatus	Receiver: 200-mL - 300 - mL Erlenmeyer flask or beaker A predetermined amount of 0.25 mol/L sulfuric acid and a few drops of methyl red-methylene blue mixture solution, or boric acid solution (40 g/L), several drops of methyl red - bromocresol green mixture solution
Steam distillation	Distillation rate: 5 mL/min -7 mL/min
Stop distilling	120 mL – 160 mL distillate
← Water (wash the part of the distillation apparatus that came in contact with the solution in the receiver)	
Titration	0.1 mol/L-0.2 mol/L sodium hydroxide solution (until the solution becomes gray-green), or 0.25 mol/L sulfuric acid (until the solution becomes light red)

Figure Flow sheet (example) for total nitrogen in fertilizers.

4.1.1.b Combustion method

(1) Summary

This test method is applicable to fertilizers.

Thermally decompose nitrogen compounds in an analytical sample using a total nitrogen analyzer by the combustion method to produce nitrogen gas and nitroxide gas. Reduce the nitroxide gas to nitrogen, and measure the nitrogen gas content with a thermal conductivity detector. This test method is also referred to as a modified Dumas' method.

(2) Instruments: Instruments are as shown below:

a) Total nitrogen analyzer by the combustion method: A total nitrogen analyzer configured on the basis of the principle of the combustion method (modified Dumas' method).

1) Turn on the total nitrogen analyzer by the combustion method (1), and adjust so that stable indicated values can be obtained.

(i) Combustion gas: Oxygen having purity no less than 99.99 % (volume percentage)

(ii) Carrier gas: Helium having purity no less than 99.99 % (volume percentage)

(3) Measurement: Conduct measurement as shown below. However, confirm in advance using an analytical sample that there is no difference from the measured value of total nitrogen obtained according to **4.1.1.a, 4.1.1.c, 4.1.1.d** or **4.1.1.e.**

a) Measurement conditions for the total nitrogen analyzer by the combustion method: Set up the measurement conditions for the total nitrogen analyzer considering the following:

Combustion temperature: No less than 870 ºC

b) Calibration curve preparation

1) Turn on the total nitrogen analyzer by the combustion method (1), and adjust so that stable indicated values can be obtained.

2) Weigh a predetermined amount of the standard for calibration curves (2) to the order of 0.1 mg into a combustion vessel.

3) Insert the combustion vessel into the total nitrogen analyzer by the combustion method, and read the indicated value.

4) Conduct the procedure in **3)** for another combustion vessel for a blank test, and read the indicated value.

5) Prepare a curve for the relationship between the nitrogen content and the indicated value of the standard for calibration curves and the blank test for calibration curves.

c) Sample measurement

1) Weigh a predetermined amount of an analytical sample to the order of 0.1 mg into a combustion vessel.

2) Insert the combustion vessel containing the analytical sample to the total nitrogen analyzer by the combustion method, and read the indicated value.

3) Obtain the nitrogen content from the calibration curve, and calculate total nitrogen in the analytical sample.

Note (1) The setup of the program and the parameter of the analyzer are according to the specification and the operation method of the total nitrogen analyzer by the combustion method used.

(2) Standard for calibration curves: DL-Aspartic acid (purity no less than 99 % (mass fraction)), EDTA (purity no less than 99 % (mass fraction)), hippuric acid (purity no less than 98 % (mass fraction)) or other reagents having equivalent purity recommended by the total nitrogen analyzer by the combustion method used.

Comment 1 Sample an analytical sample from a test sample prepared in **2.2.3 Grinding (3) Procedure (3.1) b)** by grinding with a mill until it completely passes through a sieve of 500 µm aperture. Additionally, the sampling amount of an analytical sample is as shown in Table 1. Additionally, set the sampling amount of analytical samples considering the estimated content of total nitrogen in the test sample and the measurement range of the total nitrogen analyzer by the combustion method.

Table 1 Sampling amount of analytical samples

Type of fertilizers	Sampling amount (g)
Mixed fertilizers and designated blended fertilizers	0.02 - 0.5
Organic fertilizers and compost	0.05 - 0.5
Sludge fertilizers	0.05 - 0.5

Comment 2 Compound fertilizers, designated blended fertilizers and nitrolime may have high contents of phosphate (P_2O_5), alkali metals (Na, K), alkaline earth metals (Ca, Mg), etc., causing contamination of packing or damage in quartz parts, etc. To avoid their influences, it is recommended to add tungsten oxide (elemental analysis reagent or heat-treated reagent) to completely cover the analytical sample.

Comment 3 When a sample with a low content of organic compounds, such as mixed fertilizers and designated blended fertilizers etc., and thus with low combustion efficiency is measured, it is recommended to add sucrose to the analytical sample so that the carbon content will be comparable to the standard for calibration curves. Additionally, confirm in advance that sucrose to be used has a nitrogen content that does not affect the measured value of total nitrogen of the analytical sample.

Comment 4 Table 2 shows results from replicate testing conducted with mixed fertilizers, designated blended fertilizers, nitrogenous fertilizers, fluid fertilizers, organic fertilizers, and compost and sludge fertilizers.

Additionally, Table 3 shows results and analysis results from a collaborative study for test method validation.

Additionally, the minimum limit of quantification of this test method is about 0.01 % (mass fraction) for fluid fertilizers for home gardening, and 0.05 % (mass fraction) for the other fertilizers.

Table 2　Replicate testing on total nitrogen in fertilizers by the combustion method.

Type of fertilizer	Number of samples	Mean [1] (%)[2]		Standard deviation (%)[2]		Relative standard (%)	
		Minimum [3]	Maximum [4]	Minimum [3]	Maximum [4]	Minimum [3]	Maximum [4]
Mixed fertilizers	15	3.92	18.30	0.01	0.33	0.1	2.0
Designated blended fertilizers	7	5.20	11.06	0.02	0.17	0.3	1.5
Nitrogenous fertilizers	8	15.65	46.56	0.01	0.05	0.03	0.2
Fluid fertilizers	6	0.60	16.08	0.002	0.03	0.1	0.4
Organic fertilizers and compost	5	1.09	10.83	0.01	0.04	0.1	0.7
Sludge fertilizers	5	0.61	8.09	0.004	0.05	0.1	3.1

1) Mean of triplicates.
2) Mass fraction
3) Minimum of values obtained from samples.
4) Maximum of values obtained from samples

Table 3 Analysis results of results from a collaborative study for the validation of the total nitrogen test method.

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Compound fertilizer (containing nitrate nitrogen)	11	9.32	0.07	0.8	0.25	2.7
Compound fertilizer (containing urea)	11	18.34	0.06	0.3	0.45	2.5
Designated blended fertilizer (containing organic fertilizer)	12	14.06	0.12	0.9	0.42	3.0
Nitrolime	8	19.96	0.07	0.4	0.17	0.8
Fish meal	10	8.34	0.04	0.4	0.10	1.3
Steamed wool waste	11	13.42	0.10	0.7	0.26	2.0
Rape seed meal and powdered rape seed meal	11	6.21	0.07	1.1	0.25	4.0
Composted sludge fertilizer A	13	6.20	0.02	0.3	0.09	1.4
Composted sludge fertilizer B	12	2.36	0.01	0.6	0.04	1.8
Human waste sludge fertilizer	11	4.44	0.02	0.4	0.06	1.3
Industrial sludge fertilizer	11	8.06	0.03	0.4	0.07	0.9
Calcined sludge fertilizer	13	0.80	0.02	2.8	0.03	4.3

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories x number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Mariko AIZAWA, Yasushi SUGIMURA, Yuichi TAKAHASHI, Jun OKI, Yukio FUKUCHI, Yuji SHIRAI and Norio HIKICHI: Validation of a Combustion Method for Determination of Total Nitrogen Content in Sludge Fertilizer. Research Report of Fertilizer Vol.1, 2008, (12 - 17)

2) Mariko AIZAWA and Yuji SHIRAI: Determination of Total Nitrogen content in Sludge Fertilizer by a Combustion Method: A Collaborative Study. Research Report of Fertilizer Vol.1, 2008, (18 - 24)

3) Mariko AIZAWA and Yuji SHIRAI: Validation of a Combustion Method for Determination of Total Nitrogen Content in Organic Fertilizer. Research Report of Fertilizer Vol.2, 2009, (6 - 11)

4) Mariko AIZAWA and Yuji SHIRAI: Validation of a Combustion Method for Determination of Total Nitrogen Content in Inorganic Fertilizer. Research Report of Fertilizer Vol.3, 2010, (1 - 10)

5) Mariko AIZAWA, Yuko SEKINE and Yuji SHIRAI: Determination of Total Nitrogen Content in Fertilizer by a Combustion Method: A Collaborative Study. Research Report of Fertilizer Vol.3, 2010, (11 - 18)

6) Kazumi UCHIYAMA and Yoshio MAEBASHI: Effective Analysis of Organic Trace Element, p.99, Mimizuku-sha, Tokyo (2008)

(4) Flow sheet for total nitrogen: The flow sheet for total nitrogen in fertilizers is shown below:

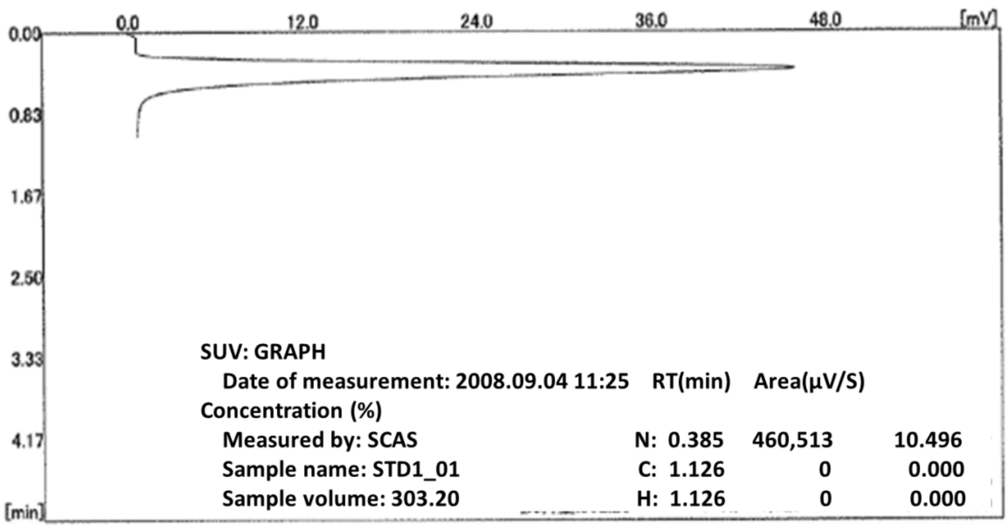
Analytical sample — Weigh to the order of 1 mg into a combustion vessel

↓

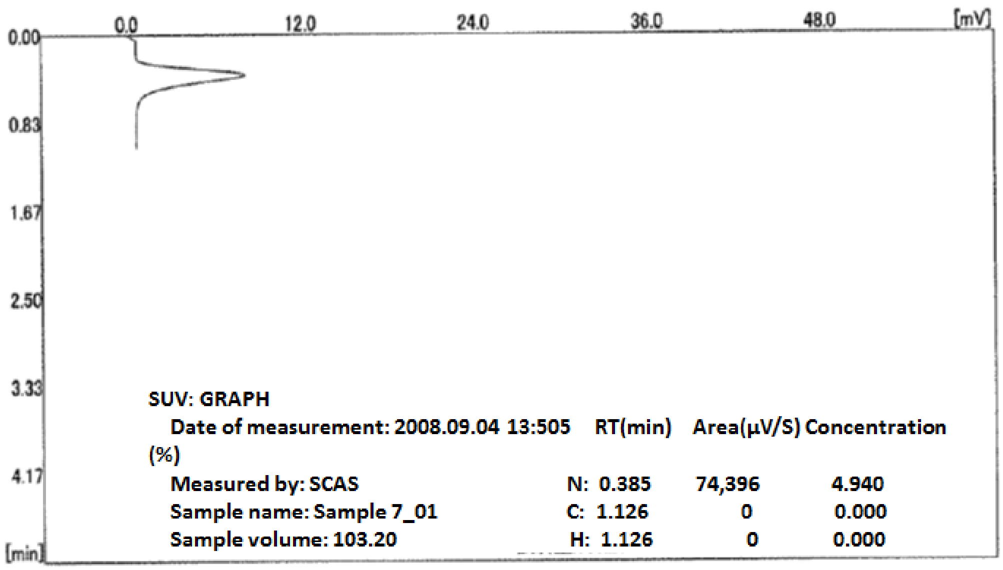
Total nitrogen analyzer by the combustion method

Figure Flow sheet for total nitrogen in fertilizers by the combustion method.

Reference: Chromatograms of the standard for calibration curves and an analytical sample are shown below:



1) Standard for calibration curves (DL-aspartic acid)



2) Analytical sample (sludge fertilizer)

Reference figures Chromatograms of total nitrogen.

Measurement conditions for total nitrogen analyzer by the combustion method

Combustion gas: Highly pure oxygen, purity no less than 99.9999 % (volume fraction), flow rate 200 mL/min

Carrier gas: Highly pure helium, purity no less than 99.9999 % (volume fraction), flow rate 80 mL/min

Separation column: A silica gel stainless column

Detector: Thermal conductivity detector (TCD)

Measurement cycle: Purge time = 60 seconds, circulation combustion time = 200 seconds, measurement time = 100 seconds

Current value of Detector: 160 mA

Temperature conditions: Reaction furnace temperature: 870 ºC
Reduction furnace temperature: 600 ºC
Column oven temperature: 70 ºC
Detector temperature: 100 ºC

4.1.1.c Devarda's alloy - Kjeldahl method

(1) Summary

The method is applicable to the fertilizers that contain nitrate nitrogen (N-N) and guarantee total nitrogen.

Add hydrochloric acid (1+1) and tin (II) chloride dihydrate to an analytical sample and further add devarda's alloy to reduce nitrate nitrogen (N-N), and then add sulfuric acid (1+1), pretreat by Kjeldahl method to change total nitrogen (T-N) to ammonium ion and add sodium hydroxide to subject to steam distillation. Measure separated ammonium ion by neutralization titration to obtain total nitrogen (T-N) in the analytical sample. This test method corresponds to the sulfuric acid method in the Official Methods of Analysis of Fertilizers (1992).

(2) Reagents: Reagents are as shown below:

a) 0.1mol/L - 0.2 mol/L sodium hydroxide solution(1)**:** Transfer about 30 mL of water to a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4 - 5 days. Transfer 5.5 mL - 11 mL of the supernatant to a ground-in stoppered storage container, and add 1,000 mL of water containing no carbonic acid.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then transfer about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Transfer a predetermined amount of the solution to a 200-mL - to 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 mg/100 mL) as an indicator, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)
$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C_1)$

W_1: Mass (g) of sulfamic acid sampled
A: Purity (% (mass fraction)) of sulfamic acid
V_1: Volume (mL) of sulfamic acid solution transferred
V_2: Constant volume (250 mL) of sulfamic acid solution
V_3: Volume (mL) of 0.1 mol/L-0.2 mol/L sodium hydroxide solution needed for titration
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) Sulfuric acid: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) 0.25 mol/L sulfuric acid(1) (2)**:** Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water added in advance, stir well, and add water to make 1,000 mL.

Standardization: Transfer a predetermined amount (3) of 0.25 mol/L sulfuric acid to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red-methylene blue mixture solution, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green(4). Calculate the volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to1 mL of 0.25 mol/L sulfuric acid, by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
$= V_4/V_5$ ………. (1)

Factor of 0.25 mol/L sulfuric acid (f_2)
$= (f_1 \times C_1 \times V_4/V_5) / (C_2 \times 2)$ ……….. (2)

V_4: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
V_5: Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
C_2: Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) Boric acid solution (40 g/L): Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1,000 mL.
e) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
f) Tin (II) chloride dihydrate: A JIS Guaranteed Reagent specified in JIS K 8136 or a reagent of mercury analysis grade or equivalent quality.
g) Devarda's alloy: A reagent of nitrogen analysis grade specified in JIS K 8653 or a reagent of equivalent quality.
h) Sodium hydroxide solution (200 g/L - 500 g/L)(1): Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.
i) Bromothymol blue solution (0.1 g/100 mL): Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.
j) Methyl red solution (0.1 g/100 mL): Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
k) Methylene blue solution (0.1 g/100 mL): Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.
l) Methyl red - methylene blue mixture solution: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).
m) Bromocresol green solution (0.5 g/100 mL): Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.
n) Methyl red - bromocresol green mixture solution: To methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).
(3) 5 mL - 10 mL
(4) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

(3) Apparatus and instruments: Apparatus and instruments are as shown below:
a) Steam distillation apparatus
b) Digestion flask: Kjeldahl flask
c) Distillation flask: A Kjeldahl flask or round bottom flask that can be connected to a steam distillation apparatus.

(4) Test procedures

(4.1) Reduction and Kjeldahl method: Conduct reduction and digestion as shown below:

- **a)** Weigh 0.5 g - 1 g (no more than the equivalents of N-N 50 mg) of an analytical sample to the order of 1 mg, and put it in a 300-mL- 500-mL digestion flask (5)
- **b)** Add 60 mL of hydrochloric acid (1+1) and 2 g of tin (II) chloride dihydrate, and shake to mix and leave at rest for about 20 minutes.
- **c)** Add 3.5 g of devarda's alloy and leave at rest for about 40 minutes while sometimes shaking to mix.
- **d)** Add 70 mL of sulfuric acid (1+1) and one boiling stone as necessary, and heat at low temperature (6).
- **e)** As soon as white smoke start evolving, strengthen heating gradually and further continue heating for about 90 minutes.
- **f)** After standing to cool, add 100 mL - 200 mL of water, mix well by shaking, transfer to a 250-mL - 500-mL volumetric flask with water, and further mix by shaking (7).
- **g)** After standing to cool, add water up to the marked line to make the digestion solution.

Note **(5)** In the case of direct distillation, a 500-mL Kjeldahl flask connectable to a steam distillation apparatus is preferable.

(6) If the bubbles foam strongly and excessively, suspend heating for a little while.

(7) It is not necessary to fill up if a whole sample solution is used for the measurement.

(4.2) Distillation: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

- **a)** Transfer a predetermined amount(8) of 0.25 mol/L sulfuric acid to an acceptor(9), add a few drops of methyl red - methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, transfer a predetermined amount(8) of boric acid solution (40 g/L) to an acceptor(9), add a few drops of methyl red - bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.
- **b)** Transfer a predetermined amount of the digestion solution to a 300-mL distillation flask, add a proper amount of sodium hydroxide solution (200 g/L – 500 g/L) (10), and immediately connect this distillation flask to the steam distillation apparatus.
- **c)** Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.
- **d)** Stop distilling when the distillate reaches 120 mL - 160 mL.
- **e)** Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note **(8)** 5 mL - 20 mL

(9) As an acceptor, use a 200-mL - 300mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or boric acid solution (40 g/L).

(10) A sufficient amount to make the solution strong alkalinity. A blue color will appear.

(4.3) Measurement: Conduct measurement as shown below.

(4.3.1) When 0.25 mol/L sulfuric acid is used in **(4.2)**,

- **a)** Titrate the distillate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).
- **b)** Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (14.007/W_2) \times (100/1,000)$
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (1.4007/W_2)$

B: Volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
V_6: Volume (mL) of 0.25 mol/L sulfuric acid transferred to the acceptor in **(4.2) a)**
V_7: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f_1: Factor of 0.1 mol/L-0.2 mol/L sodium hydroxide solution
V_8: Predetermined volume (mL) of the digestion solution in **(4.1) g)**
V_9: Transferred amount (mL) of the digestion solution subjected to distillation in **(4.2) b)**
W_2: Mass (g) of the analytical sample

(4.3.2) When boric acid solution (40 g/L) is used in **(4.2)**,

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (11).

b) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample
$= V_{10} \times C_2 \times 2 \times f_2 \times (V_{11}/V_{12}) \times (14.007/W_2) \times (100/1,000)$
$= V_{10} \times C_2 \times f_2 \times (V_{11}/V_{12}) \times (2.8014/W_2)$

V_{10}: Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
C_2: Set concentration (mol/L) of 0.25 mol/L sulfuric acid
f_2: Factor of 0.25 mol/L sulfuric acid
V_{11}: Predetermined volume (mL) of the digestion solution in **(4.1) g)**
V_{12}: Transferred amount (mL) of the digestion solution subjected to distillation in **(4.2) b)**
W_2: Mass (g) of the analytical sample

Note **(11)** The endpoint is reached when the color changes from green to light red.

Comment 1 The titration procedures in **(2) a) Standardization**, **(2) c) Standardization** and **(4.3)** can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.31-33, Yokendo, Tokyo (1988)

(5) Flow sheet for total nitrogen: The flow sheet for total nitrogen in fertilizers is shown below:

0.5 g – 1 g analytical sample	Weigh to the order of 1 mg into a 300-mL - 500-mL digestion flask.
	← 60 mL of hydrochloric acid (1+1)
	← 2 g of tin (II) chloride dihydrate
Leaving at rest	About 20 minutes
	← 3.5 g devardar's alloy
Leaving at rest	About 40 minutes
	← 70 mL sulfuric acid (1+1)
	← 1 boiling stone
Heating	Heat at low temperature, and as soon as white smoke evolves, strengthen heating gradually and further heat for about 90 minutes.
Standing to cool	
	← 100 mL - 200 mL Water
Transfer	250-mL - 500-mL volumetric flask, water
Standing to cool	Room temperature
	← Water (up to the marked line)
Aliquot (predetermined volume)	300-mL distillation flask
	← Sodium hydroxide (200 g/L - 500 g/L)
Steam distillation apparatus	Receiver: 200-mL - 300-mL Erlenmeyer flask or beaker A predetermined amount of 0.25 mol/L sulfuric acid and a few drops of methyl red-methylene blue mixture solution, or boric acid solution (40 g/L), several drops of methyl red - bromocresol green mixture solution
Steam distillation	Distillation rate: 5 mL/min - 7 mL/min
Stop distilling	120 mL – 160 mL distillate
	← Water (wash the part of the distillation apparatus that came in contact with the solution in the receiver)
Titration	0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green, or 0.25 mol/L sulfuric acid (until the solution becomes light red)

Figure Flow sheet (example) for total nitrogen in fertilizers.

4.1.1.d Reduced iron - Kjeldahl method

(1) Summary

The method is applicable to the fertilizers that contain nitrate nitrogen (N-N) and guarantee total nitrogen.

Add water, reduced iron and sulfuric acid (1+1) to an analytical sample to reduce nitrate nitrogen (N-N) and heat at low temperature, and then add sulfuric acid and pretreat by Kjeldahl method to change total nitrogen (T-N) to ammonium ion, and add sodium hydroxide to subject to steam distillation. Measure separated ammonium ion by neutralization titration to obtain total nitrogen (T-N) in the analytical sample. This test method corresponds to the sulfuric acid method in the Official Methods of Analysis of Fertilizers (1992).

(2) Reagents: Reagents are as shown below:

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution**(1): Transfer about 30 mL of water to a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Transfer 5.5 mL - 11 mL of the supernatant to a ground-in stoppered storage container, and add 1,000 mL of water containing no carbonic acid.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then transfer about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line.(1) Transfer a predetermined amount of the solution to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 mg/100 mL) as an indicator, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)
$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C_1)$

- W_1: Mass (g) of sulfamic acid sampled
- A: Purity (% (mass fraction)) of sulfamic acid
- V_1: Volume (mL) of sulfamic acid solution transferred
- V_2: Constant volume (250 mL) of sulfamic acid solution
- V_3: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
- C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sulfuric acid:** A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) **0.25 mol/L sulfuric acid**(1) (2)**:** Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1,000 mL.

Standardization: Transfer a predetermined amount (3) of 0.25 mol/L sulfuric acid to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red-methylene blue mixture solution, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green.(4) Calculate the volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
$= V_4/V_5$ …… (1)

Factor of 0.25 mol/L sulfuric acid (f_2)
$= (f_1 \times C_1 \times V_4/V_5)/ (C_2 \times 2)$ …… (2)

V_4: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
V_5: Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
C_2: Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) Boric acid solution (40 g/L): Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1,000 mL.
e) Reduced iron: Nitrogen content is no more than 0.005 % (mass fraction)
f) Catalyst (5)**:** Mix potassium sulfate specified in JIS K 8962 and copper (II) sulfate pentahydrate (6) specified in JIS K 8983 in the ratio of 9 to 1.
g) Sodium hydroxide solution (200 g/L - 500 g/L)(1)**:** Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.
h) Bromothymol blue solution (0.1 g/100 mL): Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, and add water to make 100 mL.
i) Methyl red solution (0.1 g/100 mL): Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
j) Methylene blue solution (0.1 g/100 mL): Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.
k) Methyl red - methylene blue mixture solution: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).
l) Bromocresol green solution (0.5 g/100 mL): Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.
m) Methyl red - bromocresol green mixture solution: To methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).
(3) 5 mL - 10 mL
(4) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.
(5) A tablet is commercially available.
(6) Crush into powder as appropriate.

(3) Instruments: Instruments are as shown below:
a) Steam distillation apparatus
b) Digestion flask: Kjeldahl flask
c) Distillation flask: A Kjeldahl flask or a round bottom flask that can be connected to a steam distillation apparatus.
(4) Test procedures
(4.1) Reduction and Kjeldahl method: Conduct reduction and digestion as shown below:

a) Weigh 0.5 g - 1 g of an analytical sample to the order of 1 mg, and put it in a 300-mL - 500-mL digestion flask.
b) Add 30 mL of water and mix well.
c) As soon as 5 g of reduced iron and 30mL of sulfuric acid (1+1) are added, insert a long stem funnel to a digestion flask and shake to mix gently while cooling the outside of the container under flowing water(7).
d) Leave at rest for about 5 minutes (8), boil in a low flame for about 15 minutes.
e) After standing to cool, add 5 g - 10 g of catalyst, 30 mL of sulfuric acid and, if necessary, one boiling stone, heat gradually until water evaporates and white smoke of sulfuric acid evolves(9).
f) Ignite until it is completely digested (10).
g) After standing to cool, add a small amount of water, mix well by shaking, transfer to a 250-mL - 500-mL volumetric flask with water, and further mix by shaking.
h) After standing to cool, add water up to the marked line to make the digestion solution.

Note **(7)** A sudden reaction generates heat, and unreacted nitric acid vaporizes or digests to make nitrogen oxide etc. through which process losses occur easily. Careful and efficient operation should be taken
(8) Until a sudden reaction is settled.
(9) If the bubbles foam strongly and excessively, suspend heating for a little while.
(10) After the color of the solution stops changing, heat for no less than 2 hours.

(4.2) **Distillation:** Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.
a) Transfer a predetermined amount (11) of 0.25 mol/L sulfuric acid to an acceptor (12), add a few drops of methyl red - methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, transfer a predetermined amount (11) of boric acid solution (40 g/L) to an acceptor (12), add a few drops of methyl red - bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.
b) Transfer a predetermined amount of the digestion solution to a 300-mL distillation flask, add a proper amount of sodium hydroxide solution (200 g/L - 500 g/L) (13), and immediately connect this distillation flask to the steam distillation apparatus.
c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL /min - 7 mL /min.
d) Stop distilling when the distillate reaches 120 mL - 160 mL.
e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note **(11)** 5 mL - 20 mL
(12) As an acceptor, use a 200-mL – 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or boric acid solution (40 g/L).
(13) A sufficient amount to make the solution strong alkalinity. A blue or reddish brown color will appear.

(4.3) **Measurement:** Conduct measurement as shown below.
(4.3.1) When 0.25 mol/L sulfuric acid is used in **(4.2)**,
a) Titrate the distillate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).

b) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (14.007/W_2) \times (100/1{,}000)$
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (1.4007/W_2)$

B: Volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
V_6: Volume (mL) of 0.25 mol/L sulfuric acid transferred to the acceptor in **(4.2) a)**
V_7: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f_1: Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
V_8: Predetermined volume (mL) of the digestion solution in **(4.1) e)**
V_9: Transferred amount (mL) of the digestion solution subjected to distillation in **(4.2) b)**
W_2: Mass (g) of the analytical sample

(4.3.2) When boric acid solution (40 g/L) is used in **(4.2)**,

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (14).

b) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample
$= V_{10} \times C_2 \times 2 \times f_2 \times (V_{11}/V_{12}) \times (14.007/W_2) \times (100/1{,}000)$
$= V_{10} \times C_2 \times f_2 \times (V_{11}/V_{12}) \times (2.8014/W_2)$

V_{10}: Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
C_2: Set concentration (mol/L) of 0.25 mol/L sulfuric acid
f_2: Factor of 0.25 mol/L sulfuric acid
V_{11}: Predetermined volume (mL) of the digestion solution in **(4.1) e)**
V_{12}: Transferred amount (mL) of the digestion solution subjected to distillation in **(4.2) b)**
W_2: Mass (g) of the analytical sample

Note (14) The endpoint is reached when the color changes from green to light red.

Comment 1 The titration procedures in **(2) a) Standardization**, **(2) c) Standardization** and **(4.3)** can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.33 - 34, Yokendo, Tokyo (1988)

(5) Flow sheet for total nitrogen: The flow sheet for total nitrogen in fertilizers is shown below:

0.5 g - 1 g analytical sample	Weigh to the order of 1 mg into a 300-mL digestion flask.
← 30 mL water ← 5 g reduced iron ← 30 mL sulfuric acid (1+1)	
Mixture	Immediately insert a long stem funnel to the flask and shake to mix while cooling outside the container with flowing water.
Leaving at rest	For about 5 min (Until a sudden reaction is settled.)
Heating	Boil in a low flame for about 15 minutes.
Standing to cool	
← 5 g - 10 g catalyst ← 30 mL sulfuric acid	
Heating	Heat gradually until water evaporates and the white smoke of sulfuric acid evolves and ignite to digest completely
Standing to cool	
← A small amount of water	
Transfer	250-mL - 500-mL volumetric flask, water
Standing to cool	Room temperature
← Water (up to the marked line)	
Aliquot (predetermined volume)	Distillation flask
← Sodium hydroxide (200 g/L - 500 g/L)	
Steam distillation apparatus	Receiver: 200-mL - 300-mL Erlenmeyer flask or beaker A predetermined amount of 0.25 mol/L sulfuric acid and a few drops of methyl red-methylene blue mixture solution, or boric acid solution (40 g/L), several drops of methyl red - bromocresol green mixture solution
Steam distillation	Distillation rate: 5 mL/min - 7 mL/min
Stop distilling	120 mL – 160 mL distillate
← Water (wash the part of the distillation apparatus that came in contact with the solution in the receiver)	
Titration	0.1mol/L - 0.2 mol/L sodium hydroxide solution (until the solution becomes gray-green), or 0.25 mol/L sulfuric acid (until the solution becomes light red)

Figure Flow sheet (example) for total nitrogen in fertilizers.

4.1.1.e Calculation with ammoniac nitrogen and nitrate nitrogen

(1) Summary

The calculation is applicable to the fertilizers that contain ammoniac nitrogen (A-N) and nitrate nitrogen (N-N), and that does not contain the fertilizers guaranteeing total nitrogen (T-N).

Calculate total nitrogen (T-N) by adding the ammoniac nitrogen (A-N) obtained in **4.1.2** to the nitrate nitrogen (N-N) obtained in **4.1.3**.

(2) The calculation of total nitrogen

a) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample
= (A-N) + (N-N)

A-N: Ammoniac nitrogen (% (mass fraction)) in the analytical sample [(1)] obtained by **4.1.2**

N-N: Nitrate nitrogen (% (mass fraction)) in the analytical sample (1) obtained by **4.1.3**

Note (1) A-N and N-N use raw data without rounding numerical value

4.1.2 Ammoniac nitrogen

4.1.2.a Distillation method

(1) Summary
The testing method is applicable to the fertilizers that contain ammonium salt. However, in some cases, it is not applicable to the fertilizers that contain such compounds as nitrolime that digests by heating.
Add water to an analytical sample, further add sodium hydroxide solution or magnesium oxide to make the solution alkalinity and subject it to steam distillation. Measure separated ammonium ion by neutralization titration to obtain ammoniac nitrogen (A-N) in the analytical sample.

(2) Reagents: Reagents are as shown below:

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution**(1): Transfer about 30 mL of water to a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4 - 5 days. Transfer 5.5 mL - 11 mL of the supernatant to a ground-in stoppered storage container, and add 1,000 mL of water containing no carbonic acid.
Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then transfer about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line.(1) Transfer a predetermined amount of the solution to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 mg/100 mL) as an indicator, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)
$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C_1)$

W_1: Mass (g) of sulfamic acid sampled
A: Purity (% (mass fraction)) of sulfamic acid
V_1: Volume (mL) of sulfamic acid solution transferred
V_2: Constant volume (250 mL) of sulfamic acid solution
V_3: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Magnesium oxide:** A JIS Guaranteed Reagent specified in JIS K 8432 or a reagent of equivalent quality.

c) **Sulfuric acid:** A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

d) **0.25 mol/L sulfuric acid**(1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1,000 mL.
Standardization: Transfer a predetermined amount(3) of 0.25 mol/L sulfuric acid to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red-methylene blue mixture solution, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green.(4) Calculate the volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the

following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
$= V_4/V_5$ …….. (1)

Factor of 0.25 mol/L sulfuric acid (f_2)
$= (f_1 \times C_1 \times V_4/V_5)/ (C_2 \times 2)$ …….. (2)

V_4: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
V_5: Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
C_2: Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

e) **Boric acid solution (40 g/L):** Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1,000 mL.
f) **Sodium hydroxide solution (200 g/L - 500 g/L)(1):** Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.
g) **Bromothymol blue solution (0.1 g/100 mL):** Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.
h) **Methyl red solution (0.1 g/100 mL):** Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
i) **Methylene blue solution (0.1 g/100 mL):** Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.
j) **Methyl red - methylene blue mixture solution:** To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).
k) **Bromocresol green solution (0.5 g/100 mL):** Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.
l) **Methyl red - bromocresol green mixture solution:** To methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).
(3) 5 mL - 10 mL
(4) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

(3) Apparatus and Instruments: Instruments are as shown below:
a) **Rotary shaker:** A rotary shaker that can rotate a 500-mL volumetric flask upside down at 30 - 40 revolutions/min.
b) **Steam distillation apparatus**
c) **Distillation flask:** A Kjeldahl flask or a round bottom flask that can be connected to a steam distillation apparatus.

(4) Test procedures
(4.1) Sample solution preparation: Prepare a sample solution as shown below:

a) Weigh 0.25 g - 2g [5] (the equivalents of 20 mg – 100 mg as N) of an analytical sample to the order of 1 mg, and put it in a 300-mL - 500-mL distillation flask

b) Add about 25 mL of water to make the sample solution.

Note (5) The sampling amount of the analytical sample is 5 g when there is less nitrogen content in the fertilizers such as a home garden-use fertilizer.

Comment 1 When it is a fertilizer which contains uric ammonium acid, humus acid ammonium or nitrate nitrogen, etc., or when it is not a fertilizer in which phosphate, ammonium and magnesium coexist, conduct the procedure in **(4.1) a) - c)** in **4.2.4.a** and transfer a predetermined amount of suspension (the equivalents of 20 mg – 100 mg as N) to a 300-mL - 500-mL distillation flask to make the sample solution.

(4.2) Distillation: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Transfer a predetermined amount[5] of 0.25 mol/L sulfuric acid to an acceptor [6], add a few drops of methyl red - methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, transfer a predetermined amount [5] of boric acid solution (40 g/L) to an acceptor [6], add a few drops of methyl red - bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Add a proper amount [7] of sodium hydroxide solution (200 g/L - 500 g/L) [8] to the distillation flask, and connect this distillation flask to a steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate reaches 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note (5) 5 mL - 20 mL

(6) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or boric acid solution (40 g/L).

(7) A sufficient amount to make the solution strong alkalinity.

(8) Add a small amount of silicone oil as necessary.

Comment 2 When the sample contains organic matters or urea, add no less than 2 g of magnesium oxide instead of sodium hydroxide solution.

(4.3) Measurement: Conduct measurement as shown below.

(4.3.1) When 0.25 mol/L sulfuric acid is used in **(4.2)**

a) Titrate the distillate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green [4].

b) Calculate the ammoniac nitrogen (A-N) in the analytical sample by the following formula:

Ammoniac nitrogen (A-N) (% (mass fraction)) in the analytical sample

$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (14.007/W_2) \times (100/1{,}000)$

$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (1.4007/W_2)$

B: Volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
V_6: Volume (mL) of 0.25 mol/L sulfuric acid transferred to the acceptor in **(4.2) a)**
V_7: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f_1: Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
W_2: Mass (g) of the analytical sample

(4.3.2) When boric acid solution (40 g/L) is used in **(4.2)**,

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (9).

b) Calculate the ammoniac nitrogen (A-N) in the analytical sample by the following formula:

Ammoniac nitrogen (A-N) (% (mass fraction)) in the analytical sample
$= V_8 \times C_2 \times 2 \times f_2 \times (14.007/W_2) \times (100/1{,}000)$
$= V_{10} \times C_2 \times f_2 \times (2.8014/W_2)$

V_8: Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
C_2: Set concentration (mol/L) of 0.25 mol/L sulfuric acid
f_2: Factor of 0.25 mol/L sulfuric acid
W_2: Mass (g) of the analytical sample

Note **(9)** The endpoint is reached when the color changes from green to light red.

Comment 3 If it is hard to confirm the endpoint due to the carbon dioxide resulting from carbonate in the extract when magnesium oxide is used, it is recommended to boil the extract for 1-2 minute(s) after distilling and cool, and then titrate.

Comment 4 The titration procedures in **(2) a) Standardization**, **(2) d) Standardization** and **(4.3)** can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Comment 5 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for ammonium-nitrogen hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2007	Organic compound fertilizer	143	6.20	0.09	1.4
2008	High analysis compound fertilizer	147	12.56	0.16	1.2
2009	Ordinary compound fertilizer	138	5.56	0.07	1.3
2011	High analysis compound fertilizer	130	13.50	0.17	1.3
2012	Fluid mixed fertilizer	120	2.41	0.04	1.8
2013	High analysis compound fertilizer	130	9.92	0.75	7.5

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:

$$RSD_{rob} = (NIQR/M) \times 100$$

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.36 - 37, Yokendo, Tokyo (1988)

(5) Flow sheet for ammoniac nitrogen: The flow sheet for ammoniac nitrogen in fertilizers is shown below.

0.25 g - 2 g analytical sample	Weigh the equivalents of 20 mg – 100 mg as N to the order of 1 mg, and put it in a 300-mL distillation flask
	← About 25 mL water
	← Water (up to the marked line)
	← Sodium hydroxide (200 g/L - 500 g/L) (Magnesium oxide no less than 2 g when it contains organic matters)
	← A small amount of defoaming agent as necessary
Steam distillation apparatus	Receiver: 200-mL - 300-mL Erlenmeyer flask or beaker A predetermined amount of 0.25 mol/L sulfuric acid and a few drops of methyl red-methylene blue mixture solution, or boric acid solution (40 g/L), several drops of methyl red - bromocresol green mixture solution
Steam distillation	Distillation rate: 5 mL/min - 7 mL/min
Stop distilling	120 mL – 160 mL distillate
	← Water (wash the part of the distillation apparatus that came in contact with the solution in the receiver)
Titration	0.1 mol/L - 0.2 mol/L sodium hydroxide solution (until the solution becomes gray-green), or 0.25 mol/L sulfuric acid (until the solution becomes light red)

Figure Flow sheet (example) for ammoniac nitrogen in fertilizers.

4.1.2.b Formaldehyde method

(1) Summary

The testing method is applicable to the fertilizers which do not contain a large amount of flora and fauna sample.
After adding potassium chloride solution to an analytical sample to extract ammonium ion, add aluminum chloride solution, drop potassium hydroxide solution and precipitate phosphate and excessive aluminum to make the sample solution. Adjust the sample solution to slight acidity, add formaldehyde solution and measure ammonium ion by neutralization titration to obtain ammoniac nitrogen (A-N) in the analytical sample.

(2) Reagents: Reagents are as shown below:

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution**(1): Transfer about 30 mL of water to a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4 - 5 days. Transfer 5.5 mL - 11 mL of the supernatant to a ground-in stoppered storage container, and add 1,000 mL of water containing no carbonic acid.
Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving it at rest in a desiccator at no more than 2 kPa for about 48 hours, then transfer about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve this in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Transfer a predetermined amount of the solution to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 mg/100 mL) as an indicator, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1mol/L - 0.2 mol/L sodium hydroxide solution (f)
$= (W \times A \times 0.01/97.095) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C)$

W: Mass (g) of sulfamic acid sampled
A: Purity (% (mass fraction)) of sulfamic acid
V_1: Volume (mL) of sulfamic acid solution transferred
V_2: Constant volume (250 mL) of sulfamic acid solution
V_3: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Potassium chloride solution (1 mol/L)** (1)**:** Dissolve 75 g of potassium chloride specified in JIS K 8121 in water to make 1,000 mL.
c) **Aluminum chloride solution (1 mol/L)** (1)**:** Dissolve 240 g of aluminum chloride specified in JIS K 8114 in water to make 1,000 mL.
d) **Potassium hydroxide solution (170 g/L)** (1)**:** Dissolve 170 g of potassium hydroxide in water to make 1,000 mL.
e) **Formaldehyde solution:** Add one volume of water to one volume of the 36 % (mass fraction) – 38 % (mass fraction) formaldehyde specified in JIS K 8872.
f) **Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
g) **Bromothymol blue solution (0.1 g/100 mL):** Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.

- **h) Methyl red solution (0.1 g/100 mL):** Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
- **i) Thymol blue solution (1 g/100 mL):** Dissolve 1 g of thymol blue (sodium salt) in 20 mL of ethanol (95) specified in JIS K 8102.

Note (1) This is an example of preparation. Prepare necessary amount of solution.

Comment 1 Thymol blue can be dissolved if it is sodium salt. The thymol blue specified in JIS K 8643 is slightly hard to dissolve in ethanol and hard to dissolve in water. Therefore add about 2.15 mL of sodium hydroxide solution (0.1 mol/L) per 0.1 g of thymol blue to neutralize, and then prepare the thymol blue solution (1 g/100 mL) through the same procedure as **(2) i)**.

(3) Instruments: Instruments are as shown below:

- **a) Rotary shaker:** A rotary shaker that can rotate a 500-mL volumetric flask upside down at 30 - 40 revolutions/min.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Ammonium salts

- **a)** Weigh 5g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask
- **b)** Add 400 mL of water and shake to mix at the rate of 30-40 revolutions/min for about 30 minutes.
- **c)** Add water up to the marked line.
- **d)** Filter with Type 3 filter paper to make the sample solution.

Comment 2 The procedure in **(4.1)** is the same as the procedure in **(4.1)** in **4.2.4.a**.

(4.1.2) Mixed fertilizers

- **a)** Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask
- **b)** Add about 300 mL of hydrochloric acid (1 + 20) and shake to mix at the rate of 30 - 40 revolutions/min for about 30 minutes.
- **c)** Add aluminum chloride solution (1 mol/L) (2) to the solution and add a few drops of methyl red solution (1mol/L) as an indicator. After that, add immediately potassium hydroxide (170 g/L) while shaking the flask until the color of the solution changes to light yellow (3).
- **d)** Add water up to the marked line.
- **e)** Filter with Type 3 filter paper to make the sample solution.

Note (2) Add 3 mL of aluminum chloride per 0.04 g of P or 0.1 g of P_2O_5 in the sample solution.

(3) Form precipitate of aluminum hydroxide and aluminum phosphate to separate the phosphate.

Comment 3 In the procedure of **(4.1.1) a)** and **(4.1.2) a)**, it is also allowed to weigh 2.5g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask

Comment 4 When it is not a fertilizer in which phosphate, ammonium and magnesium coexist, about 400 mL of potassium chloride solution (1 mol/L) can be used instead of about 300 mL of hydrochloric acid (1+20) in the procedure of **(4.1.2) b).**

Comment 5 In the case of the mixed fertilizers containing bentonite, filter with Type 3 filter paper after shaking to mix using about 400 mL of potassium chloride solution (1 mol/L) according to **Comment 4** in **(4.1.2) b)** and transfer 50 ml - 100 mL to a 250-mL volumetric flask, and then conduct the procedure in **(4.1.2) c) - e)**.

(4.2) Measurement: Conduct measurement as shown below.

a) Transfer a predetermined volume (up to the equivalents of 50 mg as A-N) of sample solution to a 300-mL Erlenmeyer flask (4).

b) Add water to the solution to make about 100 mL.

c) Add one or two drop(s) of methyl red solution (0.1 g/100 mL) and add hydrochloric acid (1+200) until the color of the solution changes to light pink.

d) Add 10 mL of formaldehyde solution.

e) Add one or two drop(s) of thymol blue solution (1 g/100 mL) and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution changes to blue(5).

f) As a blank test, transfer 100 mL of water to another 300-mL Erlenmeyer flask and conduct the procedure in **c) - e)**.

g) Calculate the ammoniac nitrogen (A-N) in the analytical sample by the following formula:

Ammoniac nitrogen (A-N) (% (mass fraction) in the analytical sample

$$= (V_S - V_B) \times C \times f \times (V_1/V_2) \times (14.007/W) \times (100/1,000)$$

$$= (V_S - V_B) \times C \times f \times (V_1/V_2) \times (1.4007/W)$$

V_S: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titrate in **(4.2) e)**

V_B: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration of the blank test in **(4.2) f)**

C: Setting concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

f: Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

V_1 Predetermined volume (mL) of the sample solution in **(4.1.1) c)** or **(4.1.2) d)**

V_2: Transferred amount (mL) of the sample solution in **(4.2) a)**

W: Mass of sample solution (g)

Note **(4)** The volume to be transferred should be up to 100 mL.

(5) The endpoint is reached when the solution changes from green to blue. It is easy to observe the change of color under fluorescent light.

Comment 6 The titration procedures in **(2) a) Standardization** and **(4.2) e) - f)** can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Comment 7 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for ammonium-nitrogen hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2006	High analysis compound fertilizer	150	14.60	0.10	0.9
2010	High analysis compound fertilizer	107	11.51	0.17	1.5

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:

RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.39 - 42, Yokendo, Tokyo (1988)

(5) Flow sheet for ammoniac nitrogen: The flow sheet for ammoniac nitrogen in fertilizers is shown below.

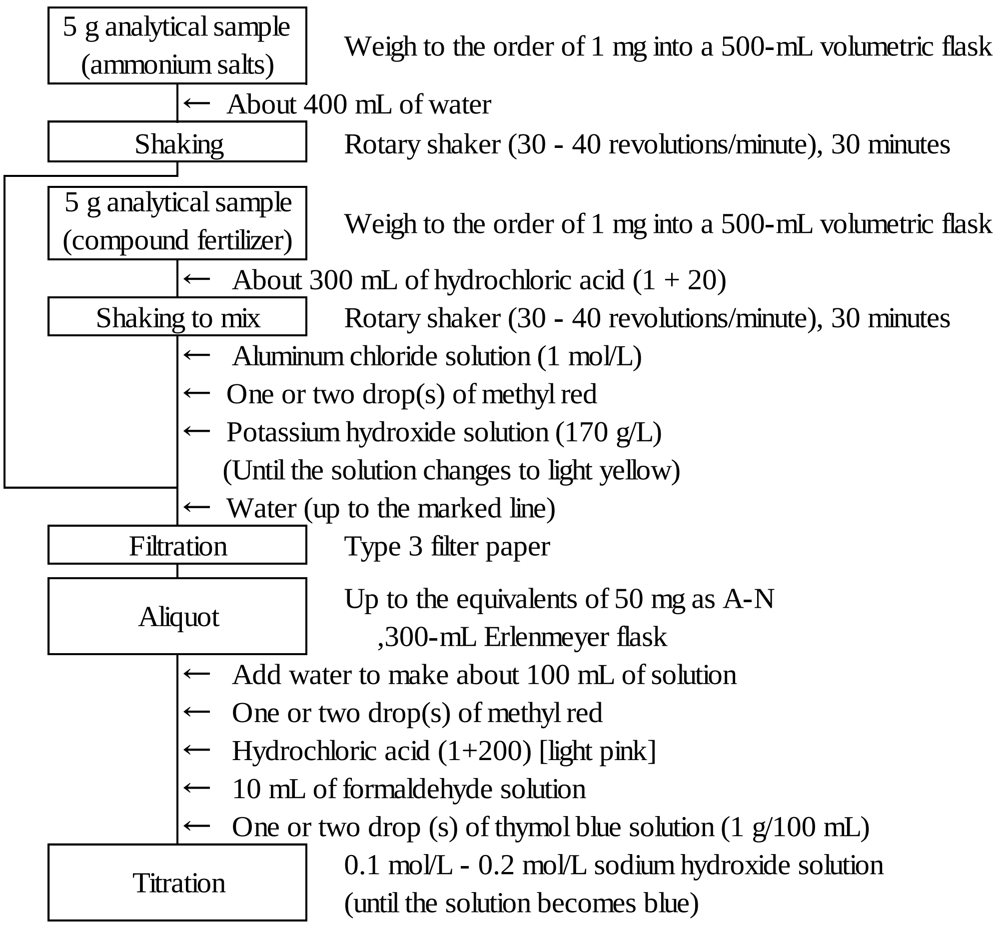


Figure The flow sheet (example) for ammoniac nitrogen in fertilizers is shown below.

4.1.3 Nitrate nitrogen

4.1.3.a Devarda's alloy - distillation method

(1) Summary

The testing method is applicable to fertilizers containing nitrate. However it is not applicable to fertilizers containing nitrolime and organic matters that digest by heating and isolate ammonia.

Add water to an analytical sample to dissolve ammoniac nitrogen (A-N) and nitrate nitrogen (N-N), and further add devarda's alloy and sodium hydroxide to reduce nitrate nitrogen (N-N) and separate ammonium ion by distillation. Measure the separated ammonium ion by neutralization titration to obtain total amount of nitrogen (N-N+A-N) in the analytical sample. Subtract separately obtained ammoniac nitrogen (A-N) to calculate nitrate nitrogen (N-N). This test method corresponds to the devarda's alloy method in the Official Methods of Analysis of Fertilizers (1992).

(2) Reagents: Reagents are as shown below:

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution**(1): Transfer about 30 mL of water to a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4 - 5 days. Transfer 5.5 mL - 11 mL of the supernatant to a ground-in stoppered storage container, and add 1,000 mL of water containing no carbonic acid.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then transfer about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Transfer a predetermined amount of the solution to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 mg/100 mL) as an indicator, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)
$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C_1)$

W_1: Mass (g) of sulfamic acid sampled
A: Purity (% (mass fraction)) of sulfamic acid
V_1: Volume (mL) of sulfamic acid solution transferred
V_2: Constant volume (250 mL) of sulfamic acid solution
V_3: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sulfuric acid:** A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) **0.25 mol/L sulfuric acid**(1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well and add water to make 1,000 mL.

Standardization: Transfer a predetermined amount (3) of 0.25 mol/L sulfuric acid to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red-methylene blue mixture solution, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green(4). Calculate the volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
$= V_4/V_5$ ………. (1)

Factor of 0.25 mol/L sulfuric acid (f_2)
$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2)$ ………. (2)

V_4: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
V_5: Volume (mL) of 0.25 mol/L sulfuric acid subjected to the standardization
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
C_2: Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) **Boric acid solution (40 g/L):** Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1,000 mL.
e) **Sodium hydroxide solution (200 g/L - 500 g/L)(1):** Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.
f) **Devarda's alloy:** A reagent of nitrogen analysis grade specified in JIS K 8653 or a reagent of equivalent quality
g) **Bromothymol blue solution (0.1 g/100 mL):** Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.
h) **Methyl red solution (0.1 g/100 mL):** Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
i) **Methylene blue solution (0.1 g/100 mL):** Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.
j) **Methyl red - methylene blue mixture solution:** To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).
k) **Bromocresol green solution (0.5 g/100 mL):** Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.
l) **Methyl red - bromocresol green mixture solution:** To methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).
(3) 5 mL - 10 mL
(4) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

(3) Instruments: Instruments are as shown below:
a) **Steam distillation apparatus**
b) **Distillation flask:** A Kjeldahl flask or a round bottom flask that can be connected to a steam distillation apparatus.

(4) Test procedures
(4.1) Preparation of sample solution: Conduct preparation of sample solution as shown below.

a) Weigh 0.25 g - 1 g [5] (the equivalents of 20 mg - 100 mg as N) of an analytical sample to the order of 1 mg, and put it in a 300-mL - 500-mL distillation flask

b) Add about 25 mL of water to make the sample solution.

Note **(5)** Conduct the procedure in **Comment 1** when there is much nitrogen content in the fertilizers such as simple salt fertilizers.

Comment 1 In the case of nitrate fertilizer, etc. containing much nitrogen content, weigh 2 g - 5g of an analytical sample to the order of 1 mg, put it into a 250- mL volumetric flask, dissolve it in water, and further add water up to the marked line. Put predetermined volume of suspension (the equivalents of 20 mg -100 mg as N) into a 300- mL - 500-mL distillation flask.

(4.2) Distillation: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Transfer a predetermined amount [6] of 0.25 mol/L sulfuric acid to an acceptor [7], add a few drops of methyl red - methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, transfer a predetermined amount [6] of boric acid solution (40 g/L) to an acceptor[7], add a few drops of methyl red - bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Add [10] no less than 3 mg of devarda's alloy and adequate volume of sodium hydroxide (200 g/L - 500 g/L) [8] [9] and connect this distillation flask to the steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate reaches 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note **(6)** 5 mL - 20 mL

(7) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or boric acid solution (40 g/L).

(8) Sudden reaction makes bubbles foam drastically and the bubbles overflow from a distillation flask. Therefore it is required to add alkali solution gradually and mix quietly.

(9) A sufficient amount to make the solution strong alkalinity.

(10) Add a small amount of silicone oil as necessary.

(4.3) Measurement: Conduct measurement as shown below.

(4.3.1) When 0.25 mol/L sulfuric acid is used in **(4.2)**,

a) Titrate the distillate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the solution becomes gray-green [4].

b) Calculate the total amount of nitrogen (N-N+A-N) in the analytical sample by the following formula:

c) Subtract separately obtained ammoniac nitrogen (A-N) from the obtained total amount of nitrogen (N-N+A-N) to calculate nitrate nitrogen (N-N) [11] [12].

Total amount of nitrogen (N-N+A-N) (% (mass fraction)) in the analytical sample

$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (14.007/W_2) \times (100/1{,}000)$

$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (1.4007/W_2)$

- B: Volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
- V_6: Volume (mL) of 0.25 mol/L sulfuric acid transferred to the acceptor in **(4.2) a)**
- V_7: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration in **(4.3) a)**
- C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
- f_1: Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
- W_2: Mass (g) of the analytical sample

Note **(11)** A-N and N-N use raw data without rounding the numerical value
(12) When no ammoniac nitrogen (A-N) is contained, the total amount of nitrogen (N-N+A-N) calculated in **(4.3) b)** is regarded as nitrate nitrogen (N-N).

(4.3.2) When boric acid solution (40 g/L) is used in **(4.2)**,

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the solution becomes light red (13).

b) Calculate the total amount of nitrogen (N-N+A-N) in the analytical sample by the following formula:

Total amount of nitrogen (N-N+A-N) (% (mass fraction)) in the analytical sample
$= V_{10} \times C_2 \times 2 \times f_2 \times (V_{11}/V_{12}) \times (14.007/W_2) \times (100/1{,}000)$
$= V_{10} \times C_2 \times f_2 \times (V_{11}/V_{12}) \times (2.8014/W_2)$

- V_{10}: Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
- C_2: Set concentration (mol/L) of 0.25 mol/L sulfuric acid
- f_2: Factor of 0.25 mol/L sulfuric acid
- V_{11}: Predetermined volume (mL) of the sample solution in **(4.1) b)**
- V_{12}: Transferred amount (mL) of the digestion solution subjected to distillation in **(4.2) b)**
- W_2: Mass (g) of the analytical sample

Note **(13)** The endpoint is reached when the color changes from green to light red.

Comment 2 The titration procedures in **(2) a) Standardization**, **(2) c) Standardization** and **(4.3)** can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Comment 3 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for nitrate nitrogen hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2009	Ordinary compound fertilizer	84	3.60	0.12	3.3
2012	Fluid mixed fertilizer	77	2.18	0.09	4.1

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
$RSD_{rob} = (NIQR/M) \times 100$

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.49 - 50, Yokendo, Tokyo (1988)

(5) Flow sheet for nitrate nitrogen: The flow sheet for nitrate nitrogen in fertilizers is shown below.

0.25 g - 1 g analytical sample — Weigh the equivalents of 20 mg - 100 mg as N to the order of 1 mg into a 300-mL distillation flask.

← 25 mL of water
← No less than 3 g of devarda's alloy
← Sodium hydroxide solution (200 g/L - 500 g/L)
← A small amount of silicone oil as necessary

Steam distillation apparatus — Receiver: 200-mL - 300-mL Erlenmeyer flask or beaker
A predetermined amount of 0.25 mol/L sulfuric acid and a few drops of methyl red-methylene blue mixture solution, or boric acid solution (40 g/L), several drops of methyl red - bromocresol green mixture solution

Steam distillation — Distillation rate: 5 mL/min - 7 mL/min

Stop distilling — 120 mL - 160 mL distillate

← Water (wash the part of the distillation apparatus that came in contact with the solution in the receiver)

Titration — 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (until the solution becomes gray-green), or 0.25 mol/L sulfuric acid (until the solution becomes light red)

Figure The flow sheet (example) for nitrate nitrogen in fertilizers

4.1.3.b Reduced iron - distillation method

(1) Summary

The testing method is applicable to the fertilizer containing nitrate. However it is not applicable to the fertilizers containing nitrolime and organic matters that digest by heating and isolate ammonia.

Add water to an analytical sample to dissolve ammoniac nitrogen (A-N) and nitrate nitrogen (N-N), and add reduced iron and sulfuric acid solution to reduce nitrate nitrogen (N-N) by lightly boiling. And further add sodium hydroxide solution to distillate. Measure the separated ammonium ion by neutralization titration to obtain total amount of nitrogen (N-N+A-N) in the analytical sample. Subtract separately obtained ammoniac nitrogen (A-N) to calculate nitrate nitrogen (N-N). This test method corresponds to the reduced iron method in the Official Methods of Analysis of Fertilizers (1992).

(2) Reagents: Reagents are as shown below:

a) 0.1 mol/L - 0.2 mol/L sodium hydroxide solution(1): Transfer about 30 mL of water to a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4 - 5 days. Transfer 5.5 mL - 11 mL of the supernatant to a ground-in stoppered storage container, and add 1,000 mL of water containing no carbonic acid.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then transfer about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Transfer a predetermined amount of the solution to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 mg/100 mL) as an indicator, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)
$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C_1)$

W_1: Mass (g) of sulfamic acid sampled
A: Purity (% (mass fraction)) of sulfamic acid
V_1: Volume (mL) of sulfamic acid solution transferred
V_2: Constant volume (250 mL) of sulfamic acid solution
V_3: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) Sulfuric acid: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) 0.25 mol/L sulfuric acid(1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1,000 mL.

Standardization: Transfer a predetermined amount (3) of 0.25 mol/L sulfuric acid to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red-methylene blue mixture solution, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green(4). Calculate the volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (A) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
$= V_4/V_5$ ………..(1)

Factor of 0.25 mol/L sulfuric acid (f_2)
$= (f_1 \times C_1 \times V_4/V_5)/ (C_2 \times 2)$ ………...(2)

V_4: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
V_5: Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
C_2: Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) **Boric acid solution (40 g/L):** Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1,000 mL.
e) **Sodium hydroxide solution (200 g/L - 500 g/L)(1):** Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.
f) **Reduced iron:** Nitrogen content is no more than 0.005 % (mass fraction).
g) **Bromothymol blue solution (0.1 g/100 mL):** Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.
h) **Methyl red solution (0.1 g/100 mL):** Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
i) **Methylene blue solution (0.1 g/100 mL):** Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.
j) **Methyl red - methylene blue mixture solution:** To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).
k) **Bromocresol green solution (0.5 g/100 mL):** Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.
l) **Methyl red - bromocresol green mixture solution:** To methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).
(3) 5 mL - 10 mL
(4) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

(3) **Instruments:** Instruments are as shown below:
a) **Steam distillation apparatus**
b) **Distillation flask:** A Kjeldahl flask or a round bottom flask that can be connected to a steam distillation apparatus.

(4) **Test procedures**
(4.1) **Preparation of sample solution:** Conduct preparation of sample solution as shown below.
a) Weigh 0.25 g - 1g (5) (the equivalents of 20 mg -100 mg as N) of an analytical sample to the order of 1 mg, and put it in a 300-mL - 500-mL distillation flask
b) Add about 30 mL of water and mix well.

c) As soon as adding 5 g of reduced iron and 10 mL of sulfuric acid (1+1), insert a long stem funnel to a distillation flask and shake to mix gently while cooling the outside of the container under flowing water(6).

d) After leaving at rest for about 5 minutes (7), heat gradually by low temperature and boil in a low flame for about 15 minutes, and then stand to cool to make the sample solution.

Note **(5)** Conduct the procedure of **Comment 1** when there is much nitrogen content in the fertilizers such as simple salt fertilizers.

(6) Sudden reaction generates heat, and unreacted nitric acid vaporizes or digests to make nitrogen oxide etc. through which process losses occur easily. Careful and efficient operation should be taken.

(7) Until strong reaction is settled.

Comment 1 In the case of nitrate fertilizers, etc. containing much nitrogen content, weigh 2 g - 5g of an analytical sample to the order of 1 mg, put it into a 250- mL volumetric flask, dissolve it with water and add water up to the marked line. Put predetermined volume of suspension (the equivalents of 20 mg - 100 mg as N) into a 300- mL - 500-mL distillation flask.

(4.2) Distillation: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Transfer a predetermined amount (8) of 0.25 mol/L sulfuric acid to an acceptor(9), add a few drops of methyl red - methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, transfer a predetermined amount(8) of boric acid solution (40 g/L) to an acceptor (9) ,add a few drops of methyl red - bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Add(10) adequate volume of sodium hydroxide (200 g/L - 500 g/L)(10) and connect this distillation flask to the steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate reaches 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note **(8)** 5 mL - 20 mL

(9) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or boric acid solution (40 g/L).

(10) A sufficient amount to make the solution strong alkalinity.

(4.3) **Measurement:** Conduct measurement as shown below.

(4.3.1) When 0.25 mol/L sulfuric acid is used in **(4.2) a)**,

a) Titrate the distillate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).

b) Calculate the total amount of nitrogen (N-N+A-N) in the analytical sample by the following formula:

c) Subtract separately obtained ammoniac nitrogen (A-N) from the obtained total amount of nitrogen (N-N+A-N) to calculate nitrate nitrogen (N-N) (11) (12).

Total amount of nitrogen (N-N+A-N) (% (mass fraction)) in the analytical sample

$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (14.007/W_2) \times (100/1{,}000)$
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (1.4007/W_2)$

- B: Volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
- V_6: Volume (mL) of 0.25 mol/L sulfuric acid transferred to the acceptor in **(4.2) a)**
- V_7: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
- C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
- f_1: Factor of 0.1 mol/L-0.2 mol/L sodium hydroxide solution
- W_2: Mass (g) of the analytical sample

Note **(11)** T-N and N-N use raw data without rounding numerical value
(12) When no ammoniac nitrogen (A-N) is contained, total amount of nitrogen (N-N+A-N) calculated in **(4.3) b)** is regarded as nitrate nitrogen (N-N).

(4.3.2) When boric acid solution (40 g/L) is used in **(4.2) a)**,

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (13).
b) Calculate the total amount of nitrogen (N-N+A-N) in the analytical sample by the following formula:
c) Subtract separately obtained ammoniac nitrogen (A-N) from the obtained total amount of nitrogen (N-N+A-N) to calculate nitrate nitrogen (N-N) (11) (12).

Total amount of nitrogen (N-N+A-N) (% (mass fraction)) in the analytical sample
$= V_{10} \times C_2 \times 2 \times f_2 \times (14.007/W_2) \times (100/1{,}000)$
$= V_{10} \times C_2 \times f_2 \times (2.8014/W_2)$

- V_{10}: Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
- C_2: Set concentration (mol/L) of 0.25 mol/L sulfuric acid
- f_2: Factor of 0.25 mol/L sulfuric acid
- W_2: Mass (g) of the analytical sample

Note **(13)** The endpoint is reached when the color changes from green to light red.

Comment 2 The titration procedures in **(2) a) Standardization**, **(2) c) Standardization** and **(4.3)** can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Comment 3 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for nitrate nitrogen hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2009	Ordinary compound fertilizer	12	3.64	0.07	2.0

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.48 - 49, Yokendo, Tokyo (1988)

(5) Flow sheet for nitrate nitrogen: The flow sheet for nitrate nitrogen in fertilizers is shown below.

Step	Procedure
0.5 g – 1 g analytical sample	Weigh the equivalents of 20 mg - 100 mg of N to the order of 1 mg, and put it into a 300-mL distillation flask.
←About 30 mL of water	
←5 g of reduced iron	
←About 10 mL of sulfuric acid (1+1)	
Mixture	Insert a long stem funnel immediately to a distillation flask and shake to mix gently while cooling the outside of the container under flowing water
Leaving at rest	For about 5 minutes (until a strong reaction is settled)
Heating	Boil in a low flame for about 15 minutes
Standing to cool	
←Sodium hydroxide (200 g/L - 500 g/L)	
Steam distillation apparatus	Receiver: 200-mL - 300-mL Erlenmeyer flask or beaker 200-mL - 300-mL beaker. A predetermined amount of 0.25 mol/L sulfuric acid and a few drops of methyl red-methylene blue mixture solution, or boric acid solution (40 g/L), several drops of methyl red - bromocresol green mixture solution
Steam distillation	Distillation rate: 5 mL/min - 7 mL/min
Stop distilling	120 mL - 160 mL distillate
← Water (wash the part of the distillation apparatus that came in contact with the solution in the receiver)	
Titration	0.1 mol/L - 0.2 mol/L sodium hydroxide solution (until the solution becomes gray-green), or 0.25 mol/L sulfuric acid (until the solution becomes light red)

Figure The flow sheet (example) for nitrate nitrogen in fertilizers

4.1.3.c Phenol sulfuric acid method

(1) Summary

The testing method is applicable to the fertilizer containing nitrate. It is also applicable to the fertilizers containing such chemical compounds as urea, nitrolime and organic matters that digest by heating and isolate ammonia.

Add copper sulfate - silver nitrate solution, calcium hydroxide and basic magnesium carbonate to an analytical sample, extract nitrate nitrogen (N-N) as well as removing chloride and organic matters, and measure the absorbance of nitro phenol ammonium sulfate formed by the reaction with phenol sulfuric acid and ammonia solution.

(2) Reagents: Reagents are as shown below:

- **a) Nitrate standard solution (N-N 5 mg/mL):** Heat potassium nitrate (no less than 99.9 (%) (mass fraction) in purity) at 110 ºC for no less than 1 hour, and after standing to cool in a desiccator, transfer 36.09 g to a weighing dish. Dissolve it in a small amount of water, move it into a 1,000-mL volumetric flask and add water up to the marked line (1).
- **b) Nitrate standard solution (N-N 0.05 mg/mL):** Dilute predetermined volume of nitrate standard solution (N-N 5 mg/mL) with water to prepare nitrate standard solution (N-N 0.05 mg/mL).
- **c) Copper sulfuric - silver sulfuric solution (1):** Dissolve 5 g of copper (II) sulfate pentahydrate specified JIS K 8983 in 900 mL of water, and dissolve while adding 4 g of silver sulfate specified in JIS K 8965 to make 1,000 mL (2).
- **d) Phenol sulfuric acid:** Dissolve 15 g of phenol sulfuric acid specified in JIS K 8798 in 100 mL of sulfuric acid specified in JIS K 8965 while heating, and then let it stand to cool (2).
- **e) Ammonia solution:** A JIS Guaranteed (NH_3 28 % (mass fraction)) reagent specified in JIS K 8085 or a reagent of equivalent quality.
- **f) Calcium hydroxide:** A JIS Guaranteed reagent specified in JIS K 8575 or a reagent of equivalent quality.
- **g) Basic magnesium carbonate:** Basic magnesium carbonate that contains no nitrate nitrogen.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) Store in an amber bottle.

(3) Instruments: Instruments are as shown below:

- **a) Rotary shaker:** A rotary shaker that can rotate a 250-mL volumetric flask upside down at 30 - 40 revolutions/min.
- **b) Spectrophotometer:** A spectrophotometer specified in JIS K 0115
- **c) Water bath:** Water bath that can be adjusted to no less than 80 ºC

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

- **a)** Weigh 1 g of an analytical sample to the order of 1 mg and transfer to a 250-mL volumetric flask.
- **b)** Add about 200 mL of copper sulfuric - silver sulfuric solution and shake to mix at 30 - 40 revolutions /min for about 20 minutes.
- **c)** Add about 1 g of calcium hydroxide and about 1 mg of basic magnesium carbonate and shake to mix at 30 - 40 revolutions /min for about 10 minutes.
- **d)** Add water up to the marked line.
- **e)** Filter with Type 3 filter paper to make the sample solution (3).

Note **(3)** As soon as sample solution is prepared, conduct the procedure in **(4.2 a)**.

Comment 1 If the filtrate of **(4.1) e)** is colored, add no more than 0.5 g of active carbon and filter with Type 3 filter paper to make the sample solution.

(4.2) **Coloring:** Conduct coloring as shown below.

a) Transfer a predetermined volume (the equivalents of 0.01 mg - 0.1 mg as N-N) into a small evaporating dish (4).

b) Evaporate water until dry on a water bath at no less than 80 ºC.

c) After standing to cool, swiftly add 2 mL of phenol sulfuric acid (5) and then rotate the evaporating dish so that the whole residue comes in contact with the acid.

d) After leaving at rest for about 10 minutes, add 20 mL of water (6).

e) After standing to cool, transfer it with water to a 100- mL volumetric flask.

f) Add ammonia solution (1+2), until the color of solution changes to light yellow, to allow it to be weak alkalinity, and further add 3 mL of ammonia solution (1+3) (7).

g) After standing to cool, add water up to the marked line and leave at rest for 30 minutes.

Note **(4)** A round-bottom glass or porcelain evaporating dish is preferable.

(5) Add at the center of a small evaporating dish with Komagome pipet.

(6) If residue does not dissolve easily, grind it with a glass rod.

(7) As no color appears from a blank test solution for calibration curve preparation, add almost the same volume of ammonia solution (1+2) as the nitrate standard solution.

(4.3) **Measurement:** Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of spectrophotometer:** Set up the measurement conditions of spectrophotometer considering the following.

Detection wavelength: 410 nm

b) **Preparation of calibration curve**

1) Transfer 1 mL - 10 mL of nitrate standard solution (N-N 0.05 mg/mL) to small evaporating dishes (4) step-by-step.

2) Conduct the same procedure as **(4.2) b) - g)** to make the nitrate standard solution for the calibration curve preparation.

3) Transfer 40 mL of water to a 100-mL volumetric flask, and shake to mix while gently adding 2 mL of phenol sulfuric acid. Let it stand to cool and conduct the same procedure as **(4.2) f) - g)** to make the blank test solution for calibration curve.

4) Measure absorbance at wavelength 410 nm of the nitrate standard solution for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control.

5) Prepare the calibration curve of the nitrate nitrogen (N-N) and absorbance of the nitrate standard solutions for the calibration curve preparation.

c) **Measurement of a sample**

1) Regarding the solution in **(4.2) g),** measure absorbance by the same procedure as **b) 4)**.

2) Obtain the nitrate nitrogen (N-N) content from the calibration curve and calculate nitrate nitrogen (N-N) in the analytical sample.

Comment 2 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for nitrate nitrogen hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2009	Ordinary compound fertilizer	26	3.67	0.09	2.3
2012	Fluid mixed fertilizer	25	2.19	0.10	4.4

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.52 - 55, Yokendo, Tokyo (1988)

(5) Flow sheet for nitrate nitrogen: The flow sheet for nitrate nitrogen in fertilizers is shown below.

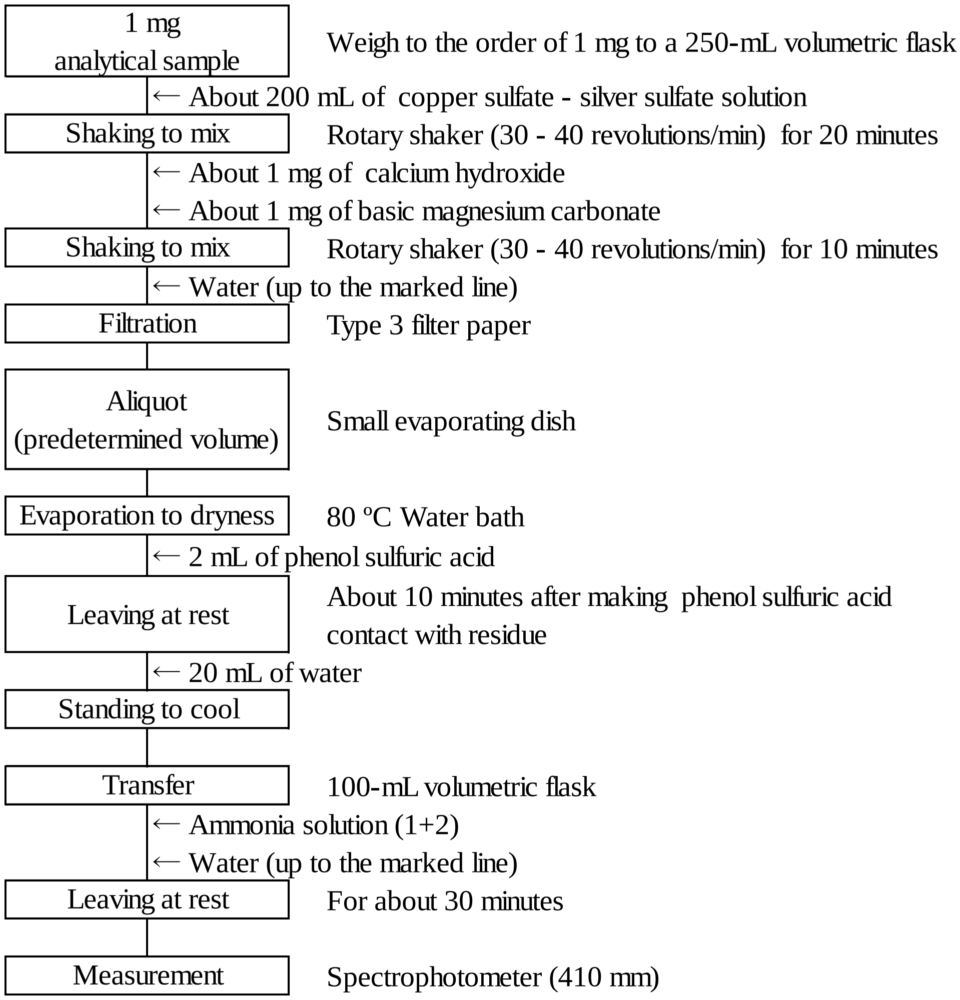


Figure The flow sheet (example) for nitrate nitrogen in fertilizers

4.2 Phosphate

4.2.1 Total phosphate

4.2.1.a Ammonium vanadomolybdate absorptiometric analysis

(1) Summary
This test method is applicable to fertilizers containing organic matters.
Add sulfuric acid, potassium sulfate and copper (II) sulfate pentahydrate to an analytical sample. Pretreat by Kjeldahl method or incineration-hydrochloric acid boiling to convert total phosphorus to phosphate ion, and measure the absorbance of phosphovanadomolybdate salt formed by the reaction with ammonium vanadate (V), hexaammonium heptamolybdate and nitric acid, to obtain total phosphate (T-P_2O_5).

(2) Reagents: Reagents are as shown below:

- **a) Sulfuric acid:** A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.
- **b) Nitric acid:** A JIS Guaranteed Reagent (HNO_3 60 % (mass fraction)) specified in JIS K 8541 or a reagent of equivalent quality.
- **c) Ammonia solution:** A JIS Guaranteed Reagent (NH_3 28 % (mass fraction)) specified in JIS K 8085 or a reagent of equivalent quality.
- **d) Catalyst(1):** Mix potassium sulfate specified in JIS K 8962 and copper (II) sulfate pentahydrate(2) specified in JIS K 8983 in the ratio of 9 to 1.
- **e) Coloring reagent solution(3) (4):** Dissolve 1.12 g of ammonium vanadate (V)(5) specified in JIS K 8747 in water, add 250 mL of nitric acid, then add 27 g of hexaammonium heptamolybdate tetrahydrate(6) specified in JIS K 8905 dissolved in water, and further add water to make 1,000 mL(7).
- **f) Phenolphthalein solution (1 g/100 mL):** Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.
- **g) Phosphate standard solution (P_2O_5 10 mg/mL)(3):** Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1,000-mL volumetric flask, add 2 mL-3 mL of nitric acid, and add water up to the marked line.
- **h) Phosphate standard solution (P_2O_5 0.5 mg/mL)(3):** Transfer 50 mL of phosphate standard solution (P_2O_5 10 mg/mL) to a 1,000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

Note **(1)** A tablet is commercially available.
(2) Crush into powder as appropriate.
(3) This is an example of preparation; prepare an amount as appropriate.
(4) This corresponds to reagent “a” solution in the Official Methods of Analysis of Fertilizers (1992).
(5) This corresponds to ammonium metavanadate in the Official Methods of Analysis of Fertilizers (1992).
(6) This corresponds to ammonium molybdate in the Official Methods of Analysis of Fertilizers (1992).
(7) Store in an amber bottle.

(3) Apparatus and instruments: Apparatus and instruments are as follows:

- **a) Spectrophotometer:** A spectrophotometer specified in JIS K 0115.
- **b) Electric furnace:** An electric furnace that can be adjusted to 550 ºC ± 5 ºC.

c) **Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.
d) **Digestion flask:** Kjeldahl flask

(4) Test procedures
(4.1) Sample solution preparation: Prepare a sample solution as shown below:
(4.1.1) Kjeldahl method
a) Weigh 0.5 g - 5 g of an analytical sample to the order of 1 mg, and put it in a 300-mL digestion flask.
b) Add 5 g - 10 g of catalyst, and further add 20 mL - 40 mL of sulfuric acid, shake to mix and heat gently.
c) After bubbles cease to form, heat until white smoke of sulfuric acid evolves.
d) Ignite until organic matters are completely digested (8).
e) After standing to cool, add a small amount of water, mix well by shaking, transfer to a 250-mL - 500-mL volumetric flask with water, and further mix by shaking.
f) After standing to cool, add water up to the marked line.
g) Filter with Type 3 filter paper to make the sample solution.

Note (8) After the change in the color of the solution ends, heat additionally for no less than 2 hours.

(4.1.2) Incineration-hydrochloric acid boiling
a) Weigh 5 g of an analytical sample to the order of 1 mg, and transfer to a 200-mL - 300-mL tall beaker.
b) Put the tall beaker in an electric furnace, and heat gently to char (9).
c) Ignite at 550 ºC ± 5 ºC for no less than 4 hours to incinerate.
d) After standing to cool, moisten the residue with a small amount of water, gradually add about 10 mL of hydrochloric acid, and further add water to make 20 mL.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.
f) After standing to cool, transfer to a 250-mL - 500-mL volumetric flask.
g) Add water up to the marked line.
h) Filter with Type 3 filter paper to make the sample solution.

Note (9) Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.

Comment 1 The procedures in **(4.1.1)** are the same as in **(4.1)** in **4.2.1.b**. Also, the procedures in **(4.1.1) a) - f)** are the same as in **(4.1)** in **4.1.1.a**.
Comment 2 The procedures for the sample solution in **(4.1.2)** are the same as in **(4.1)** in **4.3.1.a, and 4.5.1.a.**
Comment 3 The sample solution prepared in **(4.1) a) - h)** in **4.9.1.a** can also be used.

(4.2) Coloring: Conduct coloring as follows:
a) Transfer a predetermined amount of the sample solution (the equivalents of 0.5 mg - 6 mg as P_2O_5) to a 100-mL volumetric flask.
b) Add 1-2 drop(s) of phenolphthalein solution (1 g/100 mL), and neutralize by adding ammonia solution (1+1) until the color of the solution becomes light red-purple (10).
c) Add nitric acid (1+10) until the light red-purple color of the solution disappears to make it slightly acidic, and add a proper amount of water (11).

d) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes.

Note (10) It is not necessary to add phenolphthalein solution (1 g/100 mL) when determination can be done by the color change of copper ion (light blue → blue-purple).

(11) Without the addition of water, precipitate may be produced when coloring reagent solution is added.

(4.3) Measurement: Conduct measurement according to JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used in measurement.

a) Measurement conditions for the spectrophotometer: Set up the measurement conditions for the spectrophotometer considering the following:
Detection wavelength: 420 nm

b) Calibration curve preparation

1) Transfer 1 mL - 12 mL of phosphate standard solution (P_2O_5 0.5 mg/mL) to 100-mL volumetric flasks step-by-step.

2) Add a proper amount of water (11), and conduct the same procedure as **(4.2) d)** to make the P_2O_5 0.5 mg/100 mL - 6 mg/100 mL phosphate standard solutions for the calibration curve preparation.

3) Conduct the same procedures as **2)** for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Measure absorbance at a wavelength of 420 nm of the phosphate standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (12).

5) Prepare the calibration curve of the phosphate concentration and absorbance of the phosphate standard solutions for the calibration curve preparation.

c) Sample measurement

1) Regarding the solution in **(4.2) d),** measure absorbance by the same procedure as **b) 4)** (12).

2) Obtain the phosphate (P_2O_5) content from the calibration curve, and calculate total phosphate (T-P_2O_5) in the analytical sample.

Note (12) Measure within 6 hours after adding the coloring reagent solution in the procedure in **(4.2) d)**.

Comment 4 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for nitrate nitrogen hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2007	Organic compound fertilizer	140	10.35	0.10	1.0

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by th by the formula shown below:
RSD_{rob} = (NIQR/M) × 100

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details)，p.108 - 114，Yokendo, Tokyo (1988)
2) Kimie KATO, Masayuki YOSHIMOTO and Yuji SHIRAI: Systematization of Determination Methods of Major Components in Sludge Fertilizer, Compost and Organic Fertilizer, Research Report of Fertilizer, Vol.3 2010. (107 - 116)

(5) Flow sheet for total phosphate: The flow sheets for total phosphate in fertilizers are shown below:

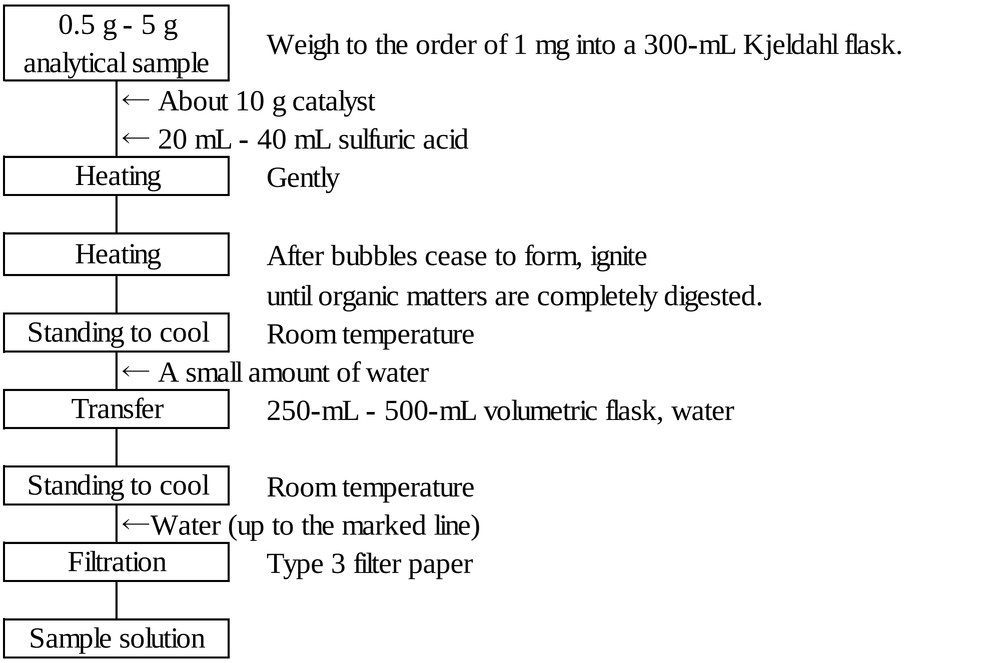


Figure 1 Flow sheet for total phosphate in fertilizers (1).
(Sample solution preparation by Kjeldahl method)

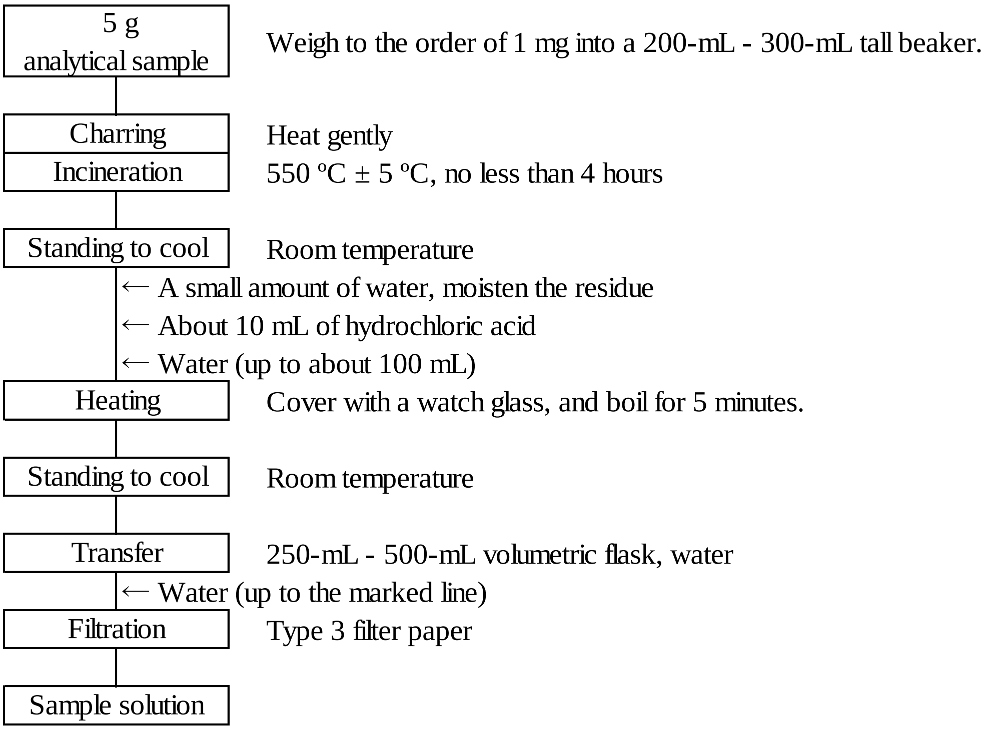


Figure 2 Flow sheet for total phosphate in fertilizers (2).
(Sample solution preparation by incineration-hydrochloric acid boiling)

Sample solution

Aliquot (predetermined amount) — 100-mL volumetric flask

← 1-2 drop(s) of phenolphthalein solution (1 g/100 mL)
← Ammonia solution (1+1) [neutralization]
← Nitric acid (1+10) [slightly acidic]
← A proper amount of water
← 20 mL of coloring reagent solution
← Water (up to the marked line)

Leaving at rest — About 30 minutes

Measurement — Spectrophotometer (420 nm)

Figure 3　Flow sheet for total phosphate in fertilizers (3).
(Measurement procedure)

4.2.1.b Quinoline gravimetric analysis

(1) Summary

This test method is applicable to fertilizers containing organic matters.
It is suitable for the fertilizers with relatively a high content of phosphate. Add sulfuric acid, potassium sulfate and copper (II) sulfate pentahydrate to an analytical sample. Pretreat by Kjeldahl method to convert total phosphate (T-P_2O_5) to phosphate ion, and measure the mass of quinonium phosphomolybdate formed by the reaction with quinoline, molybdic acid and nitric acid to obtain total phosphate (T-P_2O_5).

(2) Reagents: Reagents are as shown below:

- **a) Sulfuric acid:** A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.
- **b) Nitric acid:** A JIS Guaranteed Reagent (HNO_3 60 % (mass fraction)) specified in JIS K 8541 or a reagent of equivalent quality.
- **c) Sodium molybdate solution:** Dissolve 70 g of sodium molybdate dihydrate in 150 mL of water.
- **d) Quinoline solution:** Add 5 mL of quinolone specified in JIS K 8279 to the mixture solution of 35 mL of nitric acid and 100 mL of water.
- **e) Quimosiac solution:** Add 60 g of citric acid monohydrate specified in JIS K 8283 to the mixture solution of 85 mL nitric acid and 150 mL of water to dissolve. Add gradually total volume of the sodium molybdate solution to mix. Add gradually the total volume of the quinoline solution while mixing the solution. After leaving at rest overnight, filter the total volume with Type 3 filter paper. Add acetone specified in JIS K 8962, and further add water to make 1,000 mL.
- **f) Catalyst(1):** Mix potassium sulfate specified in JIS K 8962 and copper (II) sulfate pentahydrate(2) specified in JIS K 8983 in the ratio of 9 to 1.

Note (1) A tablet is commercially available.
(2) Crush into powder as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are as follows:

- **a) Water bath:** Water bath that can be adjusted to 60 ºC – 65 ºC.
- **b) Drying apparatus:** A drying apparatus that can be adjusted to 220 ºC±5 ºC.
- **c) Crucible type glass filter:** Put a crucible type glass filter 1G4 specified in JIS R 3503 into a drying apparatus, let it stand to cool in a desiccator after heating at 220 ºC ± 5 ºC and measure the mass to the order of 1 mg.
- **d) Digestion flask:** Kjeldahl flask

(4) Test procedures

(4.1) Kjeldahl method: Conduct digestion as shown below:

- **a)** Weigh 0.5 g - 5 g of an analytical sample to the order of 1 mg, and put it in a 300-mL digestion flask.
- **b)** Add 5 g - 10 g of catalyst, and further add 20 mL - 40 mL of sulfuric acid to shake to mix, and heat gently.
- **c)** After bubbles cease to form, heat until white smoke of sulfuric acid evolves.
- **d)** Ignite temperature until organic matters are completely digested (3).
- **e)** After standing to cool, add a small amount of water, mix well by shaking, and transfer to a 250-mL - 500-mL volumetric flask with water.
- **f)** After standing to cool, add water up to the marked line.
- **g)** Filter with Type 3 filter paper to make the sample solution.

Note (3) After the change in the color of the solution ends, heat additionally for no less than 2 hours.

Comment 1 The procedures in **(4.1)** are the same as in **(4.1.1)** in **4.2.1.a**. In addition, the sample solution prepared in **(4.1.2)** in **4.2.1.a** and **(4.1) a) - h)** in **4.9.1.a** can also be used.

(4.2) Measurement: Conduct measurement as shown below.

a) Transfer a predetermined volume (the equivalents of 10 mg - 30 mg as P_2O_5 and no more than 5 mL as sulfuric acid) of sample solution to a 300-mL tall beaker.

b) Add 5 mL of nitric acid and add water to make about 80 mL.

c) Cover with a watch glass and wash the watch glass and inside the tall beaker with water after boiling for about 3 minutes, and then add water to make about 100 mL.

d) Immediately, add 50mL of quimosiac solution, heat for about 15 minutes while sometimes mixing in a water bath at 60 ºC - 65 ºC to produce the precipitate of quinolyum molybdate.

e) After standing to cool down to room temperature while sometimes mixing, filter under reduced pressure with a crucible type glass filter, wash the tall beaker 3 times with water and transfer the whole precipitate into a crucible type glass filter, and further wash 7 - 8 times with water.

f) Transfer the precipitate together with the crucible type glass filter into a drying apparatus and heat at 220 ºC ± 5 ºC for about 30 minutes.

g) As soon as heating is complete, move it into a desiccator and let it stand to cool.

h) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.

i) Calculate total phosphate (T-P_2O_5) in the analytical sample by the following formula.

Total phosphate (T-P_2O_5) (% (mass fraction)) in an analytical sample

$$= A \times 0.03207 \times (V_1/V_2) \times (1/B) \times 100$$

A: Mass (g) of the precipitate in **h)**

B: Mass of an analytical sample

V_1: Predetermined volume (mL) of the sample solution

V_2: Volume (mL) of the sample solution transferred in **a)**

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.98 - 106, Yokendo, Tokyo (1988)

(5) Flow sheet for total phosphate: The flow sheet for total phosphate in fertilizers is shown below:

0.5 g- 5 g analytical sample	Weigh to the order of 1 mg into a 300-mL digestion flask.
	← 5 g - 10 g catalyst
	← 20 mL - 40 mL sulfuric acid
Heating	Gently
Heating	After bubbles cease to form, ignite until organic matters are completely digested
Standing to cool	Room temperature
	← Small mount of water
Transfer	250-mL - 500-mL volumetric flask, water
	← Water (up to the marked line)
Filtration	Type 3 filter paper
Aliquot (predetermined volume)	300-mL tall beaker
	← 5 mL nitrate acid
	← Water (to reach about 100 mL)
Boiling	Cover with a watch glass for 3 minutes Wash a watch glass and inside the tall beaker with water
	← Water (to be about 100 mL)
	← Quimosiac solution 50 mL
Produce precipitate	60 ºC - 65 ºC, for 15 minutes, sometimes mixing
Standing to cool	Room temperature
Filtration under reduced pressure	Crucible type glass filtering apparatus 1G4, 3 times with water
Washing	Wash 7 - 8 times with water
Drying	220 ºC ± 5 ºC for about 30 minutes
Standing to cool	Desiccator
Measurement	Measure the mass to the order of 1 mg

Figure The flow sheet for total phosphate in fertilizers

4.2.2 Soluble phosphoric acid

4.2.2.a Ammonium vanadomolybdate absorptiometric analysis

(1) Summary

This test method is applicable to fertilizers that do not contain the matter not colored by the hydrolysis with nitrate acids such as phosphonic acid.

Extract by adding water to an analytical sample, then extract by adding ammonium citric acid solution, and combine them to make ammonia alkaline ammonium citrate soluble phosphoric acid (soluble phosphoric acid (S-P_2O_5)) and further heat after adding nitric acid (1+1), hydrolyze water-soluble phosphoric acid to orthophosphate ion and measure the absorbance of phosphovanadomolybdate salt formed by the reaction with ammonium vanadate (V), hexaammonium heptamolybdate and nitric acid to obtain soluble phosphoric acid (S-P_2O_5).

(2) Reagents: Reagents are as shown below:

a) Nitric acid: A JIS Guaranteed Reagent (HNO_3 60 % (mass fraction)) specified in JIS K 8541 or a reagent of equivalent quality.

b) Ammonia solution: A JIS Guaranteed Reagent (NH_3 28 % (mass fraction)) specified in JIS K 8085 or a reagent of equivalent quality.

c) Petermans citrate solution: Add 173 g of citric acid monohydrate specified in JIS K 8283 in water to dissolve and add gradually ammonia solution equivalent to 42 g of nitrogen while cooling. After standing to cool, add water to make 1,000 mL. Additionally, check that the specific gravity of the solution is 1.082 - 1.083 (15 ºC) and the nitrogen content per 1 mL is 42 mg.

d) Coloring reagent solution(1) (2): Dissolve 1.12 g of ammonium vanadate (V)(3) specified in JIS K 8747 in water, add 150 mL of nitric acid, then add 50 g of hexaammonium heptamolybdate tetrahydrate(4) specified in JIS K 8905 while dissolving in water, and further add water to make 1,000 mL.(5)

e) Phosphate standard solution (P_2O_5 10 mg/mL)(1): Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve with a small amount of water, transfer to a 1,000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

f) Phosphate standard solution (P_2O_5 0.5 mg/mL)(1): Transfer 50 mL of phosphate standard solution (P_2O_5 10 mg/mL) to a 1,000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) This corresponds to reagent “b” solution in the Official Methods of Analysis of Fertilizers (1992).

(3) This corresponds to ammonium metavanadate in the Official Methods of Analysis of Fertilizers (1992).

(4) This corresponds to ammonium molybdate in the Official Methods of Analysis of Fertilizers (1992).

(5) Store in an amber bottle. However the reagent solution cannot tolerate long term preservation.

Comment 1 The coloring reagent solution in **d)** can also be prepared by the following method.

Dissolve 2.24 g of ammonium vanadate (V) (3) specified in JIS K 8747 in water, add 300 mL of nitric acid, and add water to make 1,000 mL. Separately, add 100 g of hexaammonium heptamolybdate tetrahydrate (4) specified in JIS

K 8905 while dissolving in water, and further add water to make 1,000 mL. In the case of usage, mix equal volumes of the two solutions.

(3) Instruments: Instruments are as follows:

a) Water bath: Water bath that can be adjusted to the test temperature ± 25 ºC.

b) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

c) Spectrophotometer: A spectrophotometer specified in JIS K 0115.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a small mortar.

b) Add about 20 mL - 25 mL of water, grind well and filter the supernatant with Type 6 filter paper into a 250-mL volumetric flask.

c) Further, after repeating procedure in **b)** 3 times, transfer non-dissolved matter in the small mortar onto a filter paper and wash with water until the filtrate becomes about 200 mL.

d) Add a small amount of nitric acid to the filtrate, and further add water up to the marked line to make the sample solution (1).

e) Transfer the non-dissolved matter on the filter paper together with the filter paper to another 250- mL volumetric flask(6), add 100 mL of Petermans citrate solution and stopple, then shake to mix until the filter paper breaks down.

f) Heat the volumetric flask in **e)** in water bath at 65 ºC ± 2 ºC for 1 hour while shaking to mix every 15 minutes.

g) After standing to cool, add water up to the marked line.

h) Filter with Type 6 filter paper to make the sample solution (2).

Note (6) It is recommended to use a 250-mL short-neck volumetric flask.

Comment 2 The procedure in **(4.1)** is the same as the procedure in **(4.1)** of **4.2.2.b**.

Comment 3 When the determination is affected by the coloring of the sample solution of **d)** and **h)**, transfer the predetermined volume (equivalent volume) (7) of the sample solution (1) and the sample solution (2) to a 100-mL volumetric flask, add a few drops of hydrochloric acid (1+1) to acidify, then add no more than 0.1 g of active carbon. After leaving at rest for a little while, add water up to the marked line and filter. The filtrate is to be the mixture solution for the sample solution of **(4.2) a)**. Additionally, as phosphorus contained in active carbon has the possibility to elute and affects the determination value, a blank test is required.

(4.2) Coloring: Conduct coloring as follows:

a) Transfer a predetermined amount (the equivalents of 0.5 mg - 6 mg as P_2O_5 and no more than the equivalents of 2 mL of Petermans citrate solution)(7) of sample solution (1) and sample solution (2) to a 100-mL tall beaker.

b) Add the solution to make Petermans citrate solution equivalent to 2 mL.

c) Add 4 mL of nitric acid (1+1) (8), and heat to boil (9).

d) After standing to cool, transfer it to a 100- mL volumetric flask with water (10).

e) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes (8).

Comment 4 In the procedure in **a)**, a 100-mL volumetric flask can be used instead of a 100-mL tall beaker. However the volumetric flask used should be distinguished as a flask to be used for phosphate coloring operation and should not be used for other purposes. Additionally, "transfer to a 100-mL volumetric flask with water" in **d)** is replaced by "add a proper amount of water (11)".

Note **(7)** The transferred volume of sample solution (1) and sample solution (2) should be equivalent.

(8) When the solution is muddled by adding nitric acid (1+1), conduct centrifugation after the procedure in **e)**.

(9) When it does not contain non-orthophosphate, the boiling operation is not necessary.

(10) The volume of solution after transferring should be up to about 60 mL.

(11) If no water is added, precipitate is produced in some cases when coloring reagent solution is added.

(4.3) **Measurement:** Conduct measurement according to JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used in measurement.

a) **Measurement conditions for the spectrophotometer:** Set up the measurement conditions for the spectrophotometer considering the following:
Detection wavelength: 420 nm

b) **Calibration curve preparation**

1) Transfer 1 mL - 12 mL of phosphate standard solution (P_2O_5 0.5 mg/mL) to 100-mL volumetric flasks step-by-step.

2) Add 2 mL of Petermans citrate solution, 4 mL of nitric acid (1+1) and a proper amount of water(11), and conduct the same procedure as **(4.2) e)** to make the P_2O_5 0.5 mg/100 mL - 6 mg/100 mL phosphate standard solutions for the calibration curve preparation.

3) Conduct the same procedures as **2)** for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Measure absorbance at a wavelength of 420 nm of the phosphate standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (12).

5) Prepare the calibration curve of the phosphate concentration and absorbance of the phosphate standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Regarding the solution of **(4.2) e),** measure the absorbance by the same procedure as **b) 4)** (12).

2) Obtain the phosphate (P_2O_5) content from the calibration curve, and calculate soluble phosphoric acid ($S\text{-}P_2O_5$) in the analytical sample.

Note **(12)** Measure within 2 hours after adding the coloring reagent solution.

Comment 5 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from a simultaneous analysis with the same samples for soluble phosphoric acid hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2006	High analysis compound fertilizer	144	10.88	0.11	1.0
2009	Ordinary compound fertilizer	124	6.37	0.12	1.9
2011	High analysis compound fertilizer	113	17.44	0.22	1.3

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
$RSD_{rob} = (NIQR/M) \times 100$

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.108 - 114, Yokendo, Tokyo (1988)

(5) Flow sheet for soluble phosphoric acid: The flow sheet for soluble phosphoric acid in fertilizers is shown below:

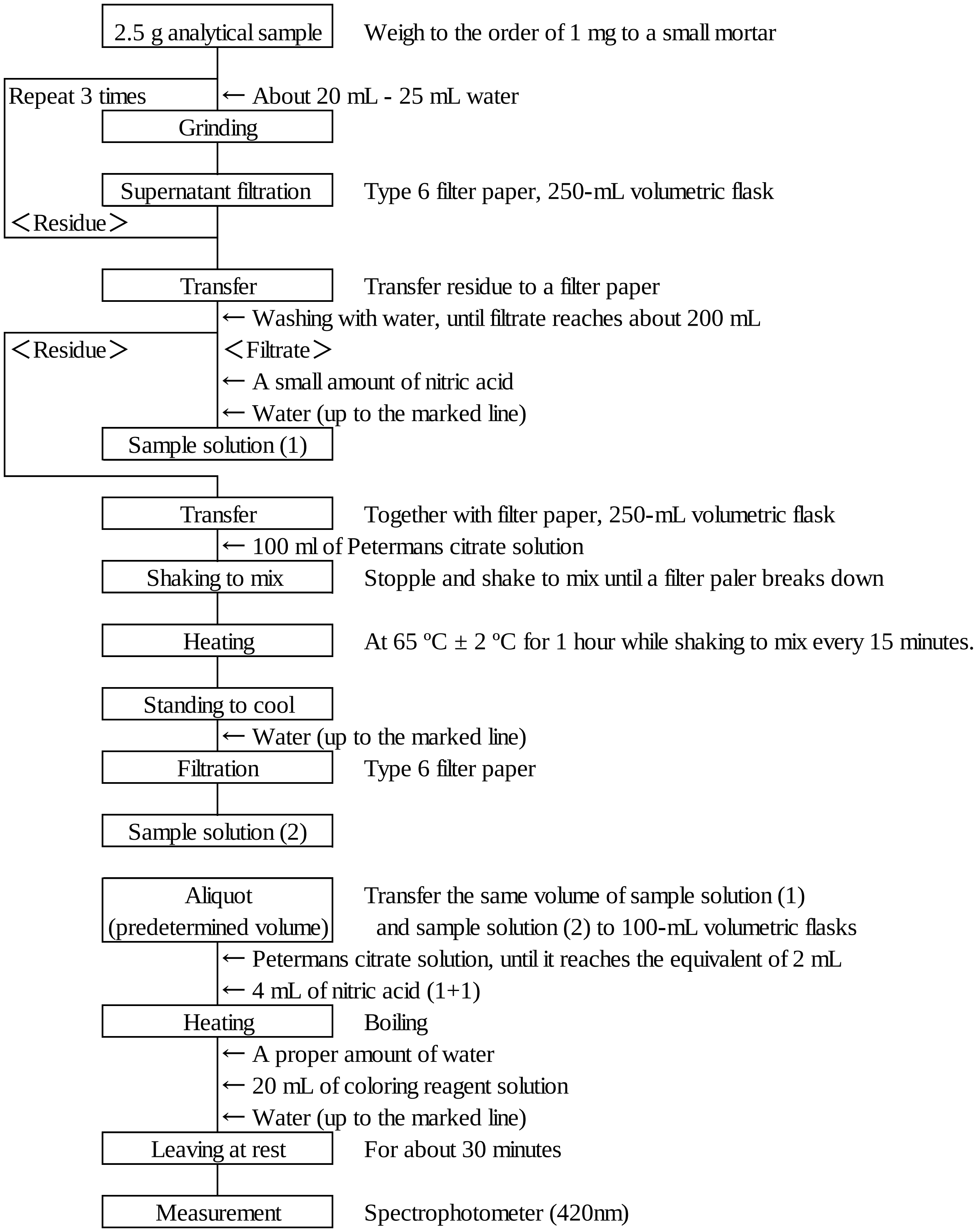


Figure The flow sheet for soluble phosphoric acid in fertilizers

4.2.2.b Quinoline gravimetric analysis

(1) Summary

This test method is applicable to fertilizers containing no phosphonic acid, etc.
It is suitable for the fertilizers containing a relatively a high content of phosphate.
Extract by adding water to an analytical sample, then extract by adding ammonium citric acid solution and combine them to make ammonia alkaline ammonium citrate soluble phosphoric acid (soluble phosphoric acid (S-P_2O_5)) and measure the mass of quinolium molybdate formed by the reaction with quinoline, molybdic acid and nitric acid to obtain soluble phosphoric acid (S-P_2O_5).

(2) Reagents: Reagents are as shown below:

a) Nitric acid: A JIS Guaranteed Reagent (HNO_3 60 % (mass fraction)) specified in JIS K 8541 or a reagent of equivalent quality.

b) Ammonia solution: A JIS Guaranteed Reagent (NH_3 28 % (mass fraction)) specified in JIS K 8085 or a reagent of equivalent quality.

c) Petermans citrate solution: Dissolve 173 g of citric acid monohydrate specified in JIS K 8283 by adding to water and add gradually ammonia solution equivalent to 42 g of nitrogen while cooling. After standing to cool, add water to make 1,000 mL. Additionally, check that the specific gravity of the solution is 1.082 -1.083 (15 ºC) and the volume of nitrogen per 1 mL is 42 mg.

d) Sodium molybdate solution: Dissolve 70 g of sodium molybdate dihydrate in 150 mL of water.

e) Quinoline solution: Add 5 mL of quinoline specified in JIS K 8279 to the mixture solution of 35 mL of nitric acid and 100 mL of water.

f) Quimosiac solution: Add 60 g of citric acid monohydrate specified in JIS K 8283 to the mixture solution of 85 mL nitric acid and 150 mL of water to dissolve. Add gradually total volume of the sodium molybdate solution to mix them. Add gradually the total volume of the quinoline solution while mixing the solution. After leaving at rest overnight, filter the total volume with Type 3 filter paper. Add 280 mL of acetone specified in JIS K 8962, and further add water to make 1,000 mL.

(3) Apparatus and instruments: Apparatus and instruments are as follows:

a) Water bath: A water bath that can be adjusted to the test temperature ± 2 ºC.

b) Drying apparatus: A drying apparatus that can be adjusted to 220 ºC ± 5 ºC.

c) Crucible type glass filter: Put a crucible type glass filter 1G4 specified in JIS R 3503 into a drying apparatus, stand to cool in a desiccator after heating at 220 ºC ± 5 ºC and measure the mass to the order of 1 mg.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a small mortar.

b) Add about 20 mL - 25 mL of water, grind well and filter the supernatant with Type 6 filter paper into a 250-mL volumetric flask.

c) Further, after repeating 3 times the procedure in **b)**, transfer non-dissolved matter in the small mortar on a filter paper and wash with water until the filtrate becomes about 200 mL.

d) Add a small amount of nitric acid to the filtrate, and further add water up to the marked line to make the sample solution (1).

e) Transfer the non-dissolved matter on the filter paper together with the filter paper to another 250- mL [(1)] volumetric flask, and add 100 mL of Petermans citrate solution and stopple. Then shake to mix until the filter paper breaks down.

f) Heat the volumetric flask in **e)** in a water bath at 65 ºC ± 2 ºC for 1 hour while shaking to mix every 15 minutes.
g) After standing to cool, add water up to the marked line.
h) Filter with Type 6 filter paper to make the sample solution (2).

Note (1) It is recommended to use a short-neck volumetric flask.

Comment 1 The procedure in**(4.1)** is the same as the procedure in **(4.1)** of **4.2.2.**a.

(4.2) Measurement: Conduct measurement as shown below.
a) Transfer a predetermined volume (the equivalents of 10 mg - 30 mg as P_2O_5 and no more than the equivalents of 8 mL of Petermans citrate solution) (2) of sample solution (1) and sample solution (2) to a 300-mL tall beaker.
b) Add 5 mL of nitric acid and add water to make about 80 mL.
c) Cover with a watch glass. After boiling for about 3 minutes, wash the watch glass and the inside of the tall beaker with water and add water to make about 100 mL.
d) Immediately, add 50 mL of quimosiac solution, heat for about 15 minutes while sometimes mixing in a water bath at 60 ºC - 65 ºC and produce the precipitate of quinolyum molybdate.
e) After standing to cool down to room temperature while sometimes mixing, filter under reduced pressure with a crucible type glass filter, wash the tall beaker 3 times with water and transfer the whole precipitate into a crucible type glass filter, further wash 7 - 8 times with water.
f) Transfer the precipitate together with the crucible type glass filter into a drying apparatus and heat at 220 ºC ± 5 ºC for about 30 minutes.
g) As soon as heating is complete, move it into a desiccator and let it stand to cool.
h) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.
i) Calculate soluble phosphoric acid (S-P_2O_5) by the following formula.

Soluble phosphoric acid (% (mass fraction)) in an analytical sample
$= A \times 0.03207 \times (V_1/V_2) \times (1/B) \times 100$

A: Mass (g) of the precipitate in **h)**
B: Mass of an analytical sample (2.5 g)
V_1: Predetermined volume (250 mL) of the sample solution
V_2: Volume (mL) of the sample solution transferred in **a)**

Note (2) The transferred volume of sample solution (1) and the sample solution (2) should be equivalent.

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.98 - 106, Yokendo, Tokyo (1988)

(5) Flow sheet for soluble phosphoric acid: The flow sheet for soluble phosphoric acid in fertilizers is shown below:

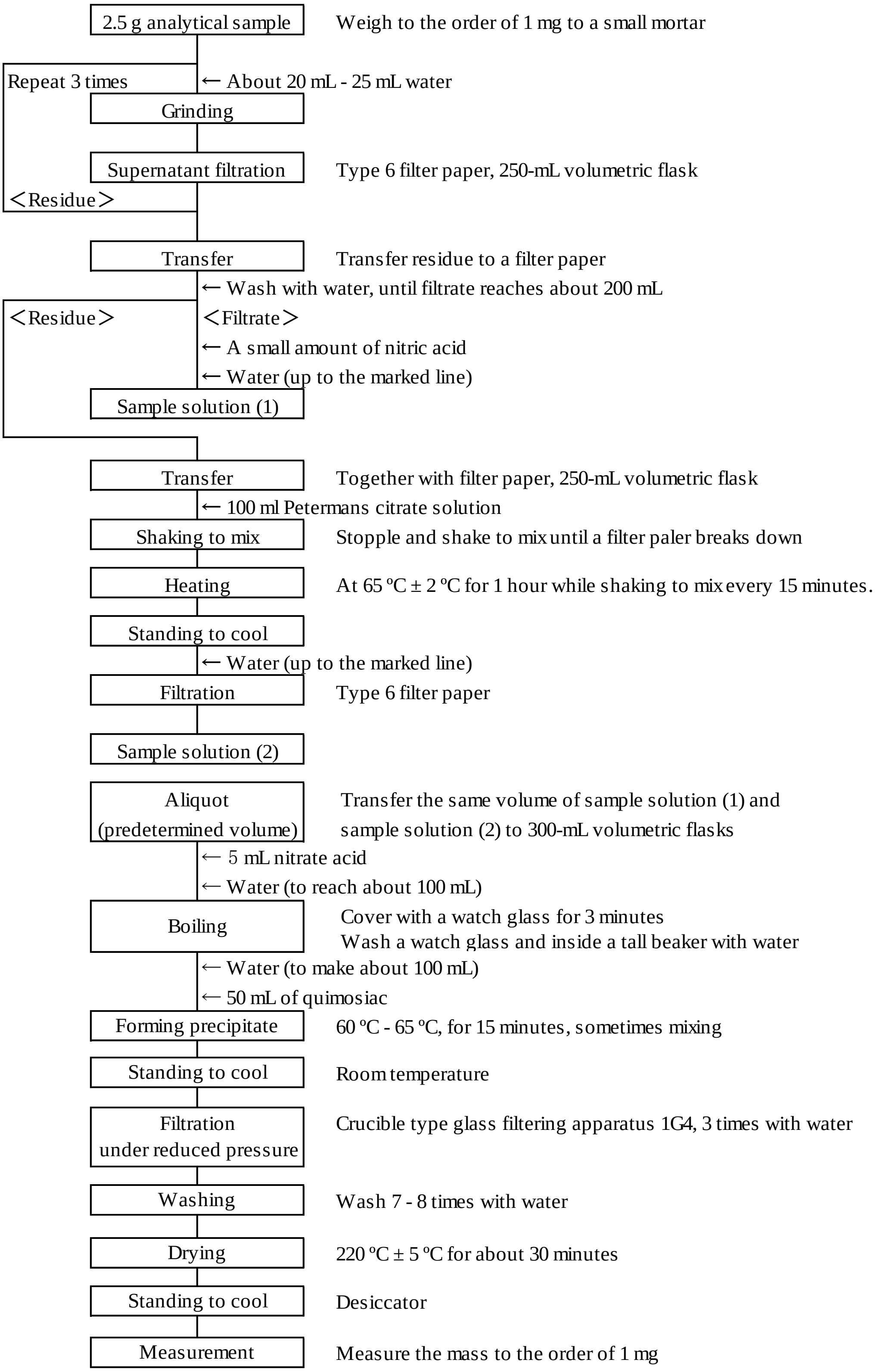


Figure The flow sheet for soluble phosphoric acid in fertilizers

4.2.3 Citrate soluble phosphoric acid

4.2.3.a Ammonium vanadomolybdate absorptiometric analysis

(1) Summary

This test method is applicable to the fertilizers that do not contain the matter not colored by the hydrolysis with nitrate acids such as phosphonic acid.

Extract by adding citric acid solution to an analytical sample, and hydrolyze citrate soluble phosphoric acid (C-P_2O_5) to orthophosphate ion and measure the absorbance of phosphovanadomolybdate salt formed by the reaction with ammonium vanadate (V), hexaammonium heptamolybdate and nitric acid to obtain citrate soluble phosphoric acid (C-P_2O_5).

(2) Reagents: Reagents are as shown below:

- **a) Nitric acid:** A JIS Guaranteed Reagent (HNO_3 60 % (mass fraction)) specified in JIS K 8541 or a reagent of equivalent quality.
- **b) Citric acid solution (1):** Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1,000 mL.
- **c) Coloring reagent solution(1) (2):** Dissolve 1.12 g of ammonium vanadate (V)(3) specified in JIS K 8747 in water, add 150 mL of nitric acid, then add 50 g of hexaammonium heptamolybdate tetrahydrate(4) specified in JIS K 8905 while dissolving in water, and further add water to make 1,000 mL(5).
- **d) Phosphate standard solution (P_2O_5 10 mg/mL)(1):** Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve with a small amount of water, transfer to a 1,000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.
- **e) Phosphate standard solution (P_2O_5 0.5 mg/mL)(1):** Transfer 50 mL of phosphate standard solution (P_2O_5 10 mg/mL) to a 1,000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(2) This corresponds to reagent “b” solution in the Official Methods of Analysis of Fertilizers (1992).

(3) This corresponds to ammonium metavanadate in the Official Methods of Analysis of Fertilizers (1992).

(4) This corresponds to ammonium molybdate in the Official Methods of Analysis of Fertilizers (1992).

(5) Store in an amber bottle. However the reagent solution does not tolerate long term preservation.

Comment 1 The coloring reagent solution in **c)** can also be prepared by the following method.

Dissolve 2.24 g of ammonium vanadate (V) (3) specified in JIS K 8747 in water, add 300 mL of nitric acid, and add water to make 1,000 mL. Separately, dissolve 100 g of hexaammonium heptamolybdate tetrahydrate (4) specified in JIS K 8905 in water, and further add water to make 1,000 mL. Mix equal volume of two solutions in the case of usage.

(3) Instruments: Instruments are as follows:

a) **Constant-temperature rotary shaker:** A constant-temperature rotary shaker that can rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, upside down at 30 - 40 revolutions/min.
b) **Hot plate:** A hot plate whose surface temperature can be adjusted up to 250 ºC.
c) **Spectrophotometer**: A spectrophotometer specified in JIS K 0115.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.
b) Add 150 mL of citric acid solution heated up to about 30 ºC, and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.
c) After standing to cool, add water up to the marked line.
d) Filter with Type 3 filter paper to make the sample solution.

Comment 2 The procedure in **(4.1)** is the same as the procedure in **(4.1)** of **4.2.3.b**, **4.3.2.a**, **4.3.2.b**, **4.6.2.a**, **4.7.2.a** and **4.8.1.a**.

Comment 3 When the determination is affected by the coloring of the sample solution of **d)**, transfer a predetermined volume of the sample solution to a 100-mL volumetric flask, add a few drops of hydrochloric acid (1+1) to make it acidity, and then add no more than 0.1 g of active carbon. After leaving at rest for a little while, add water up to the marked line and filter. The filtrate is prepared as the sample solution of **(4.2) a)**. Additionally, as phosphorus contained in active carbon has the possibility to elute and affects the determination value, a blank test is required.

Comment 4 For a by-product phosphate fertilizer or a fertilizer containing a by-product phosphate, if pH of the sample solution in **d)** is neutral or basic, prepare a sample solution anew by replacing “1 g of an analytical sample” in the procedure in **a)** with “0.5 g of an analytical sample”.

(4.2) Coloring: Conduct coloring as follows:

a) Transfer a predetermined amount (the equivalents of 0.5 mg - 6 mg as P_2O_5 and no more than the equivalents of 17 mL of the citric acid solution) of the sample solution to a 100-mL tall beaker.
b) Add the citric acid solution to make the equivalents of 17 mL of the citric acid solution (20 mg/mL).
c) Add 4 mL of nitric acid (1+1) (6), and heat to boil (7).
d) After standing to cool, transfer to a 100- mL volumetric flask with water (8).
e) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes.

Comment 5 In the procedure in **a)**, a 100-mL volumetric flask can be used instead of a 100-mL tall beaker. However the volumetric flask used should be distinguished as a flask to be used for phosphate coloring operation and should not be used for other purposes. Additionally, “transfer to a 100-mL volumetric flask with water” in **d)**” is replaced by “add a proper amount of water (9)”.

Note (6) When the solution is muddled by adding nitric acid (1+1), conduct centrifugation after the procedure in **e)**.

(7) When it does not contain non-orthophosphate, the boil operation is not necessary.
(8) The volume of solution after transferring should be up to about 60 mL.
(9) If no water is added, precipitate is produced in some cases when a coloring reagent solution is added.

(4.3) Measurement: Conduct measurement according to JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used in measurement.

a) Measurement conditions for the spectrophotometer: Set up the measurement conditions for the spectrophotometer considering the following:
Detection wavelength: 420 nm

b) Calibration curve preparation

1) Transfer 1 mL - 12 mL of phosphate standard solution (P_2O_5 0.5 mg/mL) to 100-mL volumetric flasks step-by-step.
2) Add 17 mL of citric acid solution, then add 4 mL of nitric acid (1+1), further add a proper amount of water(9) and conduct the same procedure as **(4.2) e)** to make the P_2O_5 0.5 mg/100 mL - 6 mg/100 mL phosphate standard solution for the calibration curve preparation.
3) Conduct the same procedures as **2)** for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.
4) Measure absorbance at a wavelength of 420 nm of the phosphate standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (10).
5) Prepare the calibration curve of the phosphate concentration and absorbance of the phosphate standard solutions for the calibration curve preparation.

c) Sample measurement

1) Regarding the solution in **(4.2) e),** measure the absorbance by the same procedure as **b) 4)** (10).
2) Obtain the phosphate (P_2O_5) content from the calibration curve, and calculate citrate soluble phosphoric acid ($C\text{-}P_2O_5$) in the analytical sample.

Note (10) Measure within 2 hours after adding the coloring reagent solution.

Comment 6 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for nitrate nitrogen hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2007	Organic compound fertilizer	143	9.81	0.13	1.3
2008	High analysis compound fertilizer	144	15.82	0.13	0.8
2010	High analysis compound fertilizer	140	14.59	0.18	1.2
2013	High analysis compound fertilizer	128	20.60	0.19	0.9

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.108 - 114, Yokendo, Tokyo (1988)

(5) Flow sheet for citrate soluble phosphoric acid: The flow sheet for citrate soluble phosphoric acid in fertilizers is shown below:

1 g analytical sample	Weigh to the order of 1 mg to a 250-mL volumetric flask
← 150 mL of citric acid solution [about 30 ºC]	
Shaking to mix	Constant-temperature rotary shaker (30 -40 revolutions/min) 30 ºC ± 1 ºC, for 1 hour
Standing to cool	
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined volume)	100-mL volumetric flask
← Citric acid solution, until it reaches the equivalents of 17 mL	
← 4 mL of nitric acid(1+1)	
Heating	Boiling
← A proper amount of water	
← 20 mL of coloring reagent solution	
← Water (up to the marked line)	
Leaving at rest	For about 30 minutes
Measurement	Spectrophotometer (420nm)

Figure The flow sheet for citrate soluble phosphoric acid in fertilizers

4.2.3.b Quinoline gravimetric analysis

(1) Summary

This test method is applicable to fertilizers containing no phosphonic acid, etc. It is suitable for the fertilizers containing relatively a high content of phosphate.

Extract by adding citric acid solution to an analytical sample, and measure the mass of quinonium phosphomolybdate formed by the reaction of citrate soluble phosphoric acid (C-P_2O_5) with quinoline, molybdic acid and nitric acid to obtain citrate soluble phosphoric acid (C-P_2O_5).

(2) Reagents: Reagents are as shown below:

- **a) Nitric acid:** A JIS Guaranteed Reagent (HNO_3 60 % (mass fraction)) specified in JIS K 8541 or a reagent of equivalent quality.
- **b) Citric acid solution** [(1)]**:** Dissolve citric acid monohydrate specified in JIS K 8283 in water to make 1,000 mL.
- **c) Sodium molybdate solution:** Dissolve 70 g of sodium molybdate dihydrate in 150 mL of water.
- **d) Quinoline solution:** Add 5 mL of quinoline specified in JIS K 8279 to the mixture solution of 35 mL of nitric acid and 100 mL of water.
- **e) Quimosiac solution:** Add 60 g of citric acid monohydrate specified in JIS K 8283 to the mixture solution of 85 mL of nitric acid and 150 mL of water to dissolve. Add gradually total volume of the sodium molybdate solution to mix. Add gradually the total volume of the quinoline solution while mixing the solution. After leaving at rest overnight, filter the total volume with Type 3 filter paper. Add 280 mL of acetone specified in JIS K 8962, and further add water to make 1,000 mL.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as follows:

- **a) Constant-temperature rotary shaker:** A constant-temperature rotary shaker that can rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, upside down at 30 - 40 revolutions/min.
- **b) Water bath:** Water bath that can be adjusted to 60 ºC - 65 ºC.
- **c) Drying apparatus:** A drying apparatus that can be adjusted to 220 ºC ± 5 ºC.
- **d) Crucible type glass filter:** Put a crucible type glass filter 1G4 specified in JIS R 3503 into a drying apparatus, stand to cool in a desiccator after heating at 220 ºC ± 5 ºC and measure the mass to the order of 1 mg.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

- **a)** Weigh 1 g of an analytical sample to the order of 1 mg, and transfer to a 250-mL volumetric flask.
- **b)** Add 150 mL of citric acid solution heated up to about 30 ºC, and shake to mix at 30-40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.
- **c)** After standing to cool, add water up to the marked line.
- **d)** Filter with Type 3 filter paper to make the sample solution.

Comment 1 The procedure in **(4.1)** is the same as the procedure in **(4.1)** of **4.2.3.a**.

Comment 2 For a by-product phosphate fertilizer or a fertilizer containing a by-product phosphate, if pH of the sample solution of **d)** is neutral or basic, prepare a sample solution anew by replacing "1 g of an analytical sample" in the procedure in **a)** with "0.5 g of an analytical sample".

(4.2) Measurement: Conduct measurement as shown below.

a) Transfer a predetermined volume (the equivalents of 10 mg - 30 mg as P_2O_5) of sample solution to a 300-mL tall beaker.

b) Add 5 mL of nitric acid and add water to make about 80 mL.

c) Cover with a watch glass. After boiling for about 3 minutes, wash the watch glass and the inside the tall beaker with water and add water to make about 100 mL.

d) Immediately, add 50 mL of quimosiac solution, heat for about 15 minutes while sometimes mixing in a water bath at 60 ºC - 65 ºC and produce the precipitate of quinolyum molybdate.

e) After standing to cool down to room temperature while sometimes mixing, filter under reduced pressure with a crucible type glass filter, wash the tall beaker 3 times with water and transfer the whole precipitate into a crucible type glass filter, further wash 7 - 8 times with water.

f) Transfer the precipitate together with the crucible type glass filter into a drying apparatus and heat at 220 ºC ± 5 ºC for about 30 minutes.

g) As soon as heating is complete, move into a desiccator and let it stand to cool.

h) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.

i) Calculate citrate soluble phosphoric acid (C-P_2O_5) by the following formula.

Citrate soluble phosphoric acid (C-P_2O_5) (% (mass fraction)) in an analytical sample
$= A \times 0.03207 \times (V_1/V_2) \times (1/B) \times 100$

A: Mass (g) of the precipitate in **h)**
B: Mass of an analytical sample (1 g)
V_1: Predetermined volume (250 mL) of the sample solution
V_2: Volume (mL) of the sample solution transferred in **a)**

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.98 - 106, Yokendo, Tokyo (1988)

(5) Flow sheet for citrate soluble phosphoric acid: The flow sheet for citrate soluble phosphoric acid in fertilizers is shown below:

1 g analytical sample	Weigh to the order of 1 mg to a 250-mL volumetric flask
← 150 mL of citric acid solution [about 30 ºC]	
Shaking to mix	Constant-temperature rotary shaker (30 - 40 revolutions/min) 30 ºC ± 1 ºC, for 1 hour
Standing to cool	
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined volume)	300-mL tall beaker
← 5 mL nitric acid ← Water (to reach about 100 mL)	
Boiling	Cover with a watch glass for 3 minutes Wash a watch glass and the inside the tall beaker with water
← Water (to be about 100 mL) ← 50mL of quimosiac solution	
Forming precipitation	60 ºC - 65 ºC, for 15 minutes, sometimes mixing
Standing to cool	Room temperature
Filtration under reduced pressure	Crucible type glass filtering apparatus 1G4, 3 times with water
Washing	Wash 7 - 8 times with water
Drying	220 ºC ± 5 ºC for about 30 minutes
Standing to cool	Desiccator
Measurement	Measure the mass to the order of 1 mg

Figure The flow sheet for citrate soluble phosphoric acid in fertilizers

4.2.4 Water-soluble phosphoric acid

4.2.4.a Ammonium vanadomolybdate absorptiometric analysis

(1) Summary
This test method is applicable to the fertilizers that do not contain matters not colored by hydrolysis with nitrate acids such as phosphonic acid.
Extract by adding water to an analytical sample, add nitric acid (1+1) to heat, and hydrolyze water-soluble phosphoric acid to orthophosphate ion, then measure the absorbance of phosphovanadomolybdate salt formed by the reaction with ammonium vanadate (V), hexaammonium heptamolybdate and nitric acid to obtain water-soluble phosphoric acid (W-P_2O_5).

(2) Reagents: Reagents are as shown below:

a) Nitric acid: A JIS Guaranteed Reagent (HNO_3 60 % (mass fraction)) specified in JIS K 8541 or a reagent of equivalent quality.

b) Ammonia solution: A JIS Guaranteed Reagent (NH_3 28 % (mass fraction)) specified in JIS K 8085 or a reagent of equivalent quality.

c) Coloring reagent solution(1) (2)**:** Dissolve 1.12 g of ammonium vanadate (V)(3) specified in JIS K 8747 in water, add 250 mL of nitric acid, then add 27 g of hexaammonium heptamolybdate tetrahydrate(4) specified in JIS K 8905 while dissolving in water, and further add water to make 1,000 mL(5).

d) Phenolphthalein solution (1 g/100 mL): Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

e) Phosphate standard solution (P_2O_5 10 mg/mL)(1): Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and then weigh 19.17 g to a weighing dish. Dissolve with a small amount of water, transfer to a 1,000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

f) Phosphate standard solution (P_2O_5 0.5 mg/mL)(1)**:** Transfer 50 mL of phosphate standard solution (P_2O_5 10 mg/mL) to a 1,000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.
(2) This corresponds to reagent “a” solution in the Official Methods of Analysis of Fertilizers (1992).
(3) This corresponds to ammonium metavanadate in the Official Methods of Analysis of Fertilizers (1992).
(4) This corresponds to ammonium molybdate in the Official Methods of Analysis of Fertilizers (1992).
(5) Store in an amber bottle.

(3) Instruments: Instruments are as follows:

a) Rotary shaker: A rotary shaker that can rotate a 500-mL volumetric flask upside down at 30 - 40 revolutions/min.

b) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

c) Spectrophotometer: A spectrophotometer specified in JIS K 0115.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5 g of an analytical sample to the order of 1 mg, and transfer to a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions /min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make the sample solution.

Comment 1 In the procedure in **a)**, it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it to a 250-mL volumetric flask.
Comment 2 The procedure in **(4.1)** is the same as the procedure in **(4.1)** of **4.2.4.b**, **4.2.4.c** and **4.7.3.a**, the procedure in **(4.1.1)** of **4.1.2.b**, and the procedure in **(4.1.2)** of **4.3.3.a**, **4.3.3.b** and **4.3.3.c**. In addition, the solution is also usable as the sample solution of **4.9.2.a**, **4.10.2.a**, **4.13.1.a** and **4.14.1.a**.
Comment 3 When the determination is affected by the coloring of the sample solution of **d)**, transfer the predetermined volume of the sample solution to a 100-mL volumetric flask, add a few drops of hydrochloric acid (1+1) to make it acidic, then add no more than 0.1 g of active carbon. After leaving at rest for a little while, add water up to the marked line and filter. The filtrate is prepared as the sample solution of **(4.2) a)**. Additionally, as phosphorus contained in active carbon has the possibility to elute and affects the determination value, a blank test is required.

(4.2) Coloring: Conduct coloring as follows:
a) Transfer a predetermined amount (the equivalents of 0.5 mg - 6 mg as P_2O_5) of the sample solution to a 100-mL tall beaker.
b) Add 4 mL of nitric acid (1+1) (6), and heat to boil (7).
c) After standing to cool, add 1 - 2 drop(s) of phenolphthalein solution (1 g/100 mL), and neutralize by adding ammonia solution (1+1) until the color of the solution becomes light red-purple.
d) Add nitric acid (1+10) until the light red-purple color of the solution disappears to make it slightly acidic. Transfer with water to a 100-mL volumetric flask.
e) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes (6).

Comment 4 In the procedure in **a)**, a volumetric flask can be used instead of a 100 mL tall beaker. However the volumetric flask used should be distinguished as a flask to be used for phosphoric coloring operation and should not be used for the other purposes. Additionally, “transfer to a 100 mL volumetric flask with water (8)” in **d)** is replaced by “add a proper amount of water (9)”.

Note (6) When the solution is muddled by adding nitric acid (1+1), conduct centrifugation after the procedure in **e)**.
(7) When it does not contain non-orthophosphate, the procedure in **b)** is not necessary.
(8) The volume of solution after transferring should be up to about 60 mL.
(9) If no water is added, precipitate is produced in some cases when coloring reagent solution is added.

(4.3) Measurement: Conduct measurement according to JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used in measurement.
a) Measurement conditions for the spectrophotometer: Set up the measurement conditions for the spectrophotometer considering the following:
Detection wavelength: 420 nm

b) Calibration curve preparation

1) Transfer 1 mL - 12 mL of phosphate standard solution (P_2O_5 0.5 mg/mL) to 100-mL volumetric flasks step-by-step.
2) Add a proper amount of water (9) and conduct the same procedure as **(4.2) e)** to make the P_2O_5 0.5 mg/100 mL - 6 mg/100 mL phosphate standard solution for the calibration curve preparation.
3) Conduct the same procedures as **2)** for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.
4) Measure the absorbance at a wavelength of 420 nm of the phosphate standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (10).
5) Prepare the calibration curve of the phosphate concentration and absorbance of the phosphate standard solutions for the calibration curve preparation.

c) Sample measurement

1) Regarding the solution in **(4.2) e),** measure absorbance by the same procedure as **b) 4)** (10).
2) Obtain the phosphate (P_2O_5) content from the calibration curve, and calculate water-soluble phosphoric acid (W-P_2O_5) in the analytical sample.

Note (10) Measure within 6 hours after adding the coloring reagent solution in the procedure in **(4.2) e)**.

Comment 5 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for nitrate nitrogen hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2006	High analysis compound fertilizer	157	9.02	0.12	1.3
2007	Organic compound fertilizer	143	7.02	0.23	3.3
2008	High analysis compound fertilizer	146	9.16	0.24	2.7
2009	Ordinary compound fertilizer	142	4.57	0.08	1.7
2010	High analysis compound fertilizer	143	11.56	0.52	4.5
2011	High analysis compound fertilizer	132	14.41	0.19	1.3
2012	Fluid mixed fertilizer	128	2.88	0.06	2.1
2013	High analysis compound fertilizer	133	12.08	0.19	0.9

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:

$$RSD_{rob} = (NIQR/M) \times 100$$

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.108 - 114, Yokendo, Tokyo (1988)

(5) Flow sheet for water-soluble phosphoric acid: The flow sheet for water-soluble phosphoric acid in fertilizers is shown below:

5 g analytical sample	Weigh to the order of 1 mg into a 500-mL volumetric flask
← Water, about 400 mL	
Shaking to mix	Rotary shaker (30 - 40 revolutions/min) for 30 minutes
← Water (up to the marked line)	
Filtration	Type 3 filter paper
←Water (up to the marked line)	
Aliquot (predetermined volume)	100-mL volumetric flask
← 4 mL of nitric acid(1+1)	
Heating	Boiling
Standing cool	
← 1-2 drop(s) of phenolphthalein solution (1 g/100 mL)	
← Ammonia solution (1+1) [neutralization]	
← Nitric acid (1+10) [slightly acidic]	
← A proper amount of water	
← 20 mL of coloring reagent solution	
← Water (up to the marked line)	
Leaving at rest	For about 30 minutes
Measurement	Spectrophotometer (420 nm)

Figure The flow sheet for water-soluble phosphoric acid in fertilizers

4.2.4.b Ammonium vanadomolybdate absorptiometric analysis (Fluid mixed fertilizers containing phosphonic acid or phosphite)

(1) Summary

This test method is applicable to the fertilizers containing fluid mixed fertilizers containing phosphonic acid or phosphite
Extract by adding water to an analytical sample, add hydrochloric acid - sulfuric acid to heat, and oxygenate phosphonic acid ion to orthophosphate ion, and then measure the absorbance of phosphovanadomolybdate salt formed by the reaction with ammonium vanadate (V), hexaammonium heptamolybdate and nitric acid to obtain water-soluble phosphoric acid (W-P_2O_5).

(2) Reagents: Reagents are as shown below:

a) **Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
b) **Nitric acid:** A JIS Guaranteed Reagent (HNO_3 60 % (mass fraction)) specified in JIS K 8541 or a reagent of equivalent quality.
c) **Ammonia solution:** A JIS Guaranteed Reagent (NH_3 28 % (mass fraction)) specified in JIS K 8085 or a reagent of equivalent quality.
d) **Coloring reagent solution**(1) (2)**:** Dissolve 1.12 g of ammonium vanadate (V)(3) specified in JIS K 8747 in water, add 250 mL of nitric acid, then add 27 g of hexaammonium heptamolybdate tetrahydrate(4) specified in JIS K 8905 while dissolving in water, and further add water to make 1,000 mL(5).
e) **Phenolphthalein solution (1 g/100 mL):** Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.
f) **Phosphate standard solution (P_2O_5 10 mg/mL)**(1)**:** Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve with a small amount of water, transfer to a 1,000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.
g) **Phosphate standard solution (P_2O_5 0.5 mg/mL)**(1)**:** Transfer 50 mL of phosphate standard solution (P_2O_5 10 mg/mL) to a 1,000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) This corresponds to reagent "a" solution in the Official Methods of Analysis of Fertilizers (1992).
(3) This corresponds to ammonium metavanadate in the Official Methods of Analysis of Fertilizers (1992).
(4) This corresponds to ammonium molybdate in the Official Methods of Analysis of Fertilizers (1992).
(5) Store in an amber bottle.

(3) Instruments: Instruments are as follows:

a) **Rotary shaker:** A rotary shaker that can rotate a 500-mL volumetric flask upside down at 30 - 40 revolutions/min.
b) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.
c) **Spectrophotometer**: A spectrophotometer specified in JIS K 0115.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and transfer to a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions /min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make the sample solution.

Comment 1 In the procedure in **a)**, it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it into a 250-mL volumetric flask.

Comment 2 The procedure in **(4.1)** is the same as the procedure in **(4.1)** of **4.2.4.a**.

(4.2) Coloring: Conduct coloring as shown below:

a) Transfer a predetermined amount (the equivalents of 0.5 mg - 6 mg as P_2O_5) of the sample solution to a 100-mL - 200-mL tall beaker.

b) Add 3 mL of hydrochloric acid and 1 mL of nitric acid.

c) Cover the tall beaker with a watch glass, heat on a hot plate or sand bath at 200 ºC - 250 ºC and condense (7) until the solution volume becomes 1 mL - 3 mL (6).

d) After standing to cool, transfer to a 100-mL volumetric flask with water (8).

e) Add 1 - 2 drop(s) of phenolphthalein solution (1 g/100 mL), and neutralize by adding ammonia solution (1+1) until the color of the solution becomes light red-purple.

f) Add nitric acid (1+10) until the light red-purple color of the solution disappears to make it slightly acidic.

g) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes.

Note **(6)** It is recommended to transfer 1 mL - 3 mL of water to a 100-mL - 200-mL tall beaker in advance and confirm the volume.

(7) Care should be taken not to evaporate completely. When it is evaporated completely, the determined value becomes lower than usual in some cases.

(8) The volume of solution after transferring should be up to about 60 mL.

(4.3) Measurement: Conduct measurement according to JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used in measurement.

a) **Measurement conditions for the spectrophotometer:** Set up the measurement conditions for the spectrophotometer considering the following:
Detection wavelength: 420 nm

b) **Calibration curve preparation**

1) Transfer 1mL - 12 mL of phosphate standard solution (P_2O_5 0.5 mg/mL) to 100-mL volumetric flasks step-by-step.

2) Add a proper amount of water (9) and conduct the same procedure as **(4.2) g)** to make the P_2O_5 0.5 mg/100 mL - 6 mg/100 mL phosphate standard solution for the calibration curve preparation.

3) Conduct the same procedures as **2)** for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Measure absorbance at a wavelength of 420 nm of the phosphate standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (10).

5) Prepare the calibration curve of the phosphate concentration and absorbance of the phosphate standard solutions for the calibration curve preparation.

c) Sample measurement

1) Regarding the solution in **(4.2) g)**, measure the absorbance by the same procedure as **b) 4)** (10)**.**
2) Obtain the phosphate (P_2O_5) content from the calibration curve, and calculate water-soluble phosphoric acid (W-P_2O_5) in the analytical sample.

Note (9) If no water is added, precipitate is produced in some cases when coloring reagent solution is added.

(10) Measure within 6 hours after adding the coloring reagent solution in the procedure in **(4.2) g)**.

Comment 3 As the result of a recovery testing using 7 kinds of fluid mixed fertilizers (7 samples) containing the equivalents of 0.2 % - 50 %(mass fraction) as water-soluble phosphoric acid, the average rate of recovery is 100.8 % - 102.5 % and its repeatability is 0.00 % - 0.04 % as standard deviation and 0.07 - 1.69 % as relative standard deviation.
Additionally, Table 1 shows results and analysis results from a collaborative study for test method validation. In this case, the minimum quantitation of the testing method is about 0.01 % (mass fraction).

Table 1 Results and analysis results from a collaborative study for the test method validation of water-soluble phosphoric acid

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Fluid mixed fertilizers 1	12	33.56	0.25	0.7	0.59	1.8
Fluid mixed fertilizers 2	12	17.93	0.08	0.5	0.30	1.7
Fluid mixed fertilizers 3	12	7.99	0.12	1.5	0.31	3.8
Fluid mixed fertilizers 4	11	11.93	0.13	1.1	0.33	2.8
Fluid mixed fertilizers 5	11	24.1	0.08	0.3	0.47	2.0

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories x number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Toshiaki HIROI, Masakazu SAIKI and Kimie KATO: Development and Validation of Spectrophotometry for Determination of Water-Soluble Phosphoric acid in Liquid Mixed Fertilizer Containing Phosphonic acid, Research Report of Fertilizer, Vol.1 2008. (25 - 33)
2) Toshiaki HIROI, Masakazu SAIKI and Kimie KATO: Determination of Water-Soluble Phosphoric Acid in Liquid Mixed Fertilizer Containing Phosphonic Acid by

Spectrophotometry: A Collaborative Study, Research Report of Fertilizer, Vol.1 2008. (34 - 40)

(5) Flow sheet for water-soluble phosphoric acid of the fluid mixed fertilizers containing phosphonic acid, etc.: The flow sheet for water-soluble phosphoric acid of the fluid mixed fertilizers containing phosphonic acid, etc. is shown below:

5 g analytical sample	Weigh to the order of 1 mg to a 500-mL volumetric flask
← Water, about 400 mL	
Shaking to mix	Rotary shaker (30 - 40 revolutions/min) for 30 minutes
← Water (up to the marked line)	
Filtration	Type 3 filter paper
← Water (up to the marked line)	
Aliquot (predetermined volume)	100-mL - 200-mL tall beaker
← 3 mL of hydrochloric acid	
← 1 mL of nitric acid	
Heating	Cover the tall beaker with a watch glass, heat on a hot plate or sand bath at 200 ºC - 250 ºC, condense until the solution volume reaches 1 mL – 3 mL
Standing to cool	
Transfer	100-mL volumetric flask, water
← 1-2 drop(s) of phenolphthalein solution (1 g/100 mL)	
← Ammonia solution (1+1) [neutralization]	
← Nitric acid (1+10) [slightly acidic]	
← 20 mL of coloring reagent solution	
← Water (up to the marked line)	
Leaving at rest	For about 30 minutes
Measurement	Spectrophotometer (420 nm)

Figure The flow sheet for water-soluble phosphate acid in fluid mixed fertilizers containing phosphonic acid, etc.

4.2.4.c Quinoline gravimetric analysis

(1) Summary

This test method is applicable to fertilizers containing no phosphonic acid, etc.
It is suitable for the fertilizers containing relatively a high content of phosphate.
Extract by adding water to an analytical sample, and measure the mass of quinonium phosphomolybdate formed by the reaction with quinoline, molybdic acid and nitric acid to obtain water-soluble phosphoric acid (W-P_2O_5).

(2) Reagents: Reagents are as shown below:

- **a) Nitric acid:** A JIS Guaranteed Reagent (HNO_3 60 % (mass fraction)) specified in JIS K 8541 or a reagent of equivalent quality.
- **b) Sodium molybdate solution:** Dissolve 70 g of sodium molybdate dihydrate in 150 mL of water.
- **c) Quinoline solution:** Add 5 mL of quinoline specified in JIS K 8279 to the mixture solution of 35 mL of nitric acid and 100 mL of water.
- **d) Quimosiac solution:** Add 60 g of citric acid monohydrate specified in JIS K 8283 to the mixture solution of 85 mL nitric acid and 150 mL of water to dissolve. Add gradually total volume of the sodium molybdate solution to mix them. Add gradually the total volume of the quinoline solution while mixing the solution. After leaving at rest overnight, filter the total volume with Type 3 filter paper. Add 280 mL of acetone specified in JIS K 8962, and further add water to make 1,000 mL.

(3) Instruments: Instruments are as follows:

- **a) Rotary shaker:** A rotary shaker that can rotate a 500-mL volumetric flask upside down at 30 - 40 revolutions/min.
- **b) Water bath:** Water bath that can be adjusted to the test temperature ± 2 ºC.
- **c) Drying apparatus:** A drying apparatus that can be adjusted to 220 ºC ± 5 ºC.
- **d) Crucible type glass filter:** Put a crucible type glass filter 1G4 specified in JIS R 3503 into a drying apparatus, let it stand to cool in a desiccator after heating at 220 ºC ± 5 ºC and measure the mass to the order of 1 mg.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

- **a)** Weigh 1 g of an analytical sample to the order of 1 mg, and put to a 500-mL volumetric flask.
- **b)** Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for 30 minutes.
- **c)** Add water up to the marked line.
- **d)** Filter with Type 3 filter paper to make the sample solution.

Comment 1 In the procedure in **a)**, it is allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it into a 250-mL volumetric flask.

Comment 2 The procedure in **(4.1)** is the same procedure in **(4.1)** of **4.2.4.a**.

(4.2) Measurement: Conduct measurement as shown below.

- **a)** Transfer a predetermined volume (the equivalents of 10 mg - 30 mg as P_2O_5 and no more than 20 mL as the total solution volume) of sample solution to a 300-mL tall beaker.
- **b)** Add 5 mL of nitric acid and add water to make about 80 mL.
- **c)** Cover with a watch glass. After boiling for about 3 minutes, wash the watch glass and the inside the tall beaker with water and add water to make about 100 mL.

d) Immediately, add 50 mL of quimosiac solution, heat for about 15 minutes while sometimes mixing in a water bath at 60 ºC - 65 ºC and produce precipitate of quinolyum molybdate.

e) After standing to cool down to room temperature while sometimes mixing, filter under reduced pressure with a crucible type glass filter, wash the tall beaker 3 times with water and transfer the whole precipitate into a crucible type glass filter, further wash 7 - 8 times with water.

f) Transfer the precipitate together with a crucible type glass filter into a drying apparatus and heat at 220 ºC ± 5 ºC for about 30 minutes.

g) As soon as heating is complete, move it into a desiccator and let it stand to cool.

h) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.

i) Calculate water-soluble phosphoric acid (W-P_2O_5) by the following formula.

Water-soluble phosphoric acid (W-P_2O_5) (% (mass fraction)) in an analytical sample
$= A \times 0.03207 \times (V_1/V_2) \times (1/B) \times 100$

A: Mass (g) of the precipitate in **h)**
B: Mass of an analytical sample (5 g)
V_1: Predetermined volume (500 mL) of the sample solution
V_2: Volume (mL) of the sample solution transferred in **a)**

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.98 - 114, Yokendo, Tokyo (1988)

(5) Flow sheet for water-soluble phosphoric acid: The flow sheet for water-soluble phosphoric acid in fertilizers is shown below:

5 g analytical sample	Weigh to the order of 1 mg to a 500-mL volumetric flask
← About 400 mL of water	
Shaking to mix	Rotary shaker (30 - 40 revolutions/min) for 30 minutes
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined volume)	300-mL tall beaker
← 5 mL nitrate acid	
← Water (to make about 80 mL)	
Boiling	Cover with a watch glass for 3 minutes Wash a watch glass and the inside the tall beaker with water
← Water (to make about 100 mL)	
← 50 mL quimosiac solution	
Forming precipitate	60 ºC - 65 ºC, for 15 minutes, some times mix
Standing to cool	Room temperature
Filtration under reduced pressure	Crucible type glass filtering apparatus 1G4, 3 times with water
Washing	Wash 7 - 8 times with water
Drying	220 ºC ± 5 ºC for about 30 minutes
Standing to cool	Desiccator
Measurement	Measure the mass to the order of 1 mg

Figure The flow sheet for water-soluble phosphoric acid in fertilizers

4.3 Potassium

4.3.1 Total potassium

4.3.1.a Flame atomic absorption spectrometry or flame photometry

(1) Summary
This test method is applicable to fertilizers containing organic matters.
Pretreat an analytical sample with incineration and hydrochloric acid to convert total potassium into potassium ion, add interference suppressor solution, and then spray in an acetylene-air flame, and measure the atomic absorption with potassium at a wavelength of 766.5 nm or 769.9 nm to quantify total potassium. Or, determine the intensity of the emission line at a wavelength of 766.5 nm or 769.9 nm produced in flame to quantify total potassium.

(2) Reagents: Reagents are as shown below:

- **a) Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **b) Interference suppressor solution:** Weigh 12.5 g of calcium carbonate specified in JIS K 8617 into a 2,000-mL beaker, add a small amount of water, gradually add 105 mL of hydrochloric acid, and heat for a little while. After standing to cool, add water to make 1,000 mL.
- **c) Potassium standard solution (K_2O 1 mg/mL)**(1): Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.583 g into a weighing dish. Dissolve in a small amount of water, transfer to a 1,000-mL volumetric flask, and add water up to the marked line.
- **d) Potassium standard solution (K_2O 5 µg/mL - 50 µg/mL) for the calibration curve preparation** (1)**:** Transfer 2.5 mL - 25 mL of potassium standard solution (K_2O 1 mg/mL) to 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (2), and add water up to the marked line.
- **e) Blank test solution for the calibration curve preparation** (1)**:** Transfer about 50 mL of interference suppressor solution to a 500-mL volumetric flask (2), and add water up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) Add interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of potassium standard solution (K_2O 1 mg/mL) in (**2**) **c**), potassium standard solution (K 1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiple by the conversion factor (1.2046) to use as potassium standard solution (K_2O 1.2046 mg/mL).

(3) Instruments: Instruments are as shown below:

- **a) Analytical instrument**: An atomic absorption spectrometer or a flame photometer as shown below:
- **aa) Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121.
  - **1) Light source**: A potassium hollow cathode lamp
  - **2) Gas:** Gas for heating by flame
    - (i) Fuel gas: acetylene
    - (ii) Auxiliary gas: Air sufficiently free of dust and moisture.

ab) Flame photometer:

1) Gas: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) Electric furnace: An electric furnace that can be adjusted to 550 ºC ± 5 ºC.

c) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char(3).

c) Ignite at 550 ºC ± 5 ºC for no less than 4 hours to incinerate.

d) After standing to cool, moisten the residue with a small amount of water, add gradually about 10 mL of hydrochloric acid, and further add water to make about 20 mL.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.

f) After standing to cool, transfer to a 100-mL - 500-mL volumetric flask.

g) Add water up to the marked line.

h) Filter with Type 3 filter paper to make the sample solution.

Note (3) Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.

Comment 2 The procedures in **(4.1)** are the same as in **(4.1.2)** in **4.2.1.a**.

Comment 3 The sample solution prepared in **(4.1) a) - h)** in **4.9.1.a** can also be used.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer or flame photometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer or flame photometer: Set up the measurement conditions for the atomic absorption spectrometer or flame photometer considering the following:

Analytical line wavelength: 766.5 nm or 769.9 nm

b) Calibration curve preparation

1) Spray the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 766.5 nm or 769.9 nm.

2) Prepare a curve for the relationship between the potassium concentration and the indicated value of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as K_2O) to a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (2), and add water up to the marked line.

3) Conduct similarly as in **b) 1)** to read the indicated value.

4) Obtain the potassium content from the calibration curve, and calculate total potassium in the analytical sample.

Comment 4 Replicate testing was conducted using composted sludge fertilizer (2 samples), human waste sludge fertilizer (2 samples), compost (1 sample), designated blended fertilizer containing organic fertilizer (1 sample) and compound fertilizer containing organic fertilizer (1 sample); as a result, the mean was in the range of 0.163 % - 12.40 % (mass fraction), and the standard deviation and relative standard deviation were 0.001 % - 0.03 % (mass fraction) and 0.1 % - 4.4 %, respectively.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.132 - 138, Yokendo, Tokyo (1988)

2) Kimie KATO, Masayuki YOSHIMOTO and Yuji SHIRAI: Systematization of Determination Methods of Major Components in Sludge Fertilizer, Compost and Organic Fertilizer, Research Report of Fertilizer, Vol.3 2010. (107 - 116)

(5) Flow sheet for total potassium: The flow sheet for total potassium in fertilizers is shown below:

5 g analytical sample	Weigh to the order of 1 mg into a 200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	550 ºC ± 5 ºC, no less than 4 hours
Standing to cool	Room temperature
	← A small amount of water, moisten the residue
	← About 10 mL of hydrochloric acid
	← Water (up to about 20 mL)
Heating	Cover with a watch glass, and boil for 5 minutes.
Standing to cool	Room temperature
Transfer	250-mL - 500-mL volumetric flask, water
	← Water (up to the marked line)
Filtration	Type 3 filter paper
Aliquot (predetermined amount)	100-mL volumetric flask, water
	← About 10 mL interference suppressor solution
	← Water (up to the marked line)
Measurement	Atomic absorption spectrometer or flame photometer

Figure Flow sheet for total potassium in fertilizers

4.3.1.b Sodium tetraphenylborate gravimetric analysis

(1) Summary

This test method is applicable to fertilizers containing organic matters. It is suitable for fertilizers containing relatively a high content of potassium.
Pretreat an analytical sample by incineration and hydrochloric acid to convert total potassium into potassium ion, mask co-existing ammonium and other salts with formaldehyde and ethylenediamine tetraacetate, and measure the mass of potassium tetraphenylborate formed by the reaction with tetraphenylborate to obtain total potassium (T-K_2O).

(2) Reagents: Reagents are as shown below:

- **a) Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **b) Formaldehyde solution:** A JIS Guaranteed Reagent specified in JIS K 8872 or a reagent of equivalent quality.
- **c) Sodium hydroxide solution (200 g/L)** (1)**:** Dissolve 200 g of sodium hydroxide specified in JIS K 8576 in water to make 1,000 mL.
- **d) Aluminum chloride solution** (1)**:** Dissolve 12 g of aluminum chloride (III) hexahydrate specified in JIS K 8114 in water to make 100 mL.
- **e) Tetraphenylborate solution** (1)**:** Transfer 6.1 g of sodium tetraphenylborate specified in JIS K 9521to a 250-mL volumetric flask, dissolve by adding about 200 mL of water and add 10 mL of aluminum chloride solution. Add methyl red solution (0.1 g/100 mL) as an indicator, and neutralize with sodium hydroxide solution (200 g/L) until the color of the solution changes to yellow, and then add water up to the marked line. Filter with Type 3 filter paper and add 0.5 mL of sodium hydroxide solution (200 g/L) to the total filtrate. Filter with Type 3 filter paper in the case of usage.
- **f) Tetraphenylborate washing solution** (1)**:** Dilute 40 mL of tetraphenylborate solution with water to make 1,000mL.
- **g) Ethylenediaminetetraacetate - Sodium hydroxide solution (1):** Dissolve 10 g of ethylenediaminetetraacetic acid disodium dihydrogen dihydrate specified in JIS K 8107 and 8 g of sodium hydroxide specified in JIS K 8576 in a proper amount of water. Add 6 mL - 10 mL of tetraphenylborate solution while mixing according to the potassium content coexisting as impurity after standing to cool, and then add water to make 100 mL. After leaving at rest for about 30 minutes while sometimes mixing, filter with Type 3 filter paper.

- **h) Methyl red solution (0.1 g/100 mL):** Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as shown below:

- **a) Electric furnace:** An electric furnace that can be adjusted to 550 ºC ± 5 ºC.
- **b) Drying apparatus:** A drying apparatus that can be adjusted to 120 ºC ± 2 ºC.
- **c) Crucible type glass filter:** Put a crucible type glass filter 1G4 specified in JIS R 3503 into a drying apparatus, stand to cool in a desiccator after heating at 120 ºC ± 2 ºC and measure the mass to the order of 1 mg.
- **d) Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

- **a)** Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200- mL - 300-mL tall beaker.
- **b)** Put the tall beaker in an electric furnace, and heat gently to char(2).
- **c)** Ignite at 550 ºC ± 5 ºC for no less than 4 hours to incinerate.
- **d)** After standing to cool, moisten the residue with a small amount of water, add gradually about 10 mL of hydrochloric acid, and further add water to make 20 mL.
- **e)** Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.
- **f)** After standing to cool, transfer to a 100-mL - 500-mL volumetric flask.
- **g)** Add water up to the marked line.
- **h)** Filter with Type 3 filter paper to make the sample solution.

Note (2) Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.

Comment 1 The procedure in **(4.1)** is the same as **(4.1.2)** in **4.2.1.a**. In addition, the sample solution prepared in **(4.1) a) - h)** in **4.9.1.a** can also be used.

(4.2) Measurement: Conduct measurement as shown below.

- **a)** Transfer a predetermined volume (the equivalents of 15 mg - 30 mg as K_2O) of sample solution to a 100-mL tall beaker.
- **b)** Add water to the solution to reach 50 mL when the procedure in **e)** is complete.
- **c)** Add hydrochloric acid (1+9) , so that the hydrochloric acid becomes equivalent to 0.2 mL.
- **d)** Add 5 mL of formaldehyde solution, and then add 5 mL of ethylenediamine tetraacetate - sodium hydroxide solution.
- **e)** Add necessary volume (3) of tetraphenylborate solution at the rate of one or two drop(s) per second while mixing, and further add 4 mL of the same solution in the same manner.
- **f)** Leave at rest for about 30 minutes while sometimes mixing to form the precipitate of potassium tetraphenylborate.
- **g)** Filter supernatant with a crucible type glass filter, wash the tall beaker 5 times with 5 mL of tetraphenylborate washing solution and transfer the whole precipitate to the crucible type glass filter and further wash 2 times with 2 ml of water.
- **h)** Transfer the precipitate together with the crucible type glass filter into a drying apparatus and heat at 120 ºC ± 2 ºC for 1 hour.
- **i)** After heating, move it quickly into a desiccator and let it stand to cool.
- **j)** After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.
- **k)** Calculate total potassium (T-K_2O) by the following formula.

Total potassium (T-K_2O) (% (mass fraction)) in an analytical sample
$= A \times 0.1314 \times (V_1/V_2) / W \times 100$

A: Mass (g) of the precipitate
V_1: Predetermined volume (mL) of the sample solution in **(4.1) g)**
V_2: Volume (mL) of the sample solution transferred in **(4.2) a)**
B: Mass of an analytical sample (g)

Note (3) About 3 ml of tetraphenylborate solution per 10 mg of K_2O is required to form the precipitate of potassium tetraphenylborate

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.122 - 128, Yokendo, Tokyo (1988)

(5) Flow sheet for total potassium: The flow sheet for total potassium in fertilizers is shown below:

5 g analytical sample	Weigh to the order of 1 mg into a 200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	550 ºC ± 5 ºC, no less than 4 hours
Standing to cool	Room temperature
	← A small amount of water, moisten the residue
	← About 10 mL of hydrochloric acid
	← Water (up to about 20 mL)
Heating	Cover with a watch glass, and boil for 5 minutes.
Standing to cool	Room temperature
Transfer	250-mL - 500-mL volumetric flask, water
	← Water (up to the marked line)
Filtration	Type 3 filter paper
Aliquot (predetermined amount)	100-mL tall beaker
	← Water (add tetraphenylborate solution to reach 50 mL)
	← Hydrochloric acid (1+9) (the equivalents of 0.2 mL of hydrochloric acid)
	← 5 mL of formaldehyde solution
	← 5 mL of ethylenediamine tetraacetate- sodium hydroxide solution
	← Tetraphenylborate solution (necessary volume + 4 mL)
Forming precipitate	For 30 minutes, sometimes mixing
Transfer	Crucible type glass filtering apparatus 1G4, 5 times with 5 mL of tetraphenylborate washing solution
Washing	Wash 2 times with 2 mL of water
Drying	120 ºC ± 5 ºC for 1 hour.
Standing to cool	Desiccator
Measurement	Measure the mass to the order of 1 mg

Figure Flow sheet for total potassium in fertilizers

4.3.2 Citrate soluble potassium

4.3.2.a Flame atomic absorption spectrometry or flame photometry

(1) Summary

This test method is applicable to fertilizers containing potassium silicate fertilizers, etc. Extract by adding citric acid solution to an analytical sample, add interference suppressor solution, and then spray in an acetylene-air flame, and measure the atomic absorption with potassium at a wavelength of 766.5 nm or 769.9 nm to quantify citrate soluble potassium (C-K_2O). Or, measure the intensity of the emission line at a wavelength of 766.5 nm or 769.9 nm produced in flame to quantify citrate soluble potassium (C-K_2O).

(2) Reagents: Reagents are as shown below:

a) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Citric acid solution (1)**:** Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1,000 mL.

c) Interference suppressor solution: Weigh 12.5 g of calcium carbonate specified in JIS K 8617 into a 2,000-mL beaker, add a small amount of water, gradually add 105 mL of hydrochloric acid, and heat for a little while. After standing to cool, add water to make 1,000 mL.

d) Potassium standard solution (K_2O 1 mg/mL)(1)**:** Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.583 g into a weighing dish. Dissolve with a small amount of water, transfer to a 1,000-mL volumetric flask, and add water up to the marked line.

e) Potassium standard solution (K_2O 5µg/mL - 50 µg/mL) for the calibration curve preparation (1)**:** Transfer 2.5 mL - 25 mL of potassium standard solution (K_2O 1 mg/mL) to 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (2), and add water up to the marked line.

f) Blank test solution for the calibration curve preparation (1)**:** Transfer about 50 mL of interference suppressor solution to a 500-mL volumetric flask (2), and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) Add interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of potassium standard solution (K_2O 1 mg/mL) in (**2**) **d**), potassium standard solution (K 1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiple by the conversion factor (1.2046) to use as potassium standard solution (K_2O 1.2046 mg/mL).

(3) Instruments: Instruments are as follows:

a) Constant-temperature rotary shaker: A constant-temperature rotary shaker that can rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, upside down at 30 - 40 revolutions/min.

b) Analysis instrument: An atomic absorption spectrometer or a flame photometer as shown below:

ba) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121.

1) Light source: A potassium hollow cathode lamp
2) Gas: Gas for heating by flame
(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

bb) Flame photometer:
1) Gas: Gas for heating by flame
(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and transfer to a 250-mL volumetric flask.
b) Add 150 mL of citric acid solution heated up to about 30 ºC, and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.
c) After standing to cool, add water up to the marked line.
d) Filter with Type 3 filter paper to make the sample solution.

Comment 2 The procedure in **(4.1)** is the same as the procedure in **(4.1)** of **4.2.3.a**.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer or flame photometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer or flame photometer: Set up the measurement conditions for the atomic absorption spectrometer or flame photometer considering the following:
Analytical line wavelength: 766.5 nm or 769.9 nm

b) Calibration curve preparation
1) Spray the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 766.5 nm or 769.9 nm.
2) Prepare a curve for the relationship between the potassium concentration and the indicated value of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement
1) Transfer a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as K_2O) to a 100-mL volumetric flask.
2) Add about 10 mL of interference suppressor solution (2), and add water up to the marked line.
3) Conduct similarly as in **b) 1)** to read the indicated value.
4) Obtain the potassium content from the calibration curve, and calculate citrate soluble potassium (C-K_2O) in the analytical sample.

Comment 3 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for citrate soluble potassium hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Measurement method	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2011	High analysis compound fertilizer	Atomic absorption spectrometry	50	10.35	0.15	1.4
		Flame photometry	68	10.45	0.22	2.1

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.136 - 138, Yokendo, Tokyo (1988)

(5) Flow sheet for citrate soluble potassium: The flow sheet for citrate soluble potassium in fertilizers is shown below:

1 g analytical sample — Weigh to the order of 1 mg into a 250-mL volumetric flask.
← 150 mL citrate solution [about 30 °C]
Shaking to mix — Constant temperature rotary shaker (30 - 40 revolutions/min) 30 °C ± 1 °C, 1 hour
Standing to cool
← Water (up to the marked line)
Filtration — Type 3 filter paper
Aliquot (predetermined amount) — 100-mL volumetric flask
← About 10 mL interference suppressor solution
← Water (up to the marked line)
Measurement — Atomic absorption spectrometer or flame photometer

Figure Flow sheet for citrate soluble potassium in fertilizers

4.3.2.b Sodium tetraphenylborate gravimetric analysis

(1) Summary

This test method is applicable to fertilizers containing potassium silicate fertilizer, etc. Extract by adding citric acid solution to an analytical sample, mask co-existing ammonium and other salts with formaldehyde and ethylenediamine tetraacetate and measure the mass of citrate soluble potassium (C-K_2O) and the mass of potassium tetraphenylborate formed by the reaction with tetraphenylborate to obtain citrate soluble potassium (C-K_2O).

(2) Reagents: Reagents are as shown below:

- **a) Citric acid solution** (1)**:** Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1,000 mL.
- **b) Formaldehyde solution:** A JIS Guaranteed Reagent specified in JIS K 8872 or a reagent of equivalent quality.
- **c) Sodium hydroxide solution (200 g/L)** (1)**:** Dissolve 200 g of sodium hydroxide specified in JIS K 8576 in water to make 1,000 mL.
- **d) Aluminum chloride solution** (1)**:** Dissolve 12 g of aluminum chloride (III) hexahydrate specified in JIS K 8114 in water to make 100 mL.
- **e) Tetraphenylborate solution** (1)**:** Transfer 6.1 g of Sodium tetraphenylborate specified in JIS K 9521 to a 250-mL volumetric flask, dissolve by adding about 200 mL of water and add 10 mL of aluminum chloride solution. Add methyl red solution (0.1 g/100 mL) as an indicator, and neutralize with sodium hydroxide solution (200 g/L) until the color of the solution changes to yellow, and then add water up to the marked line. Filter with Type 3 filter paper and add sodium hydroxide solution (200 g/L) to the total filtrate. Filter with Type 3 filter paper in the case of usage.
- **f) Tetraphenylborate washing solution** (1)**:** Dilute 40 mL of tetraphenylborate solution with water to make 1,000mL.
- **g) Ethylenediamine tetraacetate- Sodium hydroxide solution** (1)**:** Dissolve 10 g of ethylenediaminetetraacetic acid dihydrogen disodium dihydrate specified in JIS K 8107 and 8 g of sodium hydroxide specified in JIS K 8576 in a proper amount of water, add 6 mL - 10 mL of tetraphenylborate solution while mixing according to the volume of potassium coexisting as impurity after standing to cool and then add water to make 100 mL. After leaving at rest for about 30 minutes while sometimes mixing, filter with Type 3 filter paper.
- **h) Methyl red solution (0.1 g/100 mL):** Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are as follows:

- **a) Constant-temperature rotary shaker:** A constant-temperature rotary shaker that can rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, upside down at 30 - 40 revolutions/min.
- **b) Drying apparatus:** A drying apparatus that can be adjusted to 120 ºC ± 2 ºC.
- **c) Crucibles type glass filter:** Put a crucibles type glass filter 1G4 specified in JIS R 3503 into a drying apparatus, stand to cool in a desiccator after heating at 120 ºC ± 2 ºC and measure the mass to the order of 1 mg.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

- **a)** Weigh 1 g of an analytical sample to the order of 1 mg, and transfer to a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC, and shake to mix at 30-40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.
c) After standing to cool, add water up to the marked line.
d) Filter with Type 3 filter paper to make the sample solution.

Comment 1 The procedure **(4.1)** is the same as the procedure **(4.1)** of **4.2.3.a**.

(4.2) Measurement: Conduct measurement as shown below.
a) Transfer 20 mL of sample solution to a 100-mL tall beaker.
b) Add water to the solution to reach 50 mL when the procedure in **d)** is complete.
c) Add 5 mL of formaldehyde solution, and then add 5 mL of ethylenediamine tetraacetate- sodium hydroxide solution.
d) Add necessary volume [(2)] of tetraphenylborate solution at the rate of one or two drop(s) per second while mixing, and further add 4 mL of the same solution in the same manner.
e) Leave at rest for about 30 minutes while sometimes mixing to form the precipitate of potassium tetraphenylborate.
f) Filter supernatant with a crucible type glass filter, wash the vessel 5 times with 5 mL of tetraphenylborate washing solution and transfer the whole precipitate to the crucible type glass filter and further wash 2 times with 2 ml of water.
g) Transfer the precipitate together with the crucible type glass filter into a drying apparatus and heat at 120 ºC ± 2 ºC for 1 hour.
h) After heating, move it quickly into a desiccator and let it stand to cool.
i) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.
j) Calculate citrate soluble potassium (C-K_2O) by the following formula in the analytical sample.

Citrate soluble potassium (C-K_2O) (% (mass fraction)) in an analytical sample
$= A \times 0.1314 \times (V_1/V_2)\ /W \times 100$

A: Mass (g) of the precipitate
V_1: Predetermined volume (mL) of the sample solution in **(4.1) c)**
V_2: Transferred volume (mL) of the sample solution in **(4.2) a)**
B: Mass of an analytical sample (g)

Note (2) About 3 ml of tetraphenylborate solution per 10 mg of K_2O is required to form the precipitate of potassium tetraphenylborate

Reference
1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.122 - 128, Yokendo, Tokyo (1988)

(5) Flow sheet for the testing method: The flow sheet for citrate soluble potassium in fertilizers is shown below:

1 g analytical sample	Weigh to the order of 1 mg into a 250-mL volumetric flask.
← 150 mL citrate solution [about 30 ºC]	
Shaking to mix	Constant temperature rotary shaker (30 - 40 revolutions/min) 30 ºC ± 1 ºC, 1 hour
Standing to cool	
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined amount)	100-mL tall beaker
← Water (add tetraphenylborate solution to reach 50 mL) ← 5 mL of formaldehyde solution ← 5 mL of ethylenediamine tetraacetate - sodium hydroxide solution ← Tetraphenylborate solution (the equivalents of potassium + 4 mL)	
Forming precipitate	For 30 minutes, sometimes mixing
Transfer	Crucible type glass filtering apparatus 1G4, 5 times with 5 mL of tetraphenylborate washing solution
Washing	Wash 2 times with 2 mL of water
Drying	120 ºC ± 5 ºC for 1 hour.
Standing to cool	Desiccator
Measurement	Measure the mass to the order of 1 mg

Figure Flow sheet for citrate soluble potassium in fertilizers

4.3.2.c Sodium tetraphenylborate volumetric analysis

(1) Summary
This test method is applicable to fertilizers containing potassium silicate fertilizer, etc. but not organic matters.
Extract by adding citric acid solution to an analytical sample, mask co-existing ammonium and other salts with formaldehyde, and make potassium ion and tetraphenylborate react with each other. Conduct precipitate titration for unconsumed tetraphenylborate with benzaikonium chloride to obtain citrate soluble potassium (C-K_2O).

(2) Reagents: Reagents are as shown below:

- **a) Citric acid solution** (1)**:** Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1,000 mL.
- **b) Formaldehyde solution:** A JIS Guaranteed Reagent specified in JIS K 8872 or a reagent of equivalent quality.
- **c) Sodium hydroxide solution (120 g/L)** (1)**:** Dissolve 30 g of sodium hydroxide specified in JIS K 8576 in water to make 250 mL.
- **d) Tetraphenylborate solution** (1)**:** Transfer 12.2 g of sodium tetraphenylborate to a 1,000-mL volumetric flask, dissolve by adding about 800 mL of water and add 3 mL of sodium hydroxide (120 g/L) to the total filtrate, and further add water up to the marked line. Filter with Type 3 filter paper in the case of usage.
- **e) Benzaikonium chloride solution (3.3 g/500 mL)** (1)**:** Dissolve 3.3 g of benzaikonium chloride in 500 mL of water.
- **f) Methyl red solution (0.1 g/100 mL):** Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
- **g) Titan Yellow solution (0.04 g/100mL):** Dissolve 0.04 g of Titan Yellow in 100 mL of water in the case of usage.
- **h) Potassium standard solution (K_2O 2 mg/mL)**(1)**:** Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and then weigh 3.166 g into a weighing dish. Dissolve in a small amount of water, transfer to a 1,000-mL volumetric flask, and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as follows:

- **a) Constant-temperature rotary shaker:** A constant-temperature rotary shaker that can rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, upside down at 30 - 40 revolutions/min.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

- **a)** Weigh 1 g of an analytical sample to the order of 1 mg, and transfer to a 250-mL volumetric flask.
- **b)** Add 150 mL of citric acid solution heated up to about 30 ºC, and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.
- **c)** After standing to cool, add water up to the marked line.
- **d)** Filter with Type 3 filter paper to make the sample solution.

Comment 1 The procedure **(4.1)** is the same as the procedure **(4.1)** of **4.2.3.a**.

(4.2) Precipitate formation: Form precipitate as shown below.

a) Transfer 5 mL - 15 mL (no more than the equivalents of 30 mg as K_2O) of the extract to a 100-mL volumetric flask.
b) Add water to the solution to make about 30 mL.
c) Add about 5 mL of formaldehyde solution and add 5 mL of sodium hydroxide solution (120 g/L).
d) Add one or two drop (s) of tetraphenylborate solution per second while shaking to mix.
e) Add water up to the marked line and leave at rest for about 10 minutes.
f) Filter with Type 3 filter paper to make the sample solution.

(4.3) Measurement: Conduct measurement as shown below.

a) Calibration curve preparation

1) Transfer 1 mL - 15 mL of potassium standard solution (K_2O 2 mg/mL) to 100-mL volumetric flasks step-by-step.
2) Conduct the same procedures as **(4.2 b) - f)** to make K_2O 2 mg/100 mL - 30 mg/100 mL of the potassium standard solutions for the calibration curve preparation.
3) Conduct the same procedure as **2)** to make the blank test solution for the calibration curve preparation in another 100-mL volumetric flask.
4) Transfer 40 mL of the potassium standard solutions for a calibration curve preparation and the blank test solution for a calibration curve preparation to an Erlenmeyer flask respectively.
5) Add a few drops of Titan Yellow solution.
6) Titrate with benzaikonium chloride solution (3.3 g/500 mL) until the color of the solution changes to light red (2).
7) Prepare a curve for the relationship between the potassium concentration and the volume of the benzaikonium chloride solution (3.3 g/500 mL) required for the titration of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

b) Sample measurement

1) Transfer 40 mL of the sample solution of **(4.2) f)** to a 100-mL volumetric flask.
2) Conduct similarly as in **a) 5) - 6)** to obtain the volume of the benzaikonium chloride solution (3.3 g/500 mL) required for the titration.
3) Obtain the potassium content from the calibration curve, and calculate citrate soluble potassium (C-K_2O) in the analytical sample.

Note (2) If the solution temperature is no more than 20 ºC, the reaction does not advance in some cases. Therefore it is recommended to heat the solution up to about 30 ºC.

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.128 - 132, Yokendo, Tokyo (1988)

(5) Flow sheet for citrate soluble potassium: The flow sheet for citrate soluble potassium in fertilizers is shown below:

1 g analytical sample — Weigh to the order of 1 mg into a 250-mL volumetric flask.

← 150 mL citrate solution [about 30 ºC]

Shaking to mix — Constant temperature rotary shaker (30 - 40 revolutions/min) 30 ºC ± 1 ºC, 1 hour

Standing to cool

← Water (up to the marked line)

Filtration — Type 3 filter paper

Aliquot (5 mL - 15 mL) — 100-mL volumetric flask

← Water (the volume to reach about 30 mL)

← About 5 mL of formaldehyde solution

← 5 mL of sodium hydroxide solution (120 g/L)

← 25 mL tetraphenylborate solution (one or two drop (s) per second while shaking to mix)

← Water (up to the marked line)

Leaving at rest — 10 minutes

Filtration — Type 3 filter paper

←A few drops of Titan Yellow solution

Aliquot (40 mL) — 100-mL Erlenmeyer flask

← A few drops of titan yellow solution

Titration — Benzaikonium chloride solution (3.3 g/500 mL) (until the solution changes to light red)

Figure Flow sheet for citrate soluble potassium in fertilizers

4.3.3 Water-soluble potassium

4.3.3.a Flame atomic absorption spectrometry or flame photometry

(1) Summary

This test method is applicable to fertilizers containing potassium salts.
Extract by adding water to an analytical sample, add interference suppressor solution, and then spray in an acetylene-air flame, and measure the atomic absorption with potassium at a wavelength of 766.5 nm or 769.9 nm to quantify water-soluble potassium (W-K_2O). Or, determine the intensity of the emission line at a wavelength of 766.5 nm or 769.9 nm produced in flame to quantify water-soluble potassium (W-K_2O).

(2) Reagents: Reagents are as shown below:

- **a) Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **b) Interference suppressor solution:** Weigh 12.5 g of calcium carbonate specified in JIS K 8617 into a 2,000-mL beaker, add a small amount of water, gradually add 105 mL of hydrochloric acid, and heat for a little while. After standing to cool, add water to make 1,000 mL.
- **c) Potassium standard solution (K_2O 1 mg/mL)(1):** Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and then weigh 1.583 g into a weighing dish. Dissolve with a small amount of water, transfer to a 1,000-mL volumetric flask, and add water up to the marked line.
- **d) Potassium standard solution (K_2O 5 µg/mL - 50 µg/mL) for the calibration curve preparation (1):** Transfer 2.5 mL - 25 mL of potassium standard solution (K_2O 1 mg/mL) to 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (2), and add water up to the marked line.
- **e) Blank test solution for the calibration curve preparation (1):** Transfer about 50 mL of interference suppressor solution to a 500-mL volumetric flask (2), and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.
(2) Add interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of potassium standard solution (K_2O 1 mg/mL) in (**2**) **c**), potassium standard solution (K 1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiply by the conversion factor (1.2046) to use as potassium standard solution (K_2O 1.2046 mg/mL).

(3) Instruments: Instruments are as follows:

- **a) Rotary shaker:** A rotary shaker that can rotate a 500-mL volumetric flask upside down at 30 - 40 revolutions/min.
- **b) Analytical instrument**: An atomic absorption spectrometer or a flame photometer as shown below:
- **ba) Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121.
  - **1) Light source**: A potassium hollow cathode lamp
  - **2) Gas:** Gas for heating by flame
    - (i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

bb) Flame photometer:

1) Gas: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

c) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

(4.1.1) Mixed fertilizers containing potassium salts and magnesium potassium sulfate

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and transfer to a 300-mL tall beaker.

b) Add about 200 mL of water, and cover with a watch glass and heat on a hot plate to boil for about 15 minutes.

c) After standing to cool, transfer to a 250-mL volumetric flask with water.

d) Add water up to the marked line.

e) Filter with Type 3 filter paper to make the sample solution.

Comment 2 In the procedure in **a)**, a 250-mL volumetric flask can be used instead of a 300-mL tall beaker. However the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. Additionally, "cover with a watch glass" in **b)** is replaced by "place a long-stem funnel", and "transfer to a 250-mL volumetric flask with water" in the procedure in **c)** is skipped.

Comment 3 The procedure in **(4.1.1)** is the same as **(4.1.1)** of **4.3.3.b** and **4.3.3.c**, and **(4.1)** of **4.8.2.a**.

(4.1.2) Mixed fertilizers containing no magnesium potassium sulfate

a) Weigh 5 g of an analytical sample to the order of 1 mg, and transfer to a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make the sample solution.

Comment 4 In the procedure in **a)**, it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it into a 250-mL volumetric flask

Comment 5 The procedure in **(4.1.2)** is the same as the procedure in **(4.1)** of **4.2.4.a.**

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer or flame photometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer or flame photometer: Set up the measurement conditions for the atomic absorption spectrometer or flame photometer considering the following:
Analytical line wavelength: 766.5 nm or 769.9 nm

b) Calibration curve preparation

1) Spray the potassium standard solutions for a calibration curve preparation and the blank test solution for a calibration curve preparation into a flame, and read the indicated value at a wavelength of 766.5 nm or 769.9 nm.

2) Prepare a curve for the relationship between the potassium concentration and the indicated value of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as K_2O) to a 100-mL volumetric flask.
2) Add about 10 mL of interference suppressor solution (2), and add water up to the marked line.
3) Conduct similarly as in **b) 1)** to read the indicated value.
4) Obtain the potassium content from the calibration curve, and calculate the water-soluble potassium (W-K_2O) in the analytical sample.

Comment 6 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for water-soluble potassium hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Measurement method	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2006	High analysis compound fertilizer		156	12.38	0.22	1.8
2007	Organic compound fertilizer		145	8.43	0.15	1.8
2008	High analysis compound fertilizer	Atomic absorption spectrometry [6)]	75	11.38	0.13	1.1
		Flame photometry	57	11.42	0.16	1.4
2009	Ordinary compound fertilizer	Atomic absorption spectrometry	78	8.36	0.13	1.6
		Flame photometry	54	8.35	0.09	1.1
2010	High analysis compound fertilizer	Atomic absorption spectrometry	84	14.7	0.21	1.4
		Flame photometry	49	14.72	0.12	0.8
2011	High analysis compound fertilizer	Atomic absorption spectrometry	51	10.35	0.13	1.3
		Flame photometry	75	10.45	0.17	1.7
2012	Fluid mixed fertilizer	Atomic absorption spectrometry	47	2.44	0.04	1.8
		Flame photometry	75	2.42	0.06	2.6
2013	High analysis compound fertilizer	Atomic absorption spectrometry	57	11.72	0.93	3.3
		Flame photometry	65	11.81	0.33	2.8

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
$RSD_{rob} = (NIQR/M) \times 100$
6) Analyzed separately according to the measurement methods from 2008

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.136 - 138, Yokendo, Tokyo (1988)

(5) Flow sheet for water-soluble potassium: The flow sheet for water-soluble potassium in fertilizers is shown below:

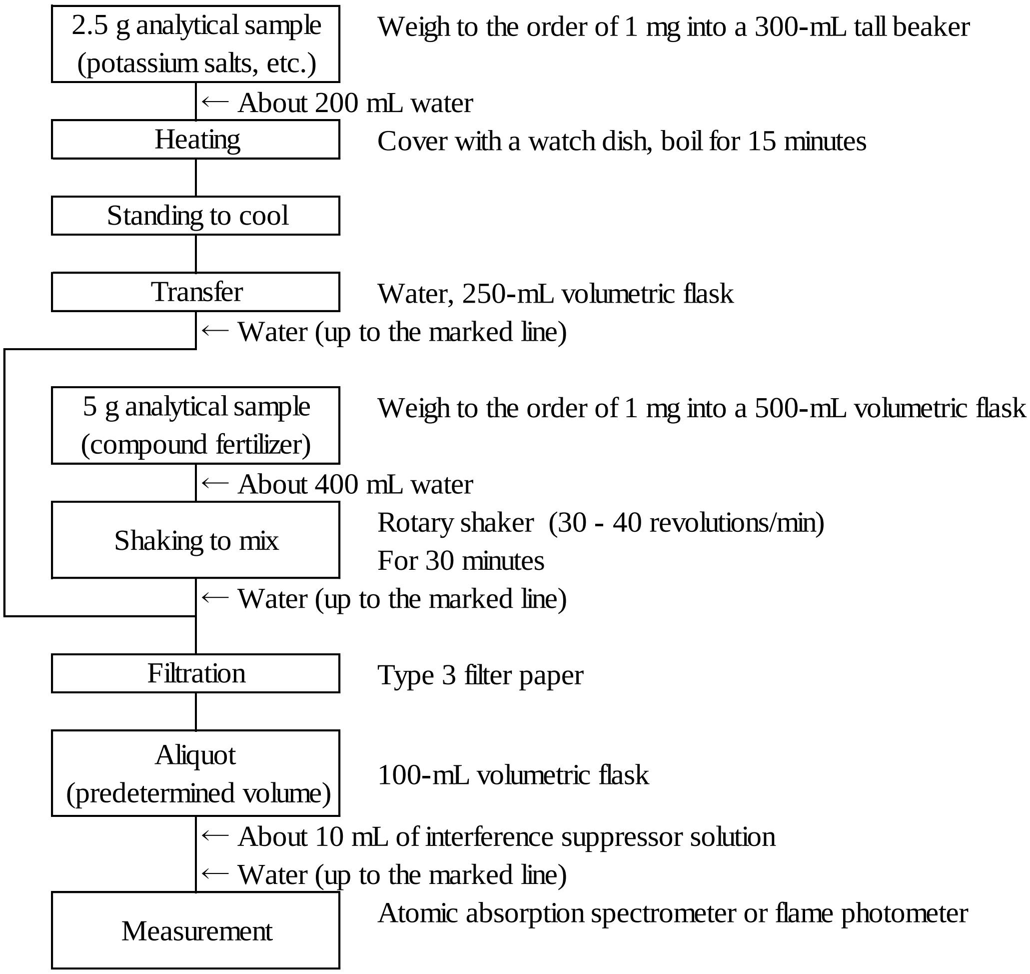


Figure Flow sheet for water-soluble potassium in fertilizers

4.3.3.b Sodium tetraphenylborate gravimetric analysis

(1) Summary

This test method is applicable to fertilizers containing potassium salts.
Extract by adding water to an analytical sample, mask co-existing ammonium and other salts with formaldehyde and ethylenediamine tetraacetate and measure the mass of potassium tetraphenylborate formed by the reaction with tetraphenylborate to obtain water-soluble potassium (W-K_2O).

(2) Reagents: Reagents are as shown below:

a) **Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
b) **Formaldehyde solution:** A JIS Guaranteed Reagent specified in JIS K 8872 or a reagent of equivalent quality.
c) **Sodium hydroxide solution (200 g/l)** (1)**:** Dissolve 200 g of sodium hydroxide specified in JIS K 8576 in water to make 1,000 mL.
d) **Aluminum chloride solution** (1)**:** Dissolve 12 g of aluminum chloride (III) hexahydrate specified in JIS K 8114 in water to make 100 mL.
e) **Tetraphenylborate solution** (1)**:** Transfer 6.1 g of Sodium tetraphenylborate to a 250-mL volumetric flask, dissolve by adding about 200 mL of water and add 10 mL of aluminum chloride solution. Add methyl red solution (0.1 g/100 mL) as an indicator, and neutralize with sodium hydroxide solution (200 g/L) until the color of the solution changes to yellow, and then add water up to the marked line. Filter with Type 3 filter paper and add sodium hydroxide solution (200 g/L) to the total filtrate. Filter with Type 3 filter paper in the case of usage.
f) **Tetraphenylborate washing solution** (1)**:** Dilute 40 mL of tetraphenylborate solution with water to make 1,000 mL.
g) **Ethylenediamine tetraacetate-sodium hydroxide solution** (1)**:** Dissolve 10 g of ethylenediaminetetraacetic acid dihydrogen disodium dihydrate specified in JIS K 8107 and 8 g of sodium hydroxide specified in JIS K 8576 in a proper amount of water, add 6 mL - 10 mL of tetraphenylborate solution while mixing according to potassium content coexisting as impurity after standing to cool and then add water to make 100 mL. After leaving at rest for about 30 minutes while sometimes mixing, filter with Type 3 filter paper.
h) **Methyl red solution (0.1 g/100 mL):** Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are as follows:

a) **Rotary shaker:** A rotary shaker that can rotate a 500-mL volumetric flask upside down at 30- 40 revolutions/min.
b) **Drying apparatus:** A drying apparatus that can be adjusted to 120 ºC ± 2 ºC.
c) **Crucible type glass filter:** Put a crucible type glass filter 1G4 specified in JIS R 3503 into a drying apparatus, stand to cool in a desiccator after heating at 120 ºC ± 2 ºC and measure the mass to the order of 1 mg.
c) **Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

(4.1.1) Mixed fertilizers containing potassium salts and magnesium potassium sulfate

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and transfer to a 300-mL tall beaker.
b) Add about 200 mL of water, and cover with a watch glass and heat on a hot plate to boil for about 15 minutes.
c) After standing to cool, transfer to a 250-mL volumetric flask with water.
d) Add water up to the marked line.
e) Filter with Type 3 filter paper to make the sample solution.

Comment 1 In the procedure in **a)**, a 250-mLvolumetric flask can be used instead of a 300-mL tall beaker. However the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. Additionally, "cover with a watch glass" in **b)** is replaced by "place a long-stem funnel", and "transfer to a 250-mL volumetric flask with water" in the procedure in **c)** is skipped.
Comment 2 The procedure in **(4.1.1)** is the same as **(4.1.1)** of **4.3.3.a**

(4.1.2) Mixed fertilizers containing no magnesium potassium sulfate
a) Weigh 5 g of an analytical sample to the order of 1 mg, and transfer to a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make the sample solution.

Comment 3 In the procedure in **a)**, it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it to a 250-mL volumetric flask
Comment 4 The procedure in **(4.1.2)** is the same as the procedure in **(4.1)** of **4.2.4.a**.

(4.2) Measurement: Conduct measurement as shown below.
a) Transfer a predetermined volume (the equivalents of 15 mg - 30 mg as K_2O) of sample solution to a 100-mL tall beaker.
b) Add water to the solution to reach 50 mL when the procedure in **e)** is complete.
c) Add 2 mL of hydrochloric acid (1+9).
d) Add 5 mL of formaldehyde solution, and then add 5 mL of ethylenediamine tetraacetate-sodium hydroxide solution.
e) Add necessary volume (2) of tetraphenylborate solution at the rate of one or two drop(s) per second while mixing, and further add 4 mL of the same solution in the same manner.
f) Leave at rest for about 30 minutes while sometimes mixing to form the precipitate of potassium tetraphenylborate.
g) Filter supernatant with a crucible type glass filter, wash the vessel 5 times with 5 mL of tetraphenylborate washing solution and transfer the whole precipitate to the crucible type glass filter and further wash 2 times with 2 mL of water.
h) Transfer the precipitate together with the crucible type glass filter into a drying apparatus adjusted to 120 ºC ± 2 ºC and heat for 1 hour.
i) After heating, move it quickly into a desiccator and let it stand to cool.
j) After standing to cool, remove a ground-in stoppered weighing bottle from the desiccator and measure the mass to the order of 1 mg.
k) Calculate water-soluble potassium (W-K_2O) in the analytical sample by the following formula.

Water-soluble potassium (W-K_2O) (% (mass fraction)) in an analytical sample

$= A \times 0.1314 \times (V_1/V_2)/W \times 100$

A: Mass (g) of precipitate
V_1: Predetermined volume (mL) of the sample solution in **(4.1.1) d)** or **(4.1.2) c)**
V_2: Volume (mL) of the sample solution transferred in **(4.2) a)**
W: Mass of an analytical sample (g)

Note **(2)** About 3 mL of tetraphenylborate solution per 10 mg of K_2O is required to form the precipitate of potassium tetraphenylborate

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.122 - 128, Yokendo, Tokyo (1988)

(5) Flow sheet for water-soluble potassium: The flow sheet for water-soluble potassium in fertilizers is shown below:

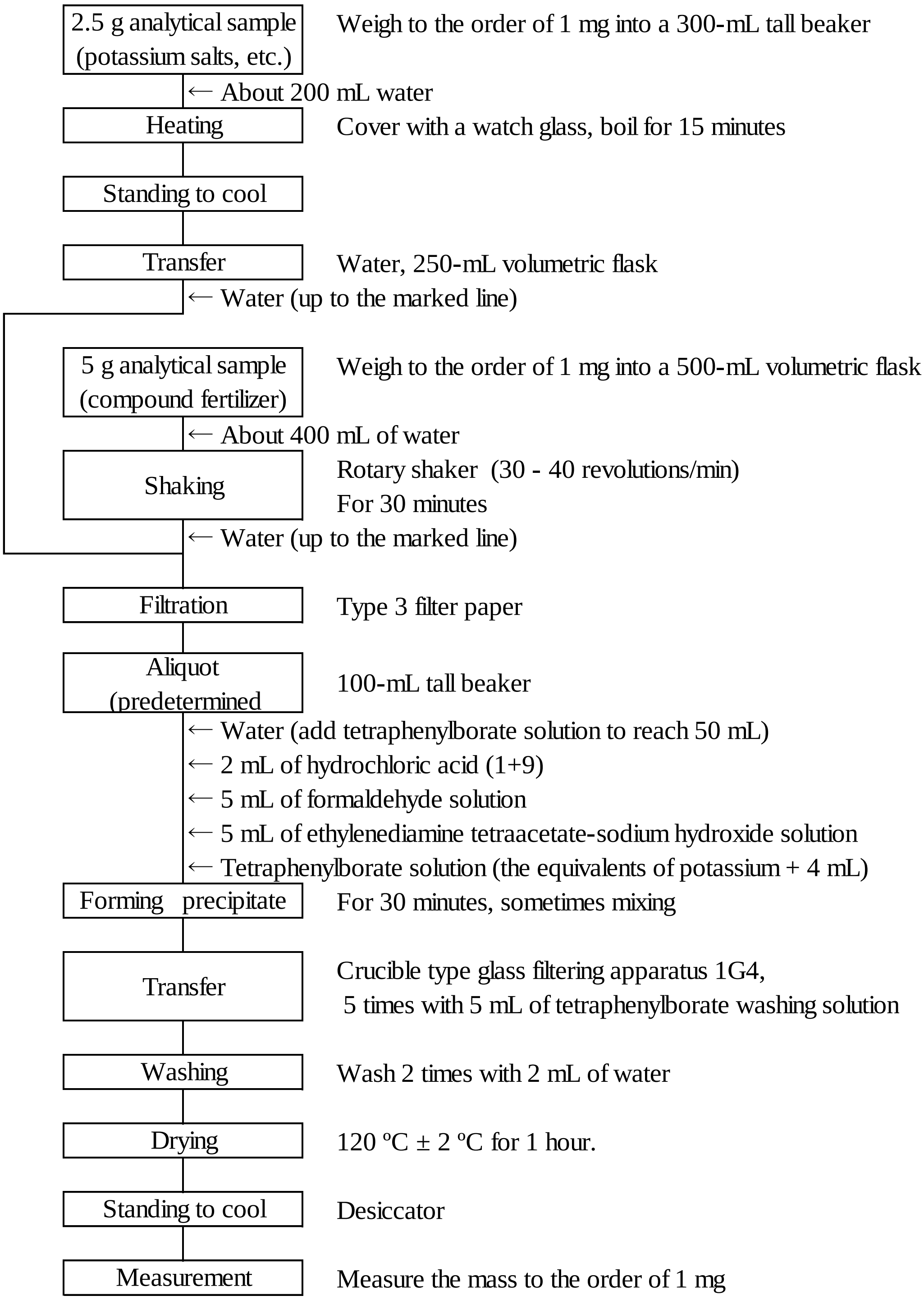


Figure Flow sheet for water-soluble potassium in fertilizers

4.3.3.c Sodium tetraphenylborate volumetric analysis

(1) Summary

This test method is applicable to fertilizers containing potassium salt but not organic matters. Extract by adding water to an analytical sample, mask co-existing ammonium and other salts with formaldehyde, and make potassium ion and tetraphenylborate react with each other. Conduct precipitate titration for unconsumed tetraphenylborate with benzaikonium chloride to obtain water-soluble potassium (W-K_2O).

(2) Reagents: Reagents are as shown below:

- **a) Formaldehyde solution:** A JIS Guaranteed Reagent specified in JIS K 8872 or a reagent of equivalent quality.
- **b) Sodium hydroxide solution (120 g/L)** (1)**:** Dissolve 30 g of sodium hydroxide specified in JIS K 8576 in water to make 250 mL.
- **c) Tetraphenylborate solution** (1)**:** Transfer 12.2 g of Sodium tetraphenylborate to a 1,000-mL volumetric flask, dissolve by adding about 800 mL of water and add 3 mL of sodium hydroxide (120g/L) to total filtrate, and further add water up to the marked line. Filter with Type 3 filter paper in the case of usage.
- **d) Benzaikonium chloride solution (3.3 g/500 mL)** (1)**:** Dissolve 3.3 g of benzaikonium chloride in 500 mL of water.
- **e) Methyl red solution (0.1 g/100 mL):** Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
- **f) Titan Yellow solution (0.04 g/100 mL):** Dissolve 0.04 g of Titan Yellow in 100 mL of water in the case of usage.
- **g) Potassium standard solution (K_2O 2 mg/mL)**(1)**:** Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 3.166 g into a weighing dish. Dissolve with a small amount of water, transfer to a 1,000-mL volumetric flask, and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are as follows:

- **a) Rotary shaker:** A rotary shaker that can rotate a 500-mL volumetric flask upside down at 30- 40 revolutions/min.
- **b) Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

(4.1.1) Mixed fertilizers containing potassium salts and magnesium potassium sulfate

- **a)** Weigh 2.5 g of an analytical sample to the order of 1 mg, and transfer to a 300-mL tall beaker.
- **b)** Add about 200 mL of water, and cover with a watch glass and heat on a hot plate to boil for about 15 minutes.
- **c)** After standing to cool, transfer to a 250-mL volumetric flask with water.
- **d)** Add water up to the marked line.
- **e)** Filter with Type 3 filter paper to make the extract.

Comment 1 In the procedure in **a)**, a 250-mL volumetric flask can be used instead of a 300-mL tall beaker. However the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. Additionally, “cover with a watch glass” in **b)** is replaced by “place a long-stem funnel", and "transfer to a 250-mL volumetric flask with water" in the procedure in **c)** is skipped.

Comment 2 The procedure of **(4.1.1)** is the same as **(4.1.1)** of **4.3.3.a**

(4.1.2) Mixed fertilizers containing no magnesium potassium sulfate

a) Weigh 5 g of an analytical sample to the order of 1 mg, and transfer to a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make the sample solution.

Comment 3 In the procedure of **a)**, it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it into a 250-mL volumetric flask

Comment 4 The procedure in **(4.1.2)** is the same as the procedure **(4.1)** of **4.2.4.a**.

(4.2) Precipitate formation: Form precipitate as shown below.

a) Transfer 5 mL - 15 mL (no more than the equivalents of 30 mg as K_2O) of the extract to a 100-mL volumetric flask.
b) Add water to the solution to make about 30 mL.
c) Add about 5 mL of formaldehyde solution and add 5 mL of sodium hydroxide (120 g/L).
d) Add 25 mL of tetraphenylborate solution at the rate of one or two drop(s) per second while shaking to mix.
e) Add water up to the marked line and leave at rest for about 10 minutes.
f) Filter with Type 3 filter paper to make the sample solution.

(4.3) Measurement: Conduct measurement as shown below.

a) Calibration curve preparation

1) Transfer 1 mL - 15 mL of potassium solution (K_2O 2 mg/mL) to 100-mL volumetric flasks step-by-step.
2) Conduct the same procedures as **(4.2 b) - f)** to make K_2O 2 mg/100 mL - 30 mg/100 mL of the potassium standard solutions for the calibration curve preparation.
3) Conduct the same procedure as **2)** to make the blank test solution for the calibration curve preparation in another 100-mL volumetric flask.
4) Transfer 40 mL of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation to an Erlenmeyer flask respectively.
5) Add a few drops of Titan Yellow solution.
6) Titrate with benzaikonium chloride solution (3.3 g/500 mL) until the color of the solution changes to light red (2).
7) Prepare a curve for the relationship between the potassium concentration and the volume of the benzaikonium chloride solution (3.3 g/500 mL) required for the titration of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

b) Sample measurement

1) Transfer 40 mL of the sample solution of **(4.2) f)** to a 100-mL volumetric flask.
2) Conduct similarly as in **a) 5) - 6)** to obtain the volume of the benzaikonium chloride solution (3.3 g/500 mL) required for the titration.

3) Obtain the potassium content from the calibration curve, and calculate water-soluble potassium (W-K_2O) in the analytical sample.

Note **(2)** If the solution temperature is no more than 20 ºC, the reaction does not advance in some cases. Therefore it is recommended to heat the solution up to about 30 ºC.

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.128 - 132, Yokendo, Tokyo (1988)

5) **Flow sheet for water-soluble potassium t:** The flow sheet for water-soluble potassium in fertilizers is shown below:

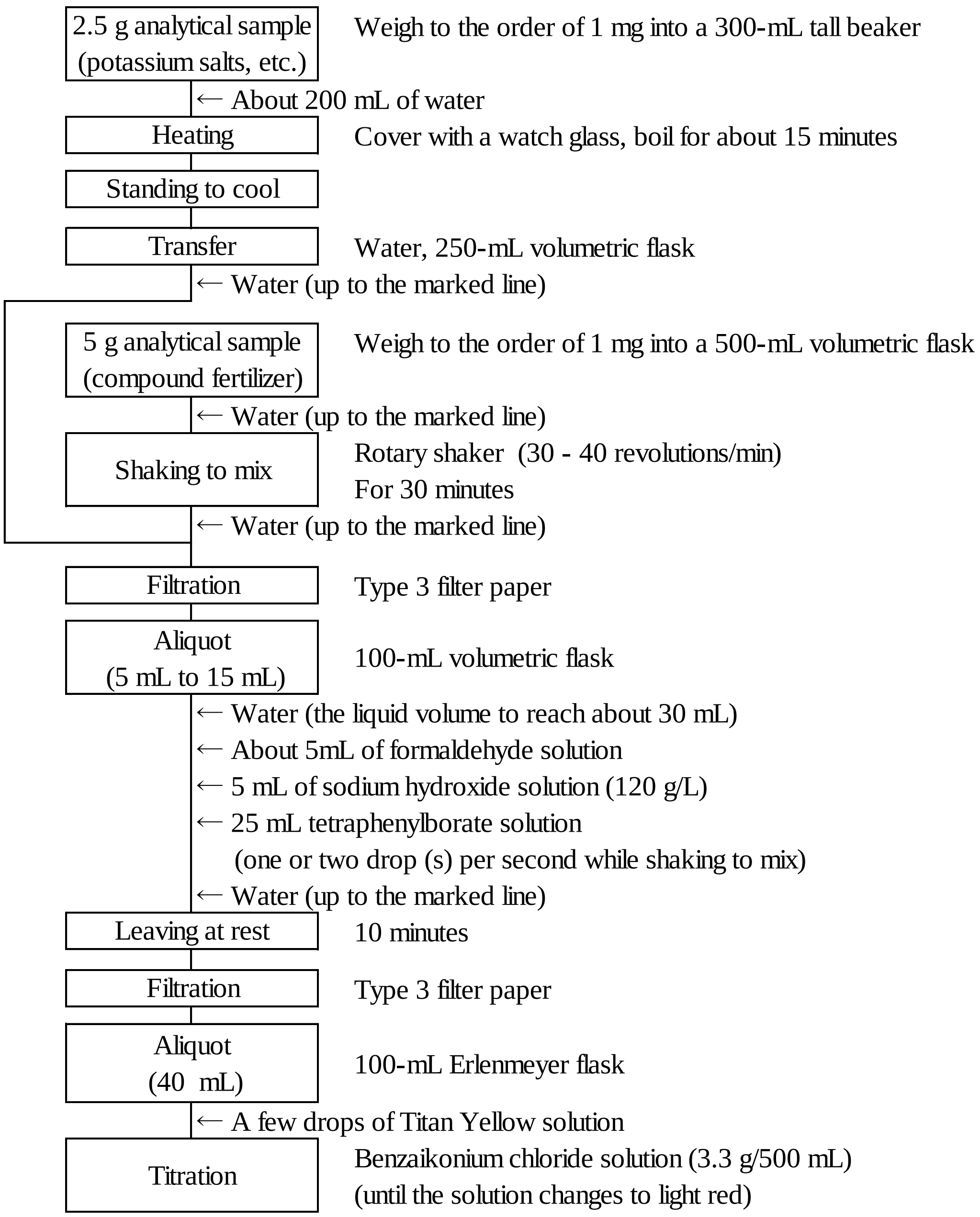


Figure Flow sheet for water soluble potassium in fertilizers

4.4 Silicic acid

4.4.1 Soluble silicic acid

4.4.1.a Potassium fluoride method

(1) Summary
This test method is applicable to fertilizers containing no silica gel fertilizers.
Extract by adding hydrochloric acid (1+23) to an analytical sample, add hydrochloric acid, potassium chloride and potassium fluoride solution and cool in a refrigerator, and then filter after forming precipitate as potassium silicofluoride. Put the precipitate in water and heat, and measure by neutralization titration to obtain the hydrochloric acid (1+23) soluble silicic acid (soluble silicic acid (S-SiO_2)) in an analytical sample.

(2) Reagents: Reagents are as shown below:

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution**(1): Transfer about 30 mL of water to a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4 - 5 days. Transfer 5.5 mL - 11 mL of the supernatant to a ground-in stoppered storage container, and add 1,000 mL of water containing no carbonic acid.
Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then transfer about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve with a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Transfer a predetermined amount of the solution to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 mg/100 mL) as an indicator, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f)
$= (W \times A \times 0.01/97.095) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C)$

W: Mass (g) of sulfamic acid sampled
A: Purity (%) of sulfamic acid
V_1: Volume (mL) of sulfamic acid solution transferred
V_2: Constant volume (250 mL) of sulfamic acid solution
V_3: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

c) **Potassium chloride:** A JIS Guaranteed Reagent specified in JIS K 8121 or a reagent of equivalent quality.

d) **Potassium chloride solution (1):** Add 250 mL of ethanol specified in JIS K 8101 to 750 mL of water to mix, and add 150 mL of potassium chloride to dissolve. Add a few drops of methyl red solution (0.1 g/100 mL) as an indicator and drop hydrochloric acid until the color of the solution becomes red to make it acidic. After leaving at rest for 1 day, neutralize with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution.

e) **Potassium fluoride solution (1):** Dissolve 58 g of potassium fluoride specified in JIS K 8815 in 1,000 mL of water (2).

f) **Methyl red solution (0.1 g/100 mL):** Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

g) **Phenolphthalein solution (1 g/100 mL):** Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) Store in a container made of polyethylene, etc. that contains no silicon.

(3) **Apparatus and instruments:** Apparatus and instruments are as follows:

a) **Constant-temperature rotary shaker:** A constant-temperature rotary shaker that can rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, upside down at 30 - 40 revolutions/min.

b) **Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedures

(4.1) **Extraction:** Conduct extraction as shown below**.**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and transfer to a 250-mL volumetric flask.

b) Add 150 mL of hydrochloric acid heated up to about 30 ºC, and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After standing to cool, add water up to the marked line.

d) Filter with Type 3 filter paper to make the sample solution.

Comment 1 The procedure in **(4.1)** is the same as the procedure in **(4.1)** of **4.4.1.d**.

(4.2) **Measurement:** Conduct measurement as shown below.

a) Transfer a predetermined volume (the equivalents of 20 mg - 50 mg as SiO_2 and no more than 25 mL of liquid volume) to a 200-mL beaker made of polyethylene.

b) Add about 10 mL of hydrochloric acid and about 15 mL of potassium fluoride solution, and further add about 2 g of potassium chloride to dissolve, and then cool in a refrigerator for 30 minutes or more (3) to form the precipitate of potassium fluoride.

c) Filter with a Gooch crucible (4) made of polyethylene topped with Type 6 filter paper, and wash the container 3 times with potassium chloride solution, then move the whole precipitate into the crucible, and further wash 6 - 7 times with a small amount of potassium chloride solution (5).

d) Move the precipitate on the filter together with the filter paper into a 300-mL tall beaker with water, and further add water to make about 200 mL and heat it up to 70 ºC - 80 ºC on a hot plate.

e) Add a few drops of phenolphthalein solution (1 g/100 mL) to the sample solution as an indicator and titrate with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes light red.

f) Calculate soluble silicic acid ($S\text{-}SiO_2$) by the following formula.

Soluble silicic acid ($S\text{-}SiO_2$) (% (mass fraction)) in an analytical sample

$$= V_S \times C \times f \times (V_4/V_5) \times (15.021/W) \times (100/1{,}000)$$

V_S: Volume of the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution required for titration

C: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

f: Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

V_4: Predetermined volume (mL) of the extract in **(4.1) c)**

V_5: Volume (mL) of the extract transferred in **(4.2) a)**
W: Mass (g) of an analytical sample

Note **(3)** To be no more than 10 °C
(4) Filter paper pulp can be stuffed to restrain precipitate from outflowing.
(5) Until the filtrate becomes neutral.

Comment 2 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for soluble silicic acid hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2008	Slag silicate fertilizer	55	33.34	0.47	1.4
2009	Slag silicate fertilizer	59	32.67	0.59	1.8
2010	Slag silicate fertilizer	52	33.50	0.59	1.8
2011	Slag silicate fertilizer	46	30.69	0.76	2.5
2012	Slag silicate fertilizer	46	35.96	0.37	1.0
2013	Slag silicate fertilizer	46	35.14	0.55	1.6

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
$RSD_{rob} = (NIQR/M) \times 100$

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.144 - 146, Yokendo, Tokyo (1988)

5) Flow sheet for soluble silicic acid: The flow sheet for soluble silicic acid in fertilizers is shown below:

1 g analytical sample	Weigh to the order of 1 mg to a 250-mL volumetric flask.
← About 150 mL hydrochloric acid (1+23) [about 30 °C]	
Shaking to mix	Rotary shaker (30 - 40 revolutions/min) 30 °C ± 1 °C, 1 hour
Standing to cool	Room temperature
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined amount)	200-mL beaker made of polyethylene
← About 10 mL hydrochloric acid ← About 15mL potassium fluoride solution ← 2 g of potassium chloride	
Cooling	For 30 minutes or more in a refrigerator
Filtration	Gooch crucible made of polyethylene Type 6 filter paper
Washing	Wash 6 - 7 times with potassium chloride solution
Transfer	300-mL tall beaker, water
← Water (until the liquid volume reaches about 200 mL)	
Heating	70 °C - 80 °C
← A few drops of phenolphthalein solution (1 g/100 mL)	
Titration	0.1 mol/L - 0.2 mol/L sodium hydroxide solution (Until the solution becomes light red)

Figure Flow sheet for soluble silicic acid in fertilizers

4.4.1.b Potassium fluoride method (Silica gel fertilizers, etc.)

(1) Summary

This test method is applicable to silica gel fertilizers and silica hydrogel fertilizers.
Extract by adding sodium hydroxide (20 g/L) to an analytical sample, add hydrochloric acid, potassium chloride and a potassium fluoride solution and cool in a refrigerator. After forming the precipitate as potassium silicofluoride, put the precipitate in water and heat , then measure by neutralization titration to obtain the sodium hydroxide (20 g/L) soluble silicic acid (soluble silicic acid (S-SiO_2)) in the analytical sample.

(2) Reagents: Reagents are as shown below:

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution**(1): Transfer about 30 mL of water to a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4 - 5 days. Transfer 5.5 mL - 11 mL of the supernatant to a ground-in stoppered storage container, and add 1,000 mL of water containing no carbonic acid.
Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then transfer about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve with a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Transfer a predetermined amount of the solution to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 mg/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)
$= (W_1 \times \mathrm{A} \times 0.01/97.095) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C_1)$

W_1: Mass (g) of sulfamic acid sampled
A: Purity (%) of sulfamic acid
V_1: Volume (mL) of sulfamic acid solution transferred
V_2: Constant volume (250 mL) of sulfamic acid solution
V_3: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sodium hydroxide:** A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.

c) **Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

d) **Potassium chloride:** A JIS Guaranteed Reagent specified in JIS K 8121 or a reagent of equivalent quality.

e) **Potassium chloride solution** (1)**:** Add 250 mL of ethanol specified in JIS K 8101 to 750 mL of water to mix, and add 150 mL of potassium chloride to dissolve. Add a few drops of methyl red solution (0.1 g/100 mL) as an indicator and drop hydrochloric acid until the color of the solution becomes red to make it acidic, and after leaving at rest for 1 day, neutralize with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution.

f) **Potassium fluoride solution** (1)**:** Dissolve 58 g of potassium fluoride specified in JIS K 8815 in 1,000 mL of water (2).

g) **Methyl red solution (0.1 g/100 mL):** Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
h) **Phenolphthalein solution (1 g/100 mL):** Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) Store in a container made of polyethylene, etc. that contains no silicon.

(3) Apparatus and instruments: Apparatus and instruments are as follows:
a) **Constant-temperature bath:** A constant-temperature bath adjustable up to 60 ºC ± 2 ºC.
b) **Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.
c) **Volumetric flask made of polymer**: A volumetric flask that is made of polyethylene, etc. of a quality of material which prevents silicic acid from eluting in the extract procedure in **(4.1)**.

(4) Test procedures
(4.1) Extraction: Conduct extraction as shown below**.**
a) Weigh 1 g of an analytical sample to the order of 1 mg, and transfer to a 250-mL volumetric flask made of polymer.
b) Add about 150 mL of sodium hydroxide solution heated up to about 65 ºC, and heat for 1 hour while shaking to mix at every 10 minutes in a water bath at 65 ºC ± 2 ºC.
c) After standing to cool, add water up to the marked line.
d) Filter with Type 3 filter paper to make the sample solution.

(4.2) Measurement: Conduct measurement as shown below.
a) Transfer a predetermined volume (the equivalents of 20 mg - 50 mg as SiO_2 and no more than 25 mL of liquid volume) to a 200-mL beaker made of polyethylene.
b) Add about 10 mL of hydrochloric acid and about 15 mL of potassium fluoride solution, and further add about 2 g of potassium chloride to dissolve, and then cool in a refrigerator for about 30 minutes (3) to form the precipitate of potassium fluoride.
c) Filter with a Gooch crucible (4) made of polyethylene topped with Type 6 filter paper, and wash the container 3 times with potassium chloride solution, then move the whole precipitate into the crucible, and further wash 6 - 7 times with a small amount of potassium chloride solution (5).
d) Move the precipitate on the filter together with the filter paper into a 300-mL tall beaker with water, and further add water to make about 200 mL and heat up to 70 ºC - 80 ºC on a hot plate.
e) Add a few drops of phenolphthalein solution (1 g/100 mL) to the sample solution as an indicator and titrate with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes light red.
f) Calculate soluble silicic acid (S-SiO_2) in the analytical sample by the following formula.

Soluble silicic acid (S-SiO_2) (% (mass fraction)) in an analytical sample
$= V_S \times C \times f \times (V_4/V_5) \times (15.021/W) \times (100/1,000)$

V_S: Volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide required for titration
C: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f: factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
V_4: Predetermined volume (mL) of the extract in **(4.1) c)**
V_5: Volume (mL) of the extract transferred in **(4.2) a)**

W: Mass (g) of an analytical sample

Note **(3)** To be no more than 10 ºC
(4) Filter paper pulp can be stuffed to restrain precipitate from outflowing.
(5) Until the filtrate becomes neutral.

Comment 1 Replicate testing was conducted using silica gel fertilizer (8 samples); as a result, the mean was in the range of 66.81 % - 91.31 % (mass fraction), and the standard deviation and relative standard deviation were 0.27 % - 0.86 % (mass fraction) and 0.3 % - 1.0 %, respectively.
Additionally, table 1 shows results and analysis results from a collaborative study for test method validation.

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Table 1 Results and analysis results from a collaborative study
for the test method validation of soluble silicic acid in silica gel fertilizers

Sample name	Number of laboratories[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	s_R[6] (%)[3]	RSD_R[7] (%)
Silica gel fertilizers 1	8	79.37	0.23	0.3	0.55	0.7
Silica gel fertilizers 2	8	84.68	0.42	0.5	0.85	1
Silica gel fertilizers 3	8	89.58	0.4	0.4	0.51	0.6
Silica gel fertilizers 4	8	84.44	0.37	0.4	0.77	0.9
Silica gel fertilizers 5	8	85.77	0.46	0.5	0.59	0.7

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Takeshi HASHIMOTO, Akira SHIMIZU and Kaori OKADA: Validation of a Method of Potassium Fluoride for Determination of Sodium Hydroxide-Soluble Silicic Acid in Silica Gel Fertilizer, Research Report of Fertilizer, Vol.3, 2010. (19 - 24)

2) Akira SHIMIZU, Shin ABE and Jun ITO: Determination of Solubility Silicic Acid in Silica gel Fertilizer and Silica gel-including Fertilizer by Potassium Fluoride Method: A Collaborative Study, Research Report of Fertilizer, Vol.5, 2012. (31 - 40)

5) Flow sheet for soluble silicic acid: The flow sheet for soluble silicic acid in silica gel fertilizers, etc. is shown below:

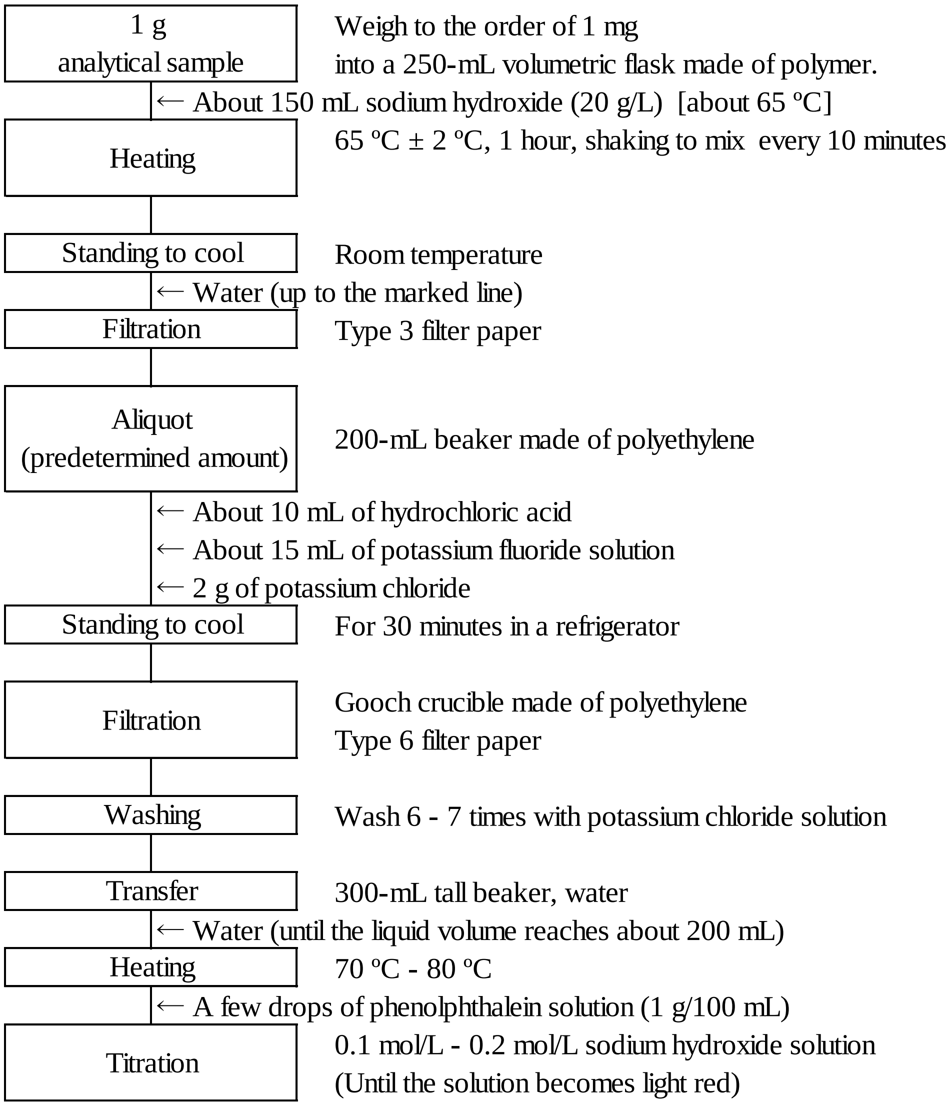


Figure Flow sheet for soluble silicic acid in silica gel fertilizers

4.4.1.c Potassium fluoride method (Fertilizers containing silica gel fertilizers)

(1) Summary

This test method is applicable to fertilizers containing silica gel fertilizers.

Mix the equivalent volumes of the extract which is extracted by adding hydrochloric acid (1+23) to an analytical sample and the liquid by extracting non-dissolved matter on a filter paper with sodium hydroxide (20 g/L), and add hydrochloric acid, potassium fluoride solution and potassium chloride. Cool it in a refrigerator, and form precipitate as potassium silicofluoride. Then transfer water in the precipitate, heat and measure by neutralization titration to obtain soluble silicic acid (S-SiO_2) in an analytical sample.

(2) Reagents: Reagents are as shown below:

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution**[(1)]: Transfer about 30 mL of water to a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4 - 5 days. Transfer 5.5 mL - 11 mL of the supernatant to a ground-in stoppered storage container, and add 1,000 mL of water containing no carbonic acid.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then transfer about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve with a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line [(1)]. Transfer a predetermined amount of the solution to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 mg/100 mL) as an indicator, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f)

$$= (W \times A \times 0.01/97.095) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C)$$

W: Mass (g) of sulfamic acid sampled
A: Purity (%) of sulfamic acid
V_1: Volume (mL) of sulfamic acid solution transferred
V_2: Constant volume (250 mL) of sulfamic acid solution
V_3: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C: Estimated concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sodium hydroxide:** A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.

c) **Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

d) **Potassium chloride:** A JIS Guaranteed Reagent specified in JIS K 8121 or a reagent of equivalent quality.

e) **Potassium chloride solution** [(1)]**:** Add 250 mL of ethanol specified in JIS K 8101 to 750 mL of water to mix, and add 150 mL of potassium chloride to dissolve. Add a few drops of methyl red solution (0.1 g/100 mL) as an indicator and drop hydrochloric acid until the color of the solution becomes red to make it acidic. After leaving at rest for 1 day, neutralize with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution.

f) **Potassium fluoride solution** [(1)]**:** Dissolve 58 g of potassium fluoride specified in JIS K 8815 in 1,000 mL of water [(2)].
g) **Methyl red solution (0.1 g/100 mL):** Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
h) **Phenolphthalein solution (1 g/100 mL):** Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) Store in a container made of polyethylene, etc. that contains no silicon.

(3) **Instruments:** Instruments are as follows:
a) **Constant-temperature bath:** A constant-temperature bath adjustable up to 80 ºC which can keep test temperature ± 2 ºC and set a 250-mL volumetric flask made of polyethylene that contains no silicon in it.
b) **Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.
c) **Volumetric flask made of polymer**: A volumetric flask that is made of polyethylene, etc. of a quality of material which prevents silicic acid from eluting in the extract procedure in **(4.1)**.

(4) Test procedures
(4.1) Extraction: Conduct extraction as shown below**.**
a) Weigh 1 g of an analytical sample to the order of 1 mg, and transfer to a 300-mL tall beaker.
b) Add 150 mL of hydrochloric acid (1+23) warmed up to about 30 ºC, and warm it in a water bath at 30 ºC ± 2 ºC while stirring every 10minutes with a glass rod for 60 minutes.
c) After standing to cool, filter with Type 6 filter paper to a 250-mL volumetric flask as an acceptor. Wash the tall beaker with water, then move the whole residue on the filter paper and add water up to the marked line to make the sample solution (1).
d) Transfer the non-dissolved matter on the filter paper together with the filter paper to a 250- mL volumetric flask made of polyethylene.
e) Add about 150 mL of sodium hydroxide solution (20 g/L) warmed up to about 65 ºC, and heat for 60 minutes while shaking to mix at every 10 minutes in a water bath at 65 ºC ± 2 ºC.
f) After standing to cool, add water up to the marked line and filter with Type 3 filter paper to make the sample solution (2).

(4.2) Measurement: Conduct measurement as shown below.
a) Transfer a predetermined volume (the equivalents of 20 mg - 50 mg as SiO_2) [(3)] of the sample solution (1) and (2) to a 200-mL beaker made of polyethylene.
b) Add about 10 mL of hydrochloric acid and about 15 mL of potassium fluoride solution, and further add about 2 g of potassium chloride to dissolve, and then cool in a refrigerator for about 30 minutes [(4)] to form the precipitate of potassium fluoride.
c) Filter with a Gooch crucible [(5)] made of polyethylene topped with Type 6 filter paper, and wash the container 3 times with potassium chloride solution, then move the whole precipitate into the crucible, and further wash 6 - 7 times with a small amount of potassium chloride solution [(6)].
d) Move the precipitate on the filter together with the filter paper into a 300-mL tall beaker with water, and further add water to make about 200 mL of solution.
e) Heat up to 70 ºC - 80 ºC on a hot plate. Then, add a few drops of phenolphthalein solution (1 g/100 mL) to the sample solution as an indicator and titrate with the 0.1

mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes light red.

f) Calculate soluble silicic acid (S-SiO_2) in the analytical sample by the following formula.

Soluble silicic acid (S-SiO_2) (%) in an analytical sample
$= V_S \times C \times f \times (V_1/V_2) \times (15.021/W) \times (100/1000)$

V_S: Volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide required for titration
C: Estimated concentration (mol/L) of sodium hydroxide solution (0.1 mol/L - 0.2 mol/L)
f: factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
V_1: Predetermined volume (250 mL) of the sample solution in **(4.1) c)**
V_2: Volume (mL) of the sample solution transferred in **(4.2) a)**
W: Mass (g) of an analytical sample

Note **(3)** The transferred volumes of the sample solution (1) and (2) should be equal.
(4) To be no more than 10 ºC
(5) Filter paper pulp can be stuffed to restrain precipitate from outflowing.
(6) Until the filtrate becomes neutral.

Comment 1 Replicate testing was conducted using fertilizers (13 samples) including a silica gel fertilizer; as a result, the mean was in the range of 12.85 % - 42.49 % (mass fraction), and the standard deviation and repeatability relative standard deviation were 0.04 % - 0.29 % (mass fraction) and 0.2 % - 1.2 %, respectively.
Additionally, table 1 shows results and analysis results from a collaborative study for test method validation.

Table 1 Results and analysis results from a collaborative study for the test method validation of soluble silicic acid in fertilizers including a silica gel fertilizer

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Mixed phosphate fertilizer 1	8.00	24.99	0.16	0.6	0.33	1.3
Mixed phosphate fertilizer 2	8.00	34.50	0.26	0.7	0.48	1.4
Compound fertilizers 1	8.00	30.30	0.13	0.4	0.60	2.0
Compound fertilizers 2	8.00	33.34	0.13	0.4	0.47	1.4
Compound fertilizers 3	8.00	15.76	0.11	0.7	0.21	1.3

1) Number of laboratories used in analysis
2) Total mean (n= number of laboratories × number of replication(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Akira SHIMIZU, Jun ITO and Shin ABE: Method Validation of Potassium Fluoride Method for Determination of Acid-Soluble and Base-Soluble Silicic Acid in Fertilizer containing Silica gel, Research Report of Fertilizer, Vol.4, 2011. (1 - 8)

2) Akira SHIMIZU: Method Validation of Potassium Fluoride Method for Determination of Acid-Soluble and Base-Soluble Silicic Acid in Fertilizer containing Silica Gel, Research Report of Fertilizer, Vol.6, 2013. (1 - 8)

5) Flow sheet for soluble silicic acid: The flow sheet for soluble silicic acid in fertilizers including silica gel fertilizers is shown below:

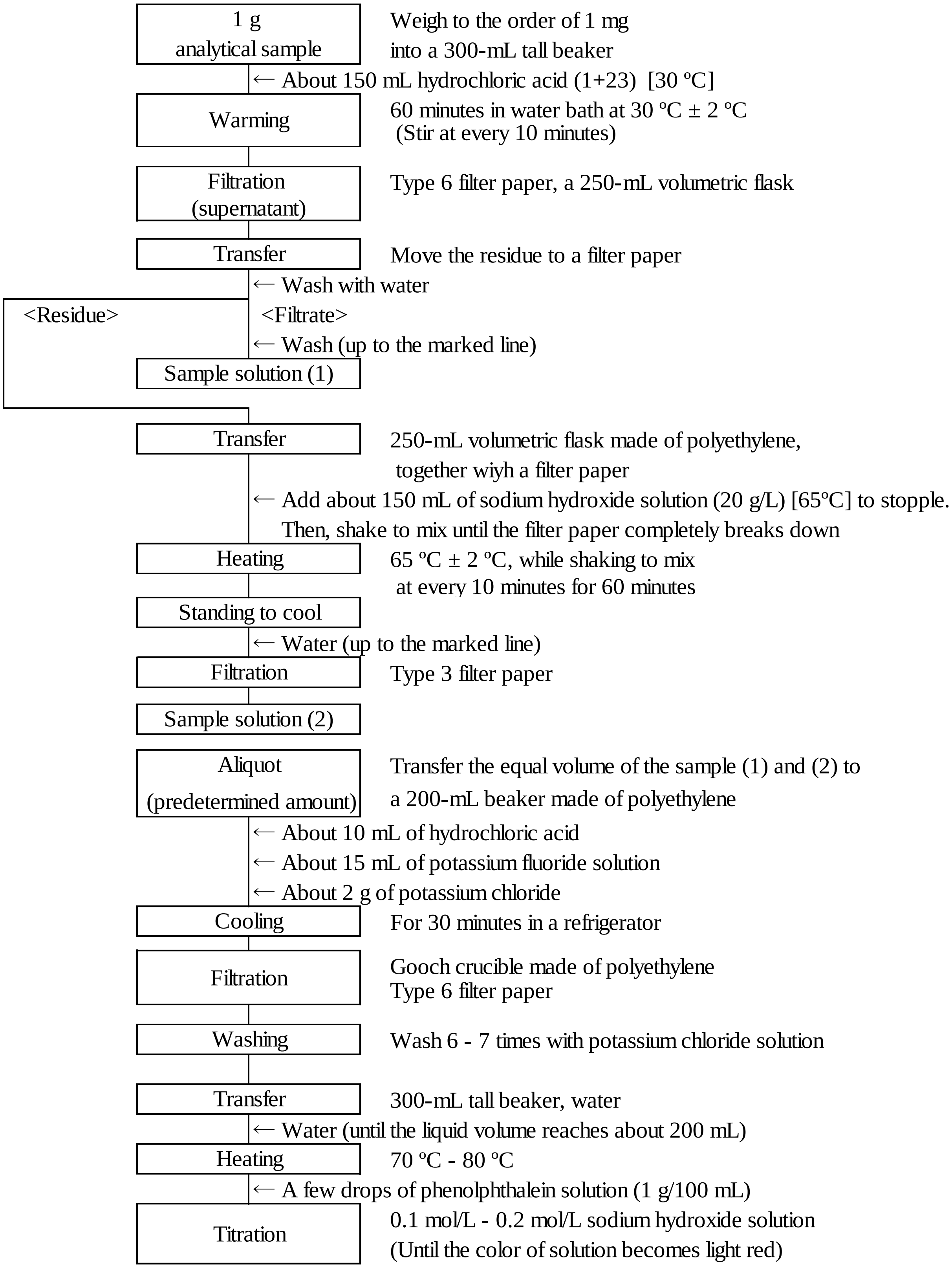


Figure Flow sheet for soluble silicic acid in fertilizers

4.4.1.d Perchloric acid method

(1) Summary

Extract by adding hydrochloric acid (1+23) to an analytical sample, add perchloric acid and heat, and then measure the formed silicic acid anhydride to obtain hydrochloric acid (1+23) soluble silicic acid (soluble silicic acid (S-SiO_2)) in the analytical sample.

(2) Reagents: Reagents are as shown below:

- **a) Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **b) Perchloric acid:** A JIS Guaranteed Reagent specified in JIS K 8223 or a reagent of equivalent quality.

(3) Apparatus and instruments: Apparatus and instruments are as follows:

- **a) Constant-temperature rotary shaker:** A constant-temperature rotary shaker that can rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, upside down at 30 - 40 revolutions/min.
- **b) Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.
- **c) Electric funnel**: An electric funnel that can be adjusted to 1,000 ºC - 1,100 ºC.
- **d) Crucible:** After heating a chemical analysis porcelain crucible specified in JIS R 1301 in an electric funnel at 1,000 ºC - 1,100 ºC, let it stand to cool in a desiccator and measure the mass to the order of 1 mg.

(4) Test procedures

(4.1) Extraction:

- **a)** Weigh 1 g of an analytical sample to the order of 1 mg, and transfer to a 250-mL volumetric flask.
- **b)** Add 150 mL of hydrochloric acid heated up to about 30 ºC, and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.
- **c)** After standing to cool, add water up to the marked line.
- **d)** Filter with Type 3 filter paper to make the sample solution.

Comment 1 The procedure in **(4.1)** is the same as the procedure in **(4.1)** of **4.4.1.a**.

(4.2) Measurement:

- **a)** Transfer a predetermined volume to a 100-mL tall beaker.
- **b)** Add about 10 mL of perchloric acid and heat.
- **c)** When white smoke from the perchloric acid starts evolving, cover with a watch glass, then heat for 15 - 20 minutes to form precipitate of silicone dioxide.
- **d)** After standing to cool, add about 50 mL of hydrochloric acid (1+4) and cover with a watch glass and heat at 70 ºC - 80 ºC on a hot plate for several minutes.
- **e)** Immediately after heating, filter with Type 5-C filter paper, wash the container with heated hydrochloric acid (1+10) and move the whole precipitate to a filter paper.
- **f)** Wash the precipitate and the filter paper 2 times with heated hydrochloric acid (1+10), and further wash several times with hot water (1).
- **g)** Put the precipitate together with the filter paper into the crucible.
- **h)** Put the crucible into a drying apparatus and dry at about 120 ºC for 1 hour.
- **i)** After standing to cool, put the crucible into an electric funnel and heat gently to char.
- **j)** Ignite at 1,000 ºC - 1,100 ºC for 1 hour.
- **k)** After ignition, move the crucible to a desiccator and let it stand to cool.
- **l)** After standing to cool, remove the crucible from the desiccator and measure the mass to the order of 1 mg.

m) Calculate soluble silicic acid (S-SiO_2) in an analytical sample by the following formula.

Soluble silicic acid (S-SiO_2) (% (mass fraction)) in an analytical sample
$= A \times (V_1/V_2)/B \times 100$

A: Mass (g) of precipitate
B: Mass (g) of an analytical sample
V_1: Predetermined volume (mL) of the sample solution in **(4.1) c)**
V_2: Volume (mL) of the sample solution transferred in **(4.2) a)**

Note (1) Wash until no reaction by chloride appears in the filtrate.

Comment 2 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for soluble silicic acid hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2008	Slag silicate fertilizer	13	33.58	0.42	1.2
2010	Slag silicate fertilizer	12	33.72	0.30	0.9
2012	Slag silicate fertilizer	13	36.09	0.63	1.7
2013	Slag silicate fertilizer	11	35.50	0.70	2.0

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
$RSD_{rob} = (NIQR/M) \times 100$

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.143 - 144, Yokendo, Tokyo (1988)

5) Flow sheet for soluble silicic acid: The flow sheet for soluble silicic acid in fertilizers is shown below:

1 g analytical sample	Weigh to the order of 1 mg into a 250-mL volumetric flask.
← About 150 mL of hydrochloric acid (1+23) [about 30 °C]	
Shaking to mix	Rotary shaker (30 - 40 revolutions/min) 30 °C ± 1 °C, 1 hour
Standing to cool	Room temperature
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined amount)	100-mL tall beaker
←About 10 mL of perchloric acid	
Heating	When white smoke of perchloric acid starts evolving, cover with a watch glass, heat for 15 - 20 minutes
Standing to cool	
←About 50 mL of hydrochloric acid (1+4)	
Heating	Cover with a watch glass, heat at 70 °C - 80 °C for several minutes
Filtration	Type 5-C filter paper
←Wash 2 times with about 10 mL of hydrochloric acid (1+10)	
←Wash several times with hot water	
Drying	Drying apparatus, about 120 °C, 1 hour
Standing to cool	
Charring / Incineration	Heat gently in an electric furnace 1,000 °C - 1,100 °C for no less than 1 hour
Standing to cool	Desiccator
Measurement	Measure the mass to the order of 1 mg

Figure Flow sheet for soluble silicic acid in fertilizers

4.4.2 Water-soluble silicic acid

4.4.2.a Potassium fluoride method

(1) Summary

This test method is applicable to liquid potassium silicate fertilizers.
Extract by adding water to an analytical sample, add hydrochloric acid, potassium chloride and potassium fluoride solution and cool in a refrigerator, and then filter after forming precipitate as potassium silicofluoride. Put the precipitate in water and heat, and measure by neutralization titration to obtain the water- soluble silicic acid (W-SiO_2) in an analytical sample.

(2) Reagents: Reagents are as shown below:

a) 0.1 mol/L - 0.2 mol/L sodium hydroxide solution(1): Transfer about 30 mL of water to a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4 - 5 days. Transfer 5.5 mL - 11 mL of the supernatant to a ground-in stoppered storage container, and add 1,000 mL of water containing no carbonic acid.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then transfer about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve with a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Transfer a predetermined amount of the solution to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 mg/100 mL) as an indicator, and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f)
$= (W \times A \times 0.01/97.095) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C)$

- W: Mass (g) of sulfamic acid sampled
- A: Purity (%) of sulfamic acid
- V_1: Volume (mL) of sulfamic acid solution transferred
- V_2: Constant volume (250 mL) of sulfamic acid solution
- V_3: Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
- C: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

c) Potassium chloride: A JIS Guaranteed Reagent specified in JIS K 8121 or a reagent of equivalent quality.

d) Potassium chloride solution (1)**:** Add 250 mL of ethanol specified in JIS K 8101 to 750 mL of water to mix, and add 150 mL of potassium chloride to dissolve. Add a few drops of methyl red solution (0.1 g/100 mL) as an indicator and drop hydrochloric acid until the color of the solution becomes red to make it acidic. After leaving at rest for 1 day, neutralize with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution.

e) Potassium fluoride solution (1)**:** Dissolve 58 g of potassium fluoride specified in JIS K 8815 in 1,000 mL of water (2).

f) Methyl red solution (0.1 g/100 mL): Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

g) Phenolphthalein solution (1 g/100 mL): Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) Store in a container made of polyethylene, etc. that contains no silicon.

(3) Apparatus and instruments: Apparatus and instruments are as follows:

a) Constant-temperature rotary shaker: A constant-temperature rotary shaker that can rotate a 500-mL volumetric flask upside down at 30 - 40 revolutions/min.

b) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below**.**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and transfer to a 500-mL volumetric flask.

b) Add 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make the sample solution.

Comment 1 In the procedure of **a)**, it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it into a 250-mL volumetric flask

Comment 2 The procedure in **(4.1)** is the same as the procedure in **(4.1.2)** of **4.3.3.a, 4.3.3.b** and **4.3.3.c**.

(4.2) Measurement: Conduct measurement as shown below.

a) Transfer a predetermined volume (the equivalents of 20 mg - 50 mg as SiO_2 and no more than 25 mL of liquid volume) to a 200-mL beaker made of polyethylene.

b) Add about 10 mL of hydrochloric acid and about 15 mL of potassium fluoride solution, and further add about 2 g of potassium chloride to dissolve, and then cool in a refrigerator for 30 minutes or more (3) to form the precipitate of potassium fluoride.

c) Filter with a Gooch crucible (4) made of polyethylene topped with Type 6 filter paper, and wash the container 3 times with potassium chloride solution, then move the whole precipitate into the crucible, and further wash 6 - 7 times with a small amount of potassium chloride solution (5).

d) Move the precipitate on the filter together with the filter paper into a 300-mL tall beaker with water, and further add water to make about 200 mL and heat it up to 70 ºC - 80 ºC on a hot plate.

e) Add a few drops of phenolphthalein solution (1 g/100 mL) to the sample solution as an indicator and titrate with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes light red.

f) Calculate soluble silicic acid (S-SiO_2) by the following formula.

Soluble silicic acid (S-SiO_2) (% (mass fraction)) in an analytical sample
$= V_S \times C \times f \times (V_4/V_5) \times (15.021/W) \times (100/1{,}000)$

V_S: Volume of the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution required for titration
C: Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f: Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
V_4: Predetermined volume (mL) of the extract in **(4.1) c)**
V_5: Volume (mL) of the extract transferred in **(4.2) a)**

W: Mass (g) of an analytical sample

Note **(3)** To be no more than 10 ºC
(4) Filter paper pulp can be stuffed to restrain precipitate from outflowing.
(5) Until the filtrate becomes neutral.

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.144 - 146, Yokendo, Tokyo (1988)

5) Flow sheet for soluble silicic acid: The flow sheet for soluble silicic acid in fertilizers is shown below:

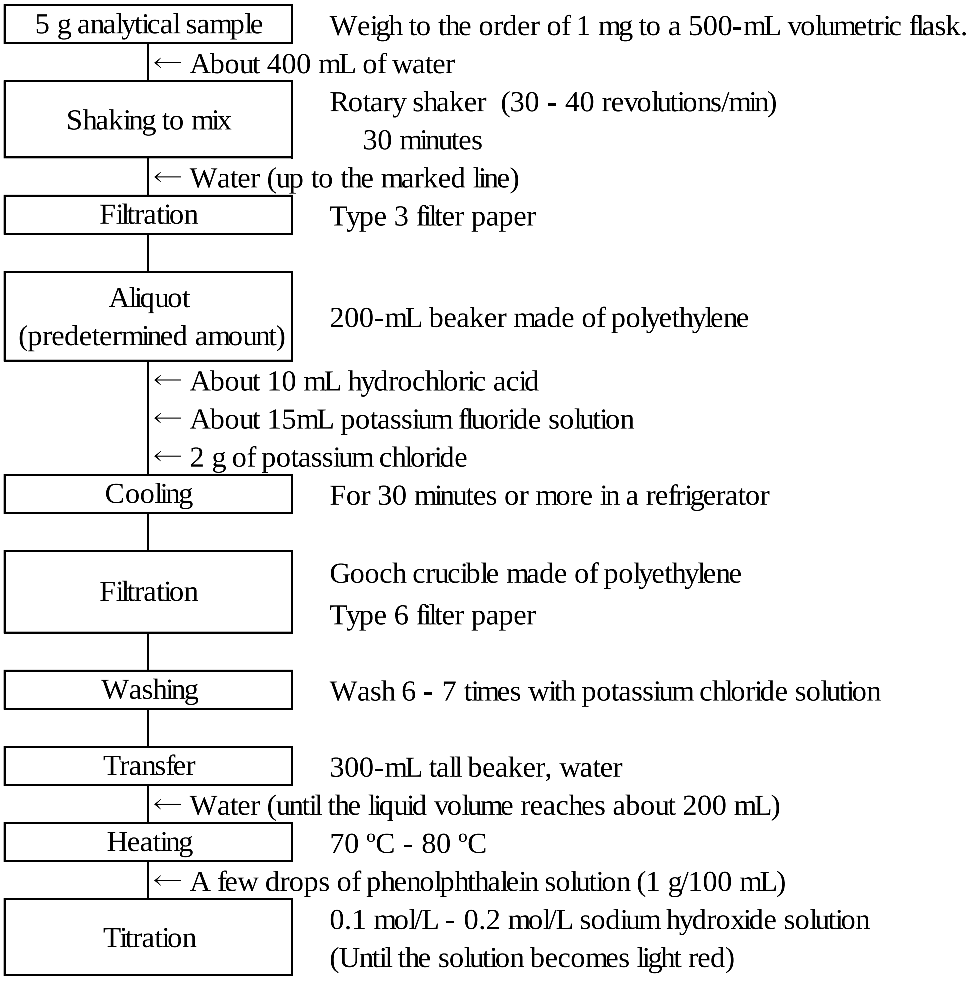


Figure Flow sheet for soluble silicic acid in fertilizers

4.5 Lime, calcium and alkalinity

4.5.1 Total lime

4.5.1.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers containing organic matters.
Pretreat an analytical sample with incineration and hydrochloric acid, add an interference suppressor solution, and then spray in an acetylene-air flame, and measure the atomic absorption with calcium at a wavelength of 422.7 nm to quantify total lime.

(2) Reagents: Reagents are as shown below:

a) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Interference suppressor solution: Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate specified in JIS K 8132(1) into a 2,000-mL beaker, add a small amount of water, gradually add 420 mL of hydrochloric acid to dissolve, and further add water to make 1,000 mL.

c) Calcium standard solution (CaO 1 mg/mL)(2): Heat calcium carbonate specified in JIS K 8617 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.785 g into a weighing dish. Transfer to a 1,000-mL volumetric flask with a small amount of water, add 20 mL of hydrochloric acid (1+3) to dissolve, and add water up to the marked line.

d) Calcium standard solution (CaO 5 µg/mL - 50 µg/mL) for the calibration curve preparation(2): Transfer 2.5 mL - 25 mL of a calcium standard solution (CaO 1 mg/mL) to 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution(3), and add water up to the marked line(4).

e) Blank test solution for the calibration curve preparation (2): Transfer about 50 mL of an interference suppressor solution to a 500-mL volumetric flask (3), and add water up to the marked line (4).

Note (1) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(2) This is an example of preparation; prepare an amount as appropriate.

(3) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

(4) For storage, use a sealable container made of materials which are not likely to dissolve calcium, such as borosilicate glass-1 specified in JIS R 3503 or Teflon.

Comment 1 Instead of a calcium standard solution (CaO 1 mg/mL) in **(2) c)**, a calcium standard solution (Ca 1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiply by the conversion factor to use as a calcium standard solution (CaO 1.3992 mg/mL).

(3) Instruments: Instruments are as shown below:

a) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A calcium hollow cathode lamp
2) **Gas:** Gas for heating by flame
   (i) Fuel gas: acetylene
   (ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) Electric furnace: An electric furnace that can be adjusted to 550 ºC ± 5 ºC.

c) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh about 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL-300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char[(5)].

c) Ignite (at 550 ºC ± 5 ºC) for no less than 4 hours to incinerate.

d) After standing to cool, moisten the residue with a small amount of water, add gradually about 10 mL of hydrochloric acid, and further add water to make about 20 mL.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.

f) After standing to cool, transfer the solution to a 250-mL - 500-mL volumetric flask with water.

g) Add water up to the marked line.

h) Filter with Type 3 filter paper to make the sample solution.

Note (5) Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.

Comment 2 The procedures in **(4.1)** are the same as in **(4.1.2)** in **4.2.1.a**.

Comment 3 The sample solution prepared in **(4.1) a) - h)** in **4.9.1.a** can also be used.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 422.7 nm

b) Calibration curve preparation

1) Spray the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 422.7 nm.

2) Prepare a curve for the relationship between the calcium concentration and the indicated value of the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as CaO) to a 100-mL volumetric flask.

2) Add about 10 mL of an interference suppressor solution [(3)], and add water up to the marked line.

3) Conduct similarly as in **b) 1)** to read the indicated value.

4) Obtain the calcium content from the calibration curve, and calculate total lime in the analytical sample.

Comment 4 Replicate testing was conducted using composted sludge fertilizer (2 samples), human waste sludge fertilizer (2 samples) and compost (1 sample), designated blended fertilizer containing organic fertilizer (1 sample) and compound fertilizer containing organic fertilizer (1 sample); as a result, the mean was in the range of 1.38 % - 2.44% (mass fraction), and the standard deviation and relative standard deviation were 0.004 % - 0.02% (mass fraction) and 0.2 % - 1.5%, respectively.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.156 – 158, Yokendo, Tokyo (1988)

2) Kimie KATO, Masayuki YOSHIMOTO and Yuji SHIRAI: Systematization of Determination Methods of Major Components in Sludge Fertilizer, Compost and Organic Fertilizer, Research Report of Fertilizer, Vol.3, 2010. (107 - 116)

(5) Flow sheet for total lime: The flow sheet for total lime in fertilizers is shown below:

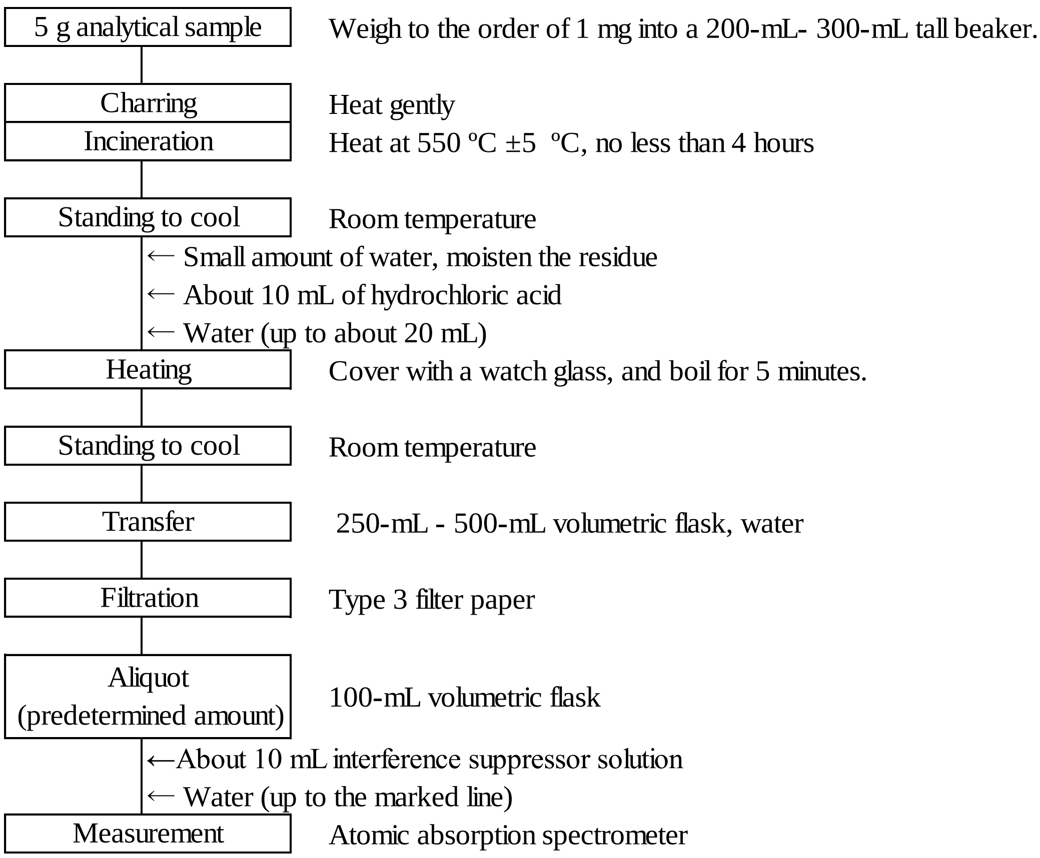


Figure Flow sheet for total lime in fertilizers.

4.5.2 Soluble lime

4.5.2.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers that guarantee alkalinity.

Add hydrochloric acid (1+23) to an analytical sample, boil to extract, and add an interference suppressor solution, and then spray in an acetylene-air flame, and measure the atomic absorption with calcium at a wavelength of 422.7 nm to quantify hydrochloric acid (1+23) soluble lime (soluble lime (S-CaO)).

(2) Reagents: Reagents are as shown below:

a) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Interference suppressor solution(2)**:** Weigh 60.9g - 152.1g of strontium chloride hexahydrate specified in JIS K 8132(1) into a 2,000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1,000 mL.

c) Calcium standard solution (CaO 1 mg/mL)(2): Put calcium carbonate specified in JIS K 8617 in a drying apparatus, heat at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.785 g into a weighing dish. Transfer to a 1,000-mL volumetric flask with a small amount of water, add about 20 mL of hydrochloric acid (1+3) to dissolve, and add water up to the marked line.

d) Calcium standard solution (CaO 5 µg/mL - 50 µg/mL) for the calibration curve preparation(2): Transfer 2.5 mL - 25 mL of a calcium standard solution (CaO 1 mg/mL) to 500-mL volumetric flasks step-by-step, add about 50 mL of an interference suppressor solution(3), and add water up to the marked line(4).

e) Blank test solution for the calibration curve preparation (2): Transfer about 50 mL of interference suppressor solution used in the procedure in **d)** to a 500-mL volumetric flask (3), and add water up to the marked line (4).

Note (1) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(2) This is an example of preparation; prepare an amount as appropriate.

(3) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

(4) For storage, use a sealable container made of materials which are not like to dissolve calcium, such as borosilicate glass-1 specified in JIS R 3503 or Teflon.

Comment 1 Instead of a calcium standard solution (CaO 1 mg/mL) in **(2) c)**, a calcium standard solution (Ca 1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiply by the conversion factor to use as the calcium standard solution (CaO 1.3992 mg/mL).

(3) Instruments: Instruments are as shown below:

a) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121.

1) Light source: A calcium hollow cathode lamp

2) Gas: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh about 2 g of an analytical sample to the order of 1 mg, and put it in a 500-mL tall beaker.

b) Add about 20 mL of hydrochloric acid (1+23), cover with a watch glass, and boil on a hot plate for about 5 minutes (5).

c) After standing to cool, transfer to a 250-mL - 500-mL volumetric flask with water.

d) Add water up to the marked line.

e) Filter with Type 3 filter paper to make the sample solution.

Note (5) Be aware that an analytical sample should not solidify in the bottom of a beaker.

Comment 2 In the case of a by-product magnesia fertilizer or a fertilizer containing a by-product magnesia, if the pH of the sample solution of **d)** is neutral or basic, prepare a sample solution anew by replacing “2 g of an analytical sample” in the procedure in **a)** with “1 g - 1.5 g of an analytical sample”.

Comment 3 In the procedure in **a)**, a 500-mL volumetric flask can be used instead of a 500-mL tall beaker. However the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. In addition, “cover with a watch glass” in **b)** is replaced by “place a long-stem funnel”, and “transfer to a 250-mL - 500 mL volumetric flask with water” in the procedure in **c)** is skipped.

Comment 4 The procedure in **(4.1)** is the same as in **(4.1)** in **4.6.1.a** and **4.7.1.a**

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 422.7 nm

b) Calibration curve preparation

1) Spray the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 422.7 nm.

2) Prepare a curve for the relationship between the calcium concentration and the indicated value of the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as CaO) to a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Conduct similarly as in **b) 1)** to read the indicated value.

4) Obtain the calcium content from the calibration curve, and calculate soluble lime (S-CaO) in the analytical sample.

Comment 5 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for soluble lime hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2009	Slag silicate fertilizer	58	32.68	0.90	2.8
2010	Slag silicate fertilizer	60	41.64	0.78	1.9
2011	Slag silicate fertilizer	55	40.78	0.79	1.9
2012	Slag silicate fertilizer	59	40.53	0.60	1.5
2013	Slag silicate fertilizer	59	30.06	0.65	2.2

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
$RSD_{rob} = (NIQR/M) \times 100$

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.167 - 169, Yokendo, Tokyo (1988)

(5) Flow sheet for soluble lime: The flow sheet for soluble lime in fertilizers is shown below:

2 g analytical sample	Weigh to the order of 1 mg into a 500-mL tall beaker.
← About 200 mL of hydrochloric acid (1+23)	
Heating	Cover with a watch glass, and boil for 5 minutes.
Standing to cool	Room temperature
Transfer	Water, 250-mL - 500-mL volumetric flask
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined amount)	100-mL volumetric flask
←About 10 mL interference suppressor solution	
← Water (up to the marked line)	
Measurement	Atomic absorption spectrometer

Figure Flow sheet for soluble lime in fertilizers.

4.5.3 Water-soluble calcium

4.5.3.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers that indicate calcium content as a response modifier.

Extract by adding water to an analytical sample, and add an interference suppressor solution, and then spray in an acetylene-air flame, and measure the atomic absorption with calcium at a wavelength of 422.7 nm to quantify water-soluble calcium (W-Ca).

(2) Reagents: Reagents are as shown below:

- **a) Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **b) Interference suppressor solution** (2)**:** Weigh 60.9 g-152.1 g of strontium chloride hexahydrate (1) specified in JIS K 8132 into a 2,000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1,000 mL.
- **c) Calcium standard solution (CaO 1 mg/mL)**(2)**:** Put calcium carbonate specified in JIS K 8617 in a drying apparatus, heat at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.785 g into a weighing dish. Transfer to a 1,000-mL volumetric flask with a small amount of water, add 20 mL of hydrochloric acid (1+3) to dissolve, and add water up to the marked line.
- **d) Calcium standard solution (CaO 5 µg/mL - 50 µg/mL) for the calibration curve preparation**(2)**:** Transfer 2.5 mL - 25 mL of a calcium standard solution (CaO 1 mg/mL) to 500-mL volumetric flasks step-by-step, add about 50 mL of an interference suppressor solution(3), and add water up to the marked line(4).
- **e) Blank test solution for the calibration curve preparation** (2)**:** Transfer about 50 mL of interference suppressor solution used in the procedure in **d)** to a 500-mL volumetric flask (3), and add water up to the marked line (4).

Note (1) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(2) This is an example of preparation; prepare an amount as appropriate.

(3) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

(4) For storage, use a sealable container made of materials which are not like to dissolve calcium, such as borosilicate glass-1 specified in JIS R 3503 or Teflon.

Comment 1 Instead of a calcium standard solution (CaO 1 mg/mL) in **(2) c)**, a calcium standard solution (Ca 1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiply by the conversion factor to use as the calcium standard solution (CaO 1.3992 mg/mL).

3) Instruments: Instruments are as shown below:

- **a) Rotary shaker:** A rotary shaker that can rotate a 500-mL volumetric flask upside down at 30 - 40 revolutions/min.
- **b) Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121.
  - **1) Light source**: A calcium hollow cathode lamp

2) Gas: Gas for heating by flame
(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh 1.00 g of an analytical sample, and put it in a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make the sample solution.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 422.7 nm

b) Calibration curve preparation

1) Spray the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 422.7 nm.

2) Prepare a curve for the relationship between the calcium concentration and the indicated value of the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as CaO) to a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution [(3)], and add water up to the marked line.

3) Conduct similarly as in **b) 1)** to read the indicated value.

4) Obtain the calcium content from the calibration curve, and calculate water-soluble calcium (W-Ca) in the analytical sample by the following formula.

Water-soluble calcium (W-Ca) (% (mass fraction)) in an analytical sample
$= A \times 0.7147$

A: Content volume of W-CaO (% (mass fraction))

(5) Flow sheet for water-soluble calcium: The flow sheet for water-soluble calcium in fertilizers is shown below:

1.00 g analytical sample	500-mL volumetric flask
← About 400 mL of water	
Shaking to mix	Rotary shaker (30 - 40 revolutions/min), for 30 minutes
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined amount)	100-mL volumetric flask
← About 10 mL interference suppressor solution	
← Water (up to the marked line)	
Measurement	Atomic absorption spectrometer (422.7 nm)

Figure Flow sheet for water-soluble calcium in fertilizers.

4.5.4 Alkalinity

4.5.4.a Ethylenediamine tetraacetate method

(1) Summary

This test method is applicable to fertilizers that guarantee alkalinity.
Add hydrochloric acid (1+23) to an analytical sample, boil to extract, and mask with 2,2',2''-nitrilotriethanol and a potassium cyanide solution, add a 0.01 mol/L ethylenediamine tetraacetate standard solution, and titrate with a 0.01 mol/L magnesium standard solution to obtain alkalinity (AL). Or after masking, titrate with an ethylenediamine tetraacetate standard solution to obtain alkalinity (AL).

(2) Reagents: Reagents are as shown below:

a) **Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Sodium hydroxide:** A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.

c) **Ascorbic acid:** A JIS Guaranteed Reagent specified in JIS K 9502 or a reagent of equivalent quality.

d) **2,2',2''-nitrilotriethanol** (1)**:** A JIS Guaranteed Reagent specified in JIS K 8663 or a reagent of equivalent quality.

e) **Acetone:** A JIS Guaranteed Reagent specified in JIS K 8034 or a reagent of equivalent quality.

f) **Ammonia solution:** A JIS Guaranteed Reagent (NH_3 28 % (mass fraction)) specified in JIS K 8085 or a reagent of equivalent quality.

g) **0.01 mol/L ethylenediamine tetraacetate standard solution:** Dissolve 3.72 g of ethylenediaminetetraacetic acid disodium dihydrate in water to make 1,000 mL.
Standardization: Wash zinc reference material for volumetric analysis specified in JIS K 8005 with hydrochloric acid (1+3), water, ethanol (99.5) specified in JIS K 8101, and diethyl ethel specified in JIS K 8103 successively, and immediately leave at rest in a desiccator for about 12 hours under no more than 2 kPa to dry, and then weigh about 0.65 mg to the order of 0.1 mg, transfer it to a 1,000-mL volumetric flask, add about 10 mL of hydrochloric acid to dissolve and then add water up to the marked line. Transfer 25 mL of the solution to a 200-mL - 300-mL Erlenmeyer flask, add about 15 mL of water and about 5 mL of ammonium chloride buffer solution, and titrate with 0.01 mol/L ethylenediamine tetraacetate standard solution, while adding Erio-chrome Black T solution as an indicator, until the color of the solution becomes blue. Calculate the factor of 0.01 mol/L ethylenediamine tetraacetate standard solution by the following formula.

Factor of 0.01 mol/L ethylenediamine tetraacetate standard solution (f_1)
$= W \times (A/100) \times (1/65.409) \times (V_1/V_2) \times (1{,}000/V_3) \times (1/C_1)$
$= W \times A \times (1/65.409) \times (0.25/V_3)$

W: Mass (g) of d zinc sampled
A: Purity (% (mass fraction)) of zinc
V_1: Volume (mL) of zinc solution transferred
V_2: Constant volume (25 mL) of zinc solution
V_3: Volume (mL) of 0.01 mol/L ethylenediamine tetraacetate standard solution needed for titration
C_1: Set concentration (0.01 mol/L) of 0.01 mol/L ethylenediamine tetraacetate standard solution

h) 0.01 mol/L magnesium standard solution: Put 0.24 g of magnesium specified in JIS K 8875 into a 1,000-mL beaker, add about 10 mL of hydrochloric acid to dissolve, add a proper amount of water, and while adding methyl red solution (0.1 g/100 mL) as an indicator, neutralize with an ammonia solution (1+3) until the color of the solution becomes achromatic, and then add water to make 1,000 mL.

Standardization: Transfer 25 mL of 0.01 mol/L magnesium standard solution to a 200-mL - 300-mL Erlenmeyer flask, add about 15 mL of water and about 5 mL of ammonium chloride buffer solution, and while adding an Erio-chrome Black T solution as an indicator, titrate with 0.01 mol/L ethylenediamine tetraacetate standard solution until the color of solution becomes blue. Calculate the factor of 0.01 mol/L magnesium standard solution by the following formula.

Factor of 0.01 mol/L magnesium standard solution (f_1)
$= (C_1 \times f_1 \times V_4) \times (1/V_5) \times (1/C_2)$
$= (f_1 \times V_4) \times (1/V_5)$

C_1: Set concentration (0.01 mol/L) of 0.01 mol/L ethylenediamine tetraacetate standard solution
C_2: Set concentration (0.01 mol/L) of 0.01 mol/L magnesium standard solution
f_1: Factor of 0.01 mol/L ethylenediamine tetraacetate standard solution
V_4: Volume (mL) of 0.01 mol/L ethylenediamine tetraacetate standard solution needed for titration
V_5: Volume (mL) of 0.01 mol/L magnesium standard solution transferred.

i) Ammonium chloride solution: Dissolve 70 g of ammonium chloride specified in JIS K 8116 and 570 mL of ammonia solution in water to make 1,000 mL.
j) 2-aminoethanol solution: Add 400 mL of water to 150 mL of 2-aminoethanol specified in JIS K 8109, add gradually hydrochloric acid to a pH 10.6.
k) Potassium cyanide solution: Dissolve 100 g of potassium cyanide specified in JIS K 8443 in water to make 1,000 mL.
l) Erio-chrome Black T solution: Dissolve 0.5 g of Erio-chrome Black T specified in JIS K 8736 and 4.5 g of hydroxyl-ammonium chloride in methanol - water (95+5) to make 100 mL.
m) Methyl red solution (0.1 g/100 mL): Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
n) Methanol: A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.

Note (1) The reagent corresponds to triethanolamine in the Official Methods of Analysis of Fertilizers (1992).

3) Instruments: Instruments are as shown below:
a) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedures
(4.1) Extraction: Conduct extraction as follows:
a) Weigh about 2 g of an analytical sample to the order of 1 mg, and put it in a 500-mL tall beaker.
b) Add about 200 mL of hydrochloric acid (1+23), cover with a watch glass, and boil on a hot plate for about 5 minutes [(2)].

c) After standing to cool, move it to a 250-mL - 500-mL volumetric flask with water.
d) Add water up to the marked line.
e) Filter with Type 3 filter paper to make the sample solution.

Note (2) Be aware that an analytical sample should not solidify in the bottom of a beaker.

Comment 1 In the case of a by-product magnesia fertilizer or a fertilizer containing a by-product magnesia, if the pH of the sample solution of **d)** is neutral or basic, prepare a sample solution anew by replacing "2 g of an analytical sample" in the procedure in **a)** with "1 g - 1.5 g of an analytical sample".
Comment 2 In the procedure in **a)**, a 500-mL volumetric flask can be used instead of a 500-mL tall beaker. However the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. In addition, "cover with a watch glass" in **b)** is replaced by "place a long-stem funnel", and "transfer to a 250-mL - 500-mL volumetric flask with water" in the procedure in **c)** is skipped.
Comment 3 The procedure in **(4.1)** is the same as in **(4.1)** in **4.5.2.a.**

(4.2) Measurement: Conduct measurement as shown below. Two examples of titration are shown as follows.
(4.2.1) Measurement (A): Titration with a magnesium standard solution (0.01 mol/L)
a) Transfer a predetermined volume (the equivalents of 5 mg - 20 mg as CaO + MgO) of sample solution to a 200-mL - 300-mL Erlenmeyer flask.
b) Add a proper amount of water, add a drop of methyl red solution as an indicator and drop sodium hydroxide (5 g/100 mL) to neutralize until the color of the solution becomes yellow.
c) Add 0.1 g of ascorbic acid, 1 ml - 10 mL of 2,2',2"-nitrilotriethanol - water (1+3) and 1 mL - 10 mL of potassium cyanide solution.
d) Add a predetermined volume of 0.01 mol/L ethylenediamine tetraacetate (3).
e) Add 20 mL of ammonium chloride solution or 2-aminoethanol solution.
f) Add several drops of Erio-chrome Black T solution, and titrate with 0.01 mol/L magnesium standard solution until the color of solution becomes red.
g) Calculate the alkalinity (AL) content in an analytical sample by the following formula.

Alkalinity (AL) (% (mass fraction)) in an analytical sample
$= ((C_1 \times f_1 \times V_6/1{,}000) - (C_2 \times f_2 \times V_7/1{,}000)) \times 56.077 \times (V_8/V_9) \times (1/W) \times 100$
$= ((f_1 \times V_1) - (f_2 \times V_2)) \times 56.077 \times (V_3/V_4) \times (1/2{,}000)$

C_1: Set concentration (0.01 mol/L) of 0.01 mol/L ethylenediamine tetraacetate standard solution
C_2: Set concentration (0.01 mol/L) of 0.01 mol/L magnesium standard solution
f_1: Factor of 0.01 mol/L ethylenediamine tetraacetate standard solution
f_2: Factor of 0.01 mol/L magnesium standard solution
V_6: Additive volume (mL) of 0.01 mol/L ethylenediamine tetraacetate standard solution
V_7: Volume (mL) of 0.01 mol/L magnesium standard solution needed for titration
V_8: Predetermined volume (mL) of the sample solution in **(4.1) d)**
V_9: Transferred volume (mL) of the sample solution subjected to titration in **(4.2) a)**.
W: Mass (2 g) of sample a solution

Note (3) Add excess volume since 1.8 mL of ethylenediamine tetraacetate standard solution (0.01 mol/L) is required for 1 mg of CaO.

(4.2.2) Measurement (B): Titration with an ethylenediamine tetraacetate standard solution (0.01 mol/L)

a) Transfer a predetermined volume (the equivalents of 5 mg - 20 mg as CaO + MgO) of sample solution to a 200-mL - 300-mL Erlenmeyer flask.

b) Add a proper amount of water, add a drop of methyl red solution as an indicator and drop sodium hydroxide (5 g/100 mL) to neutralize until the color of the solution becomes yellow.

c) Add 0.1 g of ascorbic acid, 1 ml - 10 mL of 2,2’,2”-nitrilotriethanol - water (1+3) and 1 mL - 10 mL of potassium cyanide solution.

d) Add 20 mL of ammonium chloride solution or 2-aminoethanol solution.

e) Add several drops of an Erio-chrome Black T solution, and titrate with 0.01 mol/L ethylenediamine tetraacetate standard solution until the color of solution becomes blue-green.

f) Calculate the alkalinity (AL) in an analytical sample by the following formula.

Alkalinity (AL) (% (mass fraction)) in an analytical sample

$$= (C_1 \times f_1 \times V_{10}/1{,}000) \times 56.077 \times (V_{11}/V_{12}) \times (1/W) \times 100$$

$$= (f_1 \times V_{10}) \times 56.077 \times (V_{11}/V_{12}) \times (1/2{,}000)$$

C_1: Set concentration (0.01 mol/L) of 0.01 mol/L ethylenediamine tetraacetate standard solution

f_1: Factor of 0.01 mol/L ethylenediamine tetraacetate standard solution

V_{10}: Volume (mL) of 0.01 mol/L ethylenediamine tetraacetate standard solution needed for titration

V_{11}: Predetermined volume (mL) of the sample solution in **(4.1) d)**

V_{12}: Transferred volume (mL) of the sample solution subjected to titration in **(4.2) a)**

W: Mass (2 g) of a sample solution

Comment4 Care should be fully taken in the case of using potassium cyanide and its solution in accordance with the Safety Data Sheet (SDS). In addition, observe laws and ordinances concerned such as the Poisonous and Deleterious Substance Control Law.

Criteria of the abolition in the Poisonous and Deleterious Substance Control Law (for reference):

Add sodium hydroxide solution to make it alkalinity more than pH 11, and further add oxidizer (sodium hypochlorite, bleaching powder) solution to conduct oxidative degradation processes. After dissolving CN ingredient, neutralize with sulfuric acid, and discard it after diluting it with a large amount of water. Take enough time to dissolve the CN ingredient with alkalinity.

Comment 5 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for alkalinity hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[5)]	NIQR[3)] (%)[5)]	RSD_{rob}[4)] (%)
2008	Slag silicate fertilizer	19	51.06	0.42	0.8
2009	Slag silicate fertilizer	21	39.69	1.25	3.2
2010	Slag silicate fertilizer	20	49.16	0.65	1.3
2011	Slag silicate fertilizer	16	49.44	0.59	1.2
2012	Slag silicate fertilizer	17	49.76	0.35	0.7

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:

$$RSD_{rob} = (NIQR/M) \times 100$$

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.162 - 164, Yokendo, Tokyo (1988)

(5) Flow sheet for alkalinity: The flow sheets for alkalinity in fertilizers are shown below:

2 g analytical sample — Weigh to the order of 1 mg to a 500-mL tall beaker
← About 200 mL of hydrochloric acid (1+23)
Boiling — Cover with a watch glass for 5 minutes
Cooling
Transfer — 250-mL - 500-mL volumetric flask
← Water (up to the marked line)
Filtration — Type 3 filter paper
Sample solution
Aliquot (predetermined amount) — 200-mL - 300-mL Erlenmeyer Flask
← Water (proper amount)
← A drop of methyl-red solution
Neutralization — Sodium hydroxide (5 g/100 mL) (until the solution becomes yellow)
← 0.1 g ascorbic acid
← 1 mL - 10 mL of 2,2’,2”-nitrilotriethanol - solution (1+3)
← 1 mL - 10 mL potassium cyanide solution
← Predetermined volume of 0.01 mol/L ethylenediamine tetraacetate standard solution
← 20 mL of ammonium chloride solution or 2-aminoethanol solution
← Several drops of Erio-chrome Black T solution
Titration — 0.01 mol/L magnesium standard solution (until the solution becomes red)

Figure Flow sheet for alkalinity in fertilizers (A).

2 g analytical sample — Weigh to the order of 1 mg into a 500-mL tall beaker

← About 200 mL of hydrochloric acid (1+23)

Boiling — Cover with a watch glass for 5 minutes

Cooling — Type 3 filter paper

Transfer — 250-mL - 500-mL volumetric flask

← Water (up to the marked line)

Filtration — Type 3 filter paper

Sample solution

Aliquot (predetermined amount) — 200-mL - 300-mL Erlenmeyer Flask

← Water (proper amount)

← A drop of methyl-red solution

Neutralization — Sodium hydroxide (5 g/100 mL) (until the solution becomes yellow)

← 0.1 g of ascorbic acid

← 1 mL - 10 mL of 2,2',2''-nitrilotriethanol - solution (1+3)

← 1 mL - 10 mL of potassium cyanide solution

← 20 mL of ammonium chloride solution or 2-aminoethanol solution

← Several drops of Erio-chrome Black T solution

Titration — 0.01 mol/L ethylenediamine tetraacetate standard solution (until the solution becomes blue-green)

Figure Flow sheet for alkalinity in fertilizers (B).

4.5.4.b Calculation from soluble lime and soluble magnesia

(1) Summary

This test method is applicable to fertilizers that guarantee alkalinity (AL).
Multiply the soluble magnesia (S-MgO) obtained in **4.6.1** by the factor (1.3934) and add to the soluble lime (S-CaO) obtained in **4.5.2** to calculate alkalinity (AL).

(2) Calculation of alkalinity

a) Calculate the alkalinity (AL) in a teat sample by the following formula.

Alkalinity (AL) (% (mass fraction)) in a test sample
= (S-CaO) + 1.3914 × (S-MgO)

S-CaO: Soluble lime (% (mass fraction)) (1) obtained in **4.5.2** in an analytical sample
S-MgO: Soluble magnesia (% (mass fraction)) (1) obtained in **4.6.1** in an analytical sample

Note (1) S-CaO and S-MgO use raw data without rounding numerical value

Comment 1 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for alkalinity hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2008	Slag silicate fertilizer	62	50.81	0.75	1.5
2009	Slag silicate fertilizer	59	38.82	0.90	2.3
2010	Slag silicate fertilizer	62	49.27	0.89	1.8
2011	Slag silicate fertilizer	56	49.48	0.62	1.3
2012	Slag silicate fertilizer	56	50.07	0.72	1.4
2013	Slag silicate fertilizer	59	36.56	0.86	2.4

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
RSD_{rob} = (NIQR/M) × 100

4.6 Magnesia

4.6.1 Soluble magnesia

4.6.1.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers containing by-product magnesia fertilizers and fertilizers that guarantee alkalinity.

Add hydrochloric acid (1+23) to an analytical sample, boil to extract and add an interference suppressor solution, and then spray in an acetylene-air flame, and measure the atomic absorption with magnesium at a wavelength of 285.2 nm to quantify hydrochloric acid (1+23) soluble magnesia (soluble magnesia (S-MgO)).

(2) Reagents: Reagents are as shown below:

- **a) Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **b) Interference suppressor solution** (1)**:** Weigh 60.9 g - 152.1g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2,000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1,000 mL.
- **c) Magnesium standard solution (MgO 1 mg/mL)** (1)**:** Weigh 0.603 g of guaranteed magnesium (powder) specified in JIS K 8876 into a weighing dish. Transfer to a 1,000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.
- **d) Magnesium standard solution (MgO 0.1 mg/mL):** Transfer 10 mL of magnesium standard solution (MgO 1 mg/mL) to a 100-mL volumetric flask and add water up to the marked line.
- **e) Magnesium standard solution (MgO 1 μg/mL-10 μg/mL) for the calibration curve preparation** (1)**:** Transfer 2.5 mL-25 mL of magnesium standard solution (MgO 0.1 mg/mL) to 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.
- **f) Blank test solution for the calibration curve preparation** (1)**:** Transfer about 25 mL of interference suppressor solution used in the procedure **e)** to a 250-mL volumetric flask (3), and add water up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(2) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(3) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of a magnesium standard solution (MgO 1 mg/mL) in **(2) c)**, a magnesium standard solution (Mg 1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiply by the conversion factor to use as the magnesium standard solution (MgO 1.658 mg/mL).

3) Instruments: Instruments are as shown below:

- **a) Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121.
  - **1) Light source**: A magnesium hollow cathode lamp

2) **Gas:** Gas for heating by flame
(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Hot plate:** A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) **Test procedures**

(4.1) **Extraction:** Conduct extraction as follows:

a) Weigh about 2 g of an analytical sample to the order of 1 mg, and put it in a 500-mL tall beaker.

b) Add about 200 mL of hydrochloric acid (1+23), cover with a watch glass, and boil on a hot plate for about 5 minutes (4).

c) After standing to cool, transfer to a 250-mL - 500-mL volumetric flask with water.

d) Add water up to the marked line.

e) Filter with Type 3 filter paper to make the sample solution.

Note **(4)** Be aware that an analytical sample should not solidify in the bottom of a beaker.

Comment 2 In the case of a by-product magnesia fertilizer or a fertilizer containing a by-product magnesia, if the pH of the sample solution of **d)** is neutral or basic, prepare a sample solution anew by replacing "2 g of an analytical sample" in the procedure in **a)** with "1 g - 1.5 g of an analytical sample".

Comment 3 In the procedure in **a)**, a 500-mL volumetric flask can be used instead of a 500-mL tall beaker. However the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. In addition, "cover with a watch glass" in **b)** is replaced by "place a long-stem funnel", and "transfer to a 250-mL - 500 mL volumetric flask with water" in the procedure in **c)** is skipped.

Comment 4 The procedure in **(4.1)** is the same as in **(4.1)** in **4.5.2.a.**

(4.2) **Measurement:** Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer:** Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 285.2 nm

b) **Calibration curve preparation**

1) Spray the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 285.2 nm.

2) Prepare a curve for the relationship between the magnesium concentration and the indicated value of the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MgO) to a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Conduct similarly as in **b) 1)** to read the indicated value.

4) Obtain the magnesium content from the calibration curve, and calculate the soluble magnesia (S-MgO) in the analytical sample.

Comment 5 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for soluble magnesia hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2009	Slag silicate fertilizer	58	4.48	0.10	2.3
2010	Slag silicate fertilizer	60	5.47	0.08	1.4
2011	Slag silicate fertilizer	55	6.24	0.12	1.9
2012	Slag silicate fertilizer	56	6.86	0.14	2.0
2013	Slag silicate fertilizer	59	4.62	0.15	3.2

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
$RSD_{rob} = (NIQR/M) \times 100$

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.167 - 169, Yokendo, Tokyo (1988)

(5) Flow sheet for soluble magnesia: The flow sheet for soluble magnesia in fertilizers is shown below:

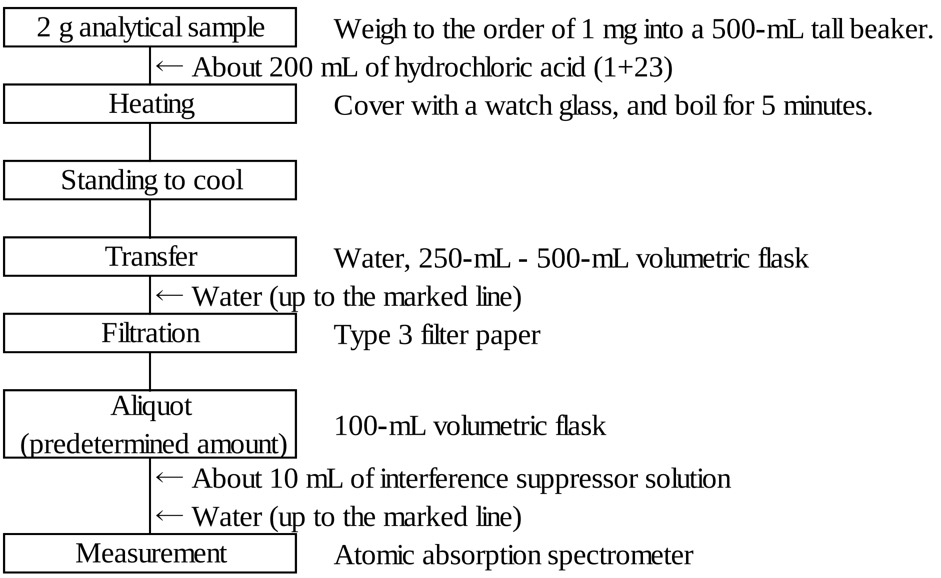


Figure Flow sheet for soluble magnesia in fertilizers.

4.6.2 Citrate soluble magnesia

4.6.2.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers containing magnesia hydroxide fertilizers, etc.

Extract by adding a citric acid solution to an analytical sample and add an interference suppressor solution, and then spray in an acetylene-air flame, and measure the atomic absorption with magnesium at a wavelength of 285.2 nm to quantify citrate soluble magnesia (C-MgO).

(2) Reagents: Reagents are as shown below:

a) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Citric acid solution (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1,000 mL.

c) Interference suppressor solution (1): Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2,000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1,000 mL.

d) Magnesium standard solution (MgO 1 mg/mL) (1): Weigh 0.603 g of the guaranteed magnesium (powder) specified in JIS K 8876 into a weighing dish. Transfer to a 1,000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

e) Magnesium standard solution (MgO 0.1 mg/mL): Transfer 10 mL of magnesium standard solution (MgO 1 mg/mL) to a 100-mL volumetric flask and add water up to the marked line.

f) Magnesium standard solution (MgO 1 µg/mL - 10 µg/mL) for the calibration curve preparation (1): Transfer 2.5 mL - 25 mL of magnesium standard solution (MgO 0.1 mg/mL) to 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.

g) Blank test solution for the calibration curve preparation (1): Transfer about 25 mL of interference suppressor solution used in the procedure in **f)** to a 250-mL volumetric flask (3), and add water up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(2) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(3) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of a magnesium standard solution (MgO 0.1 mg/mL) in **(2) e)**, a magnesium standard solution (Mg 0.1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiply by the conversion factor to use as a magnesium standard solution (MgO 0.1658 mg/mL).

(3) Instruments: Instruments are as follows:

a) Constant-temperature rotary shaker: A constant-temperature rotary shaker that can rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC±1 ºC, upside down at 30 - 40 revolutions/min.

b) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121.

1) Light source: A magnesium hollow cathode lamp

2) Gas: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample to the order of 1 mg, and transfer to a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC, and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After standing to cool, add water up to the marked line.

d) Filter with Type 3 filter paper to make the sample solution.

Comment 2 In the case of a by-product magnesia fertilizer or a fertilizer containing a by-product magnesia, if the pH of the sample solution of **d)** is neutral or basic, prepare a sample solution anew by replacing “1 g of an analytical sample” in the procedure in **a)** with “0.5 g of an analytical sample”.

Comment 3 In some slag silicate fertilizers, the variation of measurement value of citrate magnesia (C-M_gO) may be observed according to the time variation of heating state after adding citric acid solution. Therefore, in the case of slag silicate fertilizers, it is necessary to conduct the procedures of **c)** – **d)** as quickly as possible after confirming the time of shaking to mix in the procedure in **b)**.

Comment 4 The procedure in **(4.1)** is the same as the procedure in **(4.1)** in **4.2.3.a**.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 285.2 nm

b) Calibration curve preparation

1) Spray the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 285.2 nm.

2) Prepare a curve for the relationship between the magnesium concentration and the indicated value of the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MgO) to a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Conduct similarly as in **b) 1)** to read the indicated value.

4) Obtain the magnesium content from the calibration curve, and calculate the citrate soluble magnesia (C-MgO) in the analytical sample.

Comment 5 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for soluble magnesia hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2006	Slag silicate fertilizer	78	6.18	0.13	2.0
2007	Organic compound fertilizer	137	3.41	0.07	2.2
	Slag silicate fertilizer	86	3.13	0.21	6.6
2008	Organic compound fertilizer	127	4.62	0.11	2.5
	Slag silicate fertilizer	76	5.83	0.16	2.8
2009	Slag silicate fertilizer	75	2.31	0.17	7.5
2010	High analysis compound fertilizer	123	3.11	0.07	2.2
	Slag silicate fertilizer	76	5.42	0.12	2.1
2011	High analysis compound fertilizer	116	2.48	0.06	2.4
	Slag silicate fertilizer	69	3.33	0.20	6.0
2012	Slag silicate fertilizer	69	6.59	0.18	2.7
2013	High analysis compound fertilizer	116	6.17	0.13	2.3
	Slag silicate fertilizer	68	3.75	0.11	3.1

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.167 - 169, Yokendo, Tokyo (1988)

(5) Flow sheet for citrate soluble magnesia: The flow sheet for citrate soluble magnesia in fertilizers is shown below:

1 g analytical sample — Weigh to the order of 1 mg into a 250-mL tall beaker.

← About 150 mL of citrate solution [about 30 ºC]

Shaking to mix — Rotary shaker (30 - 40 revolutions/min), 30 ºC ± 1 ºC, for 1 hour

Standing to cool

← Water (up to the marked line)

Filtration — Type 3 filter paper

Aliquot (predetermined amount) — 100-mL volumetric flask

← About 10 mL of interference suppressor solution

← Water (up to the marked line)

Measurement — Atomic absorption spectrometer

Figure Flow sheet for citrate soluble magnesia in fertilizers.

4.6.3 Water-soluble magnesia

4.6.3.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers containing magnesia sulfate fertilizers, etc.

Add water to an analytical sample and boil to extract. Add an interference suppressor solution, then spray in an acetylene-air flame and measure the atomic absorption with magnesium at a wavelength of 285.2 nm to quantify water-soluble magnesia (W-MgO).

(2) Reagents: Reagents are as shown below:

a) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Interference suppressor solution (1)**:** Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2,000-mL beaker, add a small amount of water, gradually add 420 mL of hydrochloric acid to dissolve, and further add water to make 1,000 mL.

c) Magnesium standard solution (MgO 1 mg/mL) (1)**:** Weigh 0.603 g of guaranteed magnesium (powder) specified in JIS K 8876 into a weighing dish. Transfer to a 1,000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

d) Magnesium standard solution (MgO 1 mg/mL): Transfer 10 mL of magnesium standard solution (MgO 1 mg/mL) to a 100-mL volumetric flask and add water up to the marked line.

e) Magnesium standard solution (MgO 1 µg/mL-10 µg/mL) for the calibration curve preparation (1)**:** Transfer 2.5 mL - 25 mL of magnesium standard solution (MgO 0.1 mg/mL) to 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.

f) Blank test solution for the calibration curve preparation (1)**:** Transfer about 25 mL of interference suppressor solution used in the procedure in **e)** to a 250-mL volumetric flask (3), and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(3) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of a magnesium standard solution (MgO 1 mg/mL) in **(2) c)**, a magnesium standard solution (Mg 1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiply by the conversion factor to use as a magnesium standard solution (MgO 1.658 mg/mL).

(3) Apparatus and instruments: Apparatus and instruments are as shown below:

a) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A magnesium hollow cathode lamp
2) **Gas:** Gas for heating by flame
   (i) Fuel gas: acetylene
   (ii) Auxiliary gas: Air sufficiently free of dust and moisture.

- **b) Extraction flask** [(4)]**:** A 500-mL volumetric flask made of borosilicate glass
- **c) Hot plate:** A hot plate whose surface temperature can be adjusted up to 250 ºC.

Note (4) The volumetric flask used for extraction should be distinguished as an extraction flask and should not be used for the other purposes.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

- **a)** Weigh 1 g of an analytical sample to the order of 1 mg, and put it in an extraction flask.
- **b)** Add about 400 mL of water, and place a long-stem funnel to boil on a hot plate for about 30 minutes.
- **c)** After standing to cool, add water up to the marked line.
- **e)** Filter with Type 3 filter paper to make the sample solution.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

- **a) Measurement conditions for the atomic absorption spectrometer:** Set up the measurement conditions for the atomic absorption spectrometer considering the following:
  Analytical line wavelength: 285.2 nm
- **b) Calibration curve preparation**
  - **1)** Spray the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 285.2 nm.
  - **2)** Prepare a curve for the relationship between the magnesium concentration and the indicated value of the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.
- **c) Sample measurement**
  - **1)** Transfer a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MgO) to a 100-mL volumetric flask.
  - **2)** Add about 10 mL of interference suppressor solution [(3)], and add water up to the marked line.
  - **3)** Conduct similarly as in **b) 1)** to read the indicated value.
  - **4)** Obtain the magnesium content from the calibration curve, and calculate the water-soluble magnesia (W-MgO) in the analytical sample.

Comment 2 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for water-soluble magnesia hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2011	High analysis compound fertilizer	110	1.94	0.08	4.1
2012	Fluid mixed fertilizer	104	1.69	0.04	2.6
2013	High analysis compound fertilizer	109	3.80	0.63	16.6

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:

RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.167 - 169, Yokendo, Tokyo (1988)

(5) **Flow sheet for water-soluble magnesia:** The flow sheet for water-soluble magnesia in fertilizers is shown below:

1g analytical sample	Weigh to the order of 1 mg into an extraction flask.
← About 400 mL of water	
Heating	Place a long-stem funnel, boil for about 30 minutes
Standing to cool	
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined	100-mL volumetric flask
←About 10 mL of interference suppressor solution	
← Water (up to the marked line)	
Measurement	Atomic absorption spectrometer

Figure Flow sheet for water-soluble magnesia in fertilizers.

4.7 Manganese

4.7.1 Soluble manganese

4.7.1.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers containing manganese carbonate fertilizers.

Add hydrochloric acid (1+23) to an analytical sample, boil to extract and add an interference suppressor solution, and then spray into an acetylene-air flame, and measure the atomic absorption with manganese at a wavelength of 279.5 nm to quantify the hydrochloric acid (1+23) soluble manganese (soluble manganese (S-MnO)).

(2) Reagents: Reagents are as shown below:

a) **Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Interference suppressor solution** (1)**:** Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2,000-mL beaker, add a small amount of water, gradually add 420 mL of hydrochloric acid to dissolve, and further add water to make 1,000 mL.

c) **Manganese standard solution (MnO 1 mg/mL)** (1)**:** Weigh 0.775 g of manganese powder (purity no less than 99 % (mass fraction)) into a weighing dish. Transfer to a 1,000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

d) **Manganese standard solution (MnO 0.1 mg/mL):** Transfer 10 mL of manganese standard solution (MnO 1 mg/mL) to a 100-mL volumetric flask and add water up to the marked line.

e) **Manganese standard solution (MnO 1 µg/mL - 10 µg/mL) for the calibration curve preparation** (1)**:** Transfer 2.5 mL - 25 mL of manganese standard solution (MnO 0.1 mg/mL) to 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.

f) **Blank test solution for the calibration curve preparation** (1): Transfer about 25 mL of interference suppressor solution used in the procedure in **e)** to a 250-mL volumetric flask (3), and add water up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(2) 29 g of lanthanum oxide (a reagent of atomic absorption analysis grade or equivalents) can also be used.

(3) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of a manganese standard solution (MnO 0.1 mg/mL) in **(2) d)**, a manganese standard solution (Mn 0.1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiple by the conversion factor to use as a manganese standard solution (MnO 0.1291 mg/mL).

(3) Instruments: Instruments are as shown below:

a) **Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A manganese hollow cathode lamp

2) **Gas:** Gas for heating by flame

(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh about 2 g of an analytical sample to the order of 1 mg, and put it in a 500-mL tall beaker.
b) Add about 200 mL of hydrochloric acid (1+23), cover with a watch glass, and boil on a hot plate for about 5 minutes (4).
c) After standing to cool, transfer to a 250-mL - 500-mL volumetric flask with water.
d) Add water up to the marked line.
e) Filter with Type 3 filter paper to make the sample solution.

Note (4) Be aware that an analytical sample should not solidify in the bottom of a beaker.

Comment 2 In the procedure in **a)**, a 500-mL volumetric flask can be used instead of a 500-mL tall beaker. However the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. In addition, “cover with a watch glass” in **b)** is replaced by “place a long-stem funnel”, and “transfer to a 250-mL - 500-mL volumetric flask with water” in the procedure in **c)** is skipped.

Comment 3 The procedure in **(4.1)** is the same as in **(4.1)** in **4.5.2.a.**

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 279.5 nm

b) Calibration curve preparation

1) Spray the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 279.5 nm.
2) Prepare a curve for the relationship between the manganese concentration and the indicated value of the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MnO) to a 100-mL volumetric flask.
2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.
3) Conduct similarly as in **b) 1)** to read the indicated value.
4) Obtain the manganese content from the calibration curve, and calculate the soluble manganese (S-MnO) in the analytical sample.

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.176 - 177, Yokendo, Tokyo (1988)

(5) Flow sheet for soluble manganese: The flow sheet for soluble manganese in fertilizers is shown below:

2 g analytical sample	Weigh to the order of 1 mg into a 500-mL tall beaker.
← About 200 mL of hydrochloric acid (1+23)	
Heating	Cover with a watch glass, and boil for about 5 minutes.
Standing to cool	
Transfer	Water, 250-mL - 500-mL volumetric flask
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined amount)	100-mL volumetric flask
← About 10 mL of interference suppressor solution	
← Water (up to the marked line)	
Measurement	Atomic absorption spectrometer

Figure Flow sheet for soluble manganese in fertilizers.

4.7.2 Citrate soluble manganese

4.7.2.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers containing manganese carbonate fertilizers.

Extract by adding citric acid solution to an analytical sample and add an interference suppressor solution, and then spray into an acetylene-air flame, and measure the atomic absorption with manganese at a wavelength of 279.5 nm to quantify citrate soluble manganese (citrate soluble manganese(C-MnO)).

(2) Reagents: Reagents are as shown below:

- **a) Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **b) Citric acid solution** (1)**:** Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1,000 mL.
- **c) Interference suppressor solution** (1)**:** Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2,000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1,000 mL.
- **d) Manganese standard solution (MnO 1 mg/mL)** (1)**:** Weigh 0.775 g of manganese powder (purity no less than 99 % (mass fraction)) into a weighing dish. Transfer to a 1,000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.
- **e) Manganese standard solution (MnO 0.1 mg/mL):** Transfer 10 mL of manganese standard solution (MnO 1 mg/mL) to a 100-mL volumetric flask and add water up to the marked line.
- **f) Manganese standard solution (MnO 1 µg/mL - 10 µg/mL) for the calibration curve preparation** (1)**:** Transfer 2.5 mL - 25 mL of manganese standard solution (MnO 0.1 mg/mL) to 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.
- **g) Blank test solution for the calibration curve preparation** (1)**:** Transfer about 25 mL of interference suppressor solution used in the procedure in **f)** to a 250-mL volumetric flask (3), and add water up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(2) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(3) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of a manganese standard solution (MnO 0.1 mg/mL) in **(2) e)**, a manganese standard solution (Mn 0.1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiply by the conversion factor to use as a manganese standard solution (MnO 0.1291 mg/mL).

(3) Instruments: Instruments are as follows:

- **a) Constant-temperature rotary shaker:** A constant-temperature rotary shaker that can rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, upside down at 30 - 40 revolutions/min.

b) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121.

1) Light source: A manganese hollow cathode lamp

2) Gas: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample to the order of 1 mg, and transfer to a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC, and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After standing to cool, add water up to the marked line.

d) Filter with Type 3 filter paper to make the sample solution.

Comment 2 The procedure in **(4.1)** is the same as the procedure **(4.1)** in **4.2.3.a**.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 279.5 nm

b) Calibration curve preparation

1) Spray the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 279.5 nm.

2) Prepare a curve for the relationship between the manganese concentration and the indicated value of the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MnO) to a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Conduct similarly as in **b) 1)** to read the indicated value.

4) Obtain the manganese content from the calibration curve, and citrate soluble manganese (C-MnO) in the analytical sample.

Comment 3 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for citrate soluble manganese hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2009	High analysis compound fertilizer	110	0.547	0.0017	3.0
2013	High analysis compound fertilizer	102	0.513	0.013	2.5

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
$RSD_{rob} = (NIQR/M) \times 100$

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.176 - 177, Yokendo, Tokyo (1988)

(5) Flow sheet for citrate soluble manganese: The flow sheet for citrate soluble manganese in fertilizers is shown below:

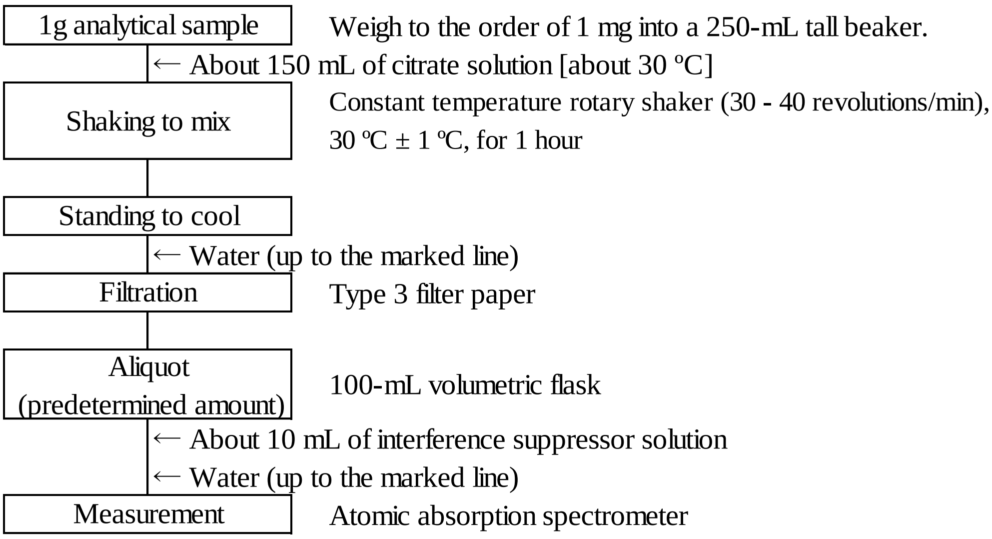


Figure Flow sheet for citrate soluble manganese in fertilizers.

4.7.3 Water-soluble manganese

4.7.3.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers containing manganese sulfate fertilizers.
Extract by adding water to an analytical sample and add an interference suppressor solution, and then spray in an acetylene-air flame, and measure the atomic absorption with manganese at a wavelength of 279.5 nm to quantify water-soluble manganese (W-MnO).

(2) Reagents: Reagents are as shown below:

a) **Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Interference suppressor solution** [(1)]**:** Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate [(2)] specified in JIS K 8132 into a 2,000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1,000 mL.

c) **Manganese standard solution (MnO 1 mg/mL)** [(1)]**:** Weigh 0.775 g of manganese powder (purity no less than 99 % (mass fraction)) into a weighing dish. Transfer to a 1,000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

d) **Manganese standard solution (MnO 0.1 mg/mL):** Transfer 10 mL of manganese standard solution (MnO 1 mg/mL) to a 100-mL volumetric flask and add water up to the marked line.

e) **Manganese standard solution (MnO 1 µg/mL - 10 µg/mL) for the calibration curve preparation** [(1)]**:** Transfer 2.5 mL - 25 mL of manganese standard solution (MnO 0.1 mg/mL) to 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution [(3)], and add water up to the marked line.

f) **Blank test solution for the calibration curve preparation** [(1)]: Transfer about 25 mL of interference suppressor solution used in the procedure **e)** to a 250-mL volumetric flask [(3)], and add water up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(2) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(3) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of a manganese standard solution (MnO 0.1 mg/mL) in **(2) d)**, a manganese standard solution (Mn 0.1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can also be used. Additionally, it is recommended to multiply by the conversion factor to use as manganese standard solution (MnO 0.1291 mg/mL).

(3) Instruments: Instruments are as follows:

a) **Rotary shaker:** A rotary shaker that can rotate upside down a 500-mL volumetric flask at 30 - 40 revolutions/min.

b) **Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A manganese hollow cathode lamp

2) **Gas:** Gas for heating by flame

(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5 g of an analytical sample to the order of 1 mg, and transfer to a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make the sample solution.

Comment 2 In the procedure in **a)**, it is also allowed to weigh 2.5 g of the analytical sample to the order of 1 mg and transfer to a 250-mL volumetric flask.

Comment 3 The procedure in **(4.1)** is the same as the procedure in **(4.1)** in **4.2.4.a**

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 279.5 nm

b) Calibration curve preparation

1) Spray the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 279.5 nm.

2) Prepare a curve for the relationship between the manganese concentration and the indicated value of the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MnO) to a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution [(3)], and add water up to the marked line.

3) Conduct similarly as in **b) 1)** to read the indicated value.

4) Obtain the manganese content from the calibration curve, and water-soluble manganese (W-MnO) in the analytical sample.

Comment 4 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for water-soluble manganese hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2012	Fluid mixed fertilizer	99	1.23	0.03	2.7

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.176 - 177, Yokendo, Tokyo (1988)

(5) Flow sheet for water-soluble manganese: The flow sheet for water-soluble manganese in fertilizers is shown below:

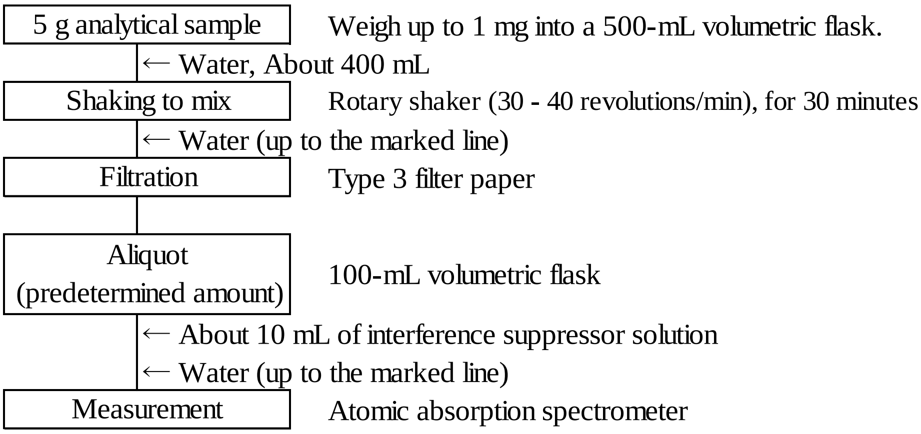


Figure Flow sheet for water-soluble manganese in fertilizers.

4.8 Boron

4.8.1 Citrate soluble boron

4.8.1.a Azomethine-H method

(1) Summary

This test method is applicable to fertilizers containing borate fertilizers, etc.
Extract by adding citric acid solution to an analytical sample, mask co-existing copper, iron and other salts with ethylenediamine tetraacetate and measure the absorbance with azomethine-H borate formed by the reaction with azomethine-H to obtain citrate soluble boron (C-B_2O_3).

(2) Reagents: Reagents are as shown below:

- **a) Citric acid solution (1):** Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1,000 mL.
- **b) Ethylenediamine tetraacetate solution (1):** Dissolve 37.2 g of ethylenediaminetetraacetic acid disodium dihydrate specified in JIS K 8107 in water to make 1,000 mL.
- **c) Ammonium acetate solution (1):** Dissolve 250 g of ammonium acetate specified in JIS K 8359 in water to make 500 mL and adjust pH with sulfuric acid (1+4) to pH 5.2 ± 0.1.
- **d) Azomethine-H solution:** Add water to 0.6 g of azomethine-H and 2 g of L (+) – ascorbic acid specified in JIS K 9502, and heat up to 35 ºC - 40 ºC to dissolve and add water after cooling to make 100 mL.
- **e) Boron standard solution (B_2O_3 2.5 mg/mL) (1):** After leaving boric acid specified in JIS K 8863 at rest in a desiccator for about 24 hours to dry, weigh 4.441 g to a weighing dish. Dissolve with a small amount of water, transfer to a 1,000-mL volumetric flask and add water up to the marked line.
- **f) Boron standard solution (B_2O_3 0.05 mg/mL):** Dilute with water exactly 50 times the predetermined volume of boron standard solution (B_2O_3 2.5 mg/mL).

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as follows:

- **a) Constant-temperature rotary shaker:** A constant-temperature rotary shaker that can rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, upside down at 30 - 40 revolutions/min.
- **b) Spectrophotometer:** A spectrophotometer specified in JIS K 0115

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

- **a)** Weigh 1 g of an analytical sample to the order of 1 mg, and transfer to a 250-mL volumetric flask.
- **b)** Add 150 mL of citric acid solution heated up to about 30 ºC, and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.
- **c)** After standing to cool, add water up to the marked line.
- **d)** Filter with Type 3 filter paper to make the sample solution.

Comment 1 The procedure in **(4.1)** is the same as the procedure in **(4.1)** in **4.2.3.a**.

(4.2) Coloring: Conduct coloring as shown below.

a) Transfer a predetermined volume (the equivalents of 0.05 mg -1 mg of B_2O_3, and no more than the equivalents of 15 mL of citric acid solution) of an analytical sample to a 100-mL volumetric flask.

b) Add the solution to make the citric acid solution equivalent to 15 mL.

c) Add 25 mL of ethylenediamine tetraacetate solution, and add 10 mL of ammonium acetate solution and then 10 mL of azomethine-H solution successively, and further add water up to the marked line, then leave at rest for about 2 hours.

(4.3) Measurement: Conduct measurement according to JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used in measurement.

a) Measurement conditions for the spectrophotometer: Set up the measurement conditions for the spectrophotometer considering the following:
Detection wavelength: 415 nm

b) Calibration curve preparation

1) Transfer 1mL - 20 mL of boron standard solution (B_2O_3 0.05 mg/mL) to 100-mL volumetric flasks step-by-step.

2) Add 15 mL of citric acid solution and conduct the same procedure as **(4.2) c)** to make the B_2O_3 0.05 mg/100 mL - 1 mg/100 mL boron standard solution for the calibration curve preparation.

3) Conduct the same procedures as **2)** for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Measure absorbance at a wavelength of 415 nm of the boron standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control.

5) Prepare a curve for the relationship between the boron concentration and absorbance of the boron standard solutions for the calibration curve preparation.

c) Sample measurement

1) Regarding the solution in **(4.2) c)**, measure the absorbance by the same procedure as **b) 4).**

2) Obtain the boron (B_2O_3) content from the calibration curve, and calculate the citrate soluble boron (C-B_2O_3) in the analytical sample.

Comment 2 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for citrate soluble boron hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2009	High analysis compound fertilizer	110	0.280	0.015	5.3
2014	High analysis compound fertilizer	95	0.243	0.014	5.6

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:

RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.184 - 187, Yokendo, Tokyo (1988)

(5) Flow sheet for citrate soluble boron: The flow sheet for citrate soluble boron in fertilizers is shown below:

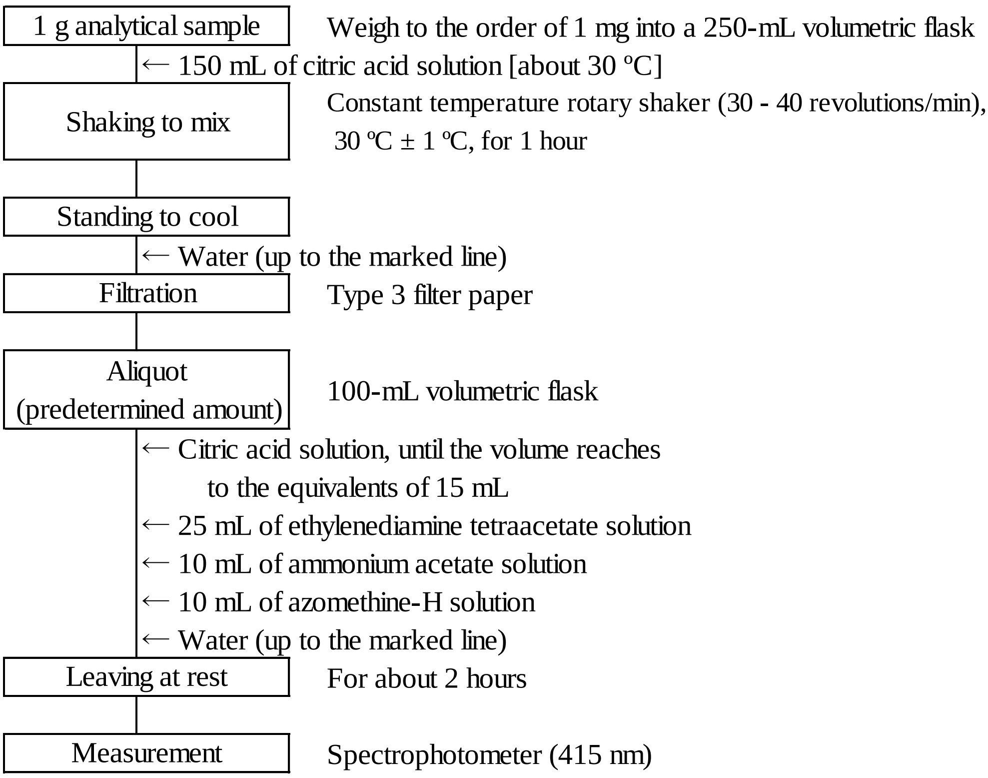


Figure Flow sheet for citrate soluble boron in fertilizers.

4.8.2 Water-soluble boron

4.8.2.a Azomethine-H method

(1) Summary

This test method is applicable to fertilizers containing borate fertilizers, etc.

Extract by adding water to an analytical sample, boil to extract, and mask co-existing copper, iron and other salts with ethylenediamine tetraacetate and measure the absorbance with azomethine-H borate formed by the reaction with azomethine-H to obtain water-soluble boron (W-B_2O_3).

(2) Reagents: Reagents are as shown below:

a) Ethylenediamine tetraacetate solution [(1)]: Dissolve 37.2 g of ethylenediaminetetraacetic acid disodium dihydrate specified in JIS K 8107 in water to make 1,000 mL.

b) Ammonium acetate solution [(1)]: Dissolve 250 g of ammonium acetate specified in JIS K 8359 in water to make 500 mL and adjust pH with sulfuric acid (1+4) to pH 5.2 ± 0.1.

c) Azomethine-H solution [(1)]: Add water to 0.6 g of azomethine-H and 2 g of L (+) – ascorbic acid specified in JIS K 9502, and heat up to 35 ºC - 40 ºC to dissolve and add water after cooling to make100 mL.

d) Boron standard solution (B_2O_3 2.5 mg/mL) [(1)]: After leaving boric acid specified in JIS K 8863 at rest in a desiccator for about 24 hours to dry, weigh 4.441 g to a weighing dish. Dissolve with a small amount of water, transfer to a 1,000-mL volumetric flask and add water up to the marked line.

e) Boron standard solution (B_2O_3 0.05 mg/mL): Dilute with water precisely 50 times the predetermined volume of boron standard solution (B_2O_3 2.5 mg/mL).

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as follows:

a) Spectrophotometer: A spectrophotometer specified in JIS K 0115

b) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh about 2.5 g of an analytical sample to the order of 1 mg, and put it in a 300-mL tall beaker.

b) Add about 200 mL of water, cover with a watch glass, and boil on a hot plate for about 15 minutes.

c) After standing to cool, transfer to a 250-mL volumetric flask with water.

d) Add water up to the marked line.

e) Filter with Type 3 filter paper to make the sample solution.

Comment 1 In the procedure in **a)**, a 250-mLvolumetric flask can be used instead of a 300-mL tall beaker. However the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. In addition, “cover with a watch glass” in **b)** is replaced by “place a long-stem funnel”, and “transfer to a 250-mL volumetric flask with water” in the procedure in **c)** is skipped.

Comment 2 The procedure in **(4.1)** is the same as in **(4.1)** in **4.3.3.a.**

(4.2) Coloring: Conduct coloring as shown below.

a) Transfer a predetermined volume (the equivalents of 0.05 mg -1 mg as B_2O_3) of an analytical sample to a 100-mL volumetric flask.

b) Add 25 mL of ethylenediamine tetraacetate solution, and add 10 mL of ammonium acetate solution and 10 mL of azomethine-H solution successively, and further add water up to the marked line, then leave at rest for about 2 hours.

Comment 3 Water-soluble boron can be measured simultaneously with citrate soluble boron by adding 15 mL of citric acid solution before the procedure in **(4.2) b)**.

(4.3) Measurement: Conduct measurement according to JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used in measurement.

a) Measurement conditions for the spectrophotometer: Set up the measurement conditions for the spectrophotometer considering the following:
Detection wavelength: 415 nm

b) Calibration curve preparation

1) Transfer 1mL - 20 mL of boron standard solution (B_2O_3 0.05 mg/mL) to 100-mL volumetric flasks step-by-step.

2) Conduct the same procedure as **(4.2) b)** to make the B_2O_3 0.05 mg/100 mL - 1 mg/100 mL boron standard solution for the calibration curve preparation.

3) Conduct the same procedures as **2)** for another a 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Measure the absorbance at a wavelength of 415 nm of the boron standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control .

5) Prepare a curve for the relationship between the boron concentration and absorbance of the boron standard solutions for the calibration curve preparation.

c) Sample measurement

1) Regarding the solution in **(4.2) b)**, measure the absorbance by the same procedure as **b) 4).**

2) Obtain the boron (B_2O_3) content from the calibration curve, and calculate the water-soluble boron (W-B_2O_3) in the analytical sample.

Comment 4 Water-soluble boron can be measured simultaneously with citrate soluble boron by adding 15 mL of citric acid solution before the procedure in **(4.3) b) 2)**.

Comment 5 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for water-soluble boron hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2006	High analysis compound fertilizer	132	0.40	0.02	4.2
2008	High analysis compound fertilizer	113	0.26	0.01	2.9
2010	High analysis compound fertilizer	109	0.291	0.009	3.1
2012	Fluid mixed fertilizer	92	0.240	0.008	3.2

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
$RSD_{rob} = (NIQR/M) \times 100$

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.184 - 187, Yokendo, Tokyo (1988)

(5) Flow sheet for water-soluble boron: The flow sheet for water-soluble boron in fertilizers is shown below:

2.5 g analytical sample	Weigh to the order of 1 mg into a 300-mL tall beaker.
← About 200 mL of Water	
Heating	Cover with a watch glass, boil for 15 minutes
Standing to cool	
Transfer	Water, 250-mL volumetric flask
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined amount)	100-mL volumetric flask
← 25 mL of ethylenediamine tetraacetate solution	
← 10 mL of ammonium acetate solution	
← 10 mL of azomethine-H solution	
← Water (up to the marked line)	
Leaving at rest	For about 2 hours
Measurement	Spectrophotometer (415 nm)

Figure Flow sheet for water-soluble boron in fertilizers.

4.9 Zinc

4.9.1 Total zinc

4.9.1.a Flame atomic absorption spectrometry

(1) Summary
This test method is applicable to fertilizers.
Pretreat an analytical sample with incineration and nitric acid-hydrochloric acid (1+3), and then spray in an acetylene-air flame, and measure the atomic absorption with zinc at a wavelength of 213.9 nm to quantify total zinc.

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Zinc standard solution (0.1 mg/mL):** Zinc standard solution (0.1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **e) Zinc standard solutions (0.5 µg - 5 µg/mL) for the calibration curve preparation(1):** Transfer 2.5 mL - 25 mL of zinc standard solution (0.1 mg/mL) to 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.
- **f) Blank test solution for the calibration curve preparation (1):** Hydrochloric acid (1+23) used in the procedure in **e**).

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as shown below:

- **a) Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121 with the background correction (2) function.
  - **1) Light source**: A zinc hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)
  - **2) Gas:** Gas for heating by flame
    - (i) Fuel gas: acetylene
    - (ii) Auxiliary gas: Air sufficiently free of dust and moisture.
- **b) Electric furnace:** An electric furnace that can be adjusted to 450 ºC ± 5 ºC.
- **c) Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note (2) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

- **a)** Weigh 5.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.
- **b)** Put the tall beaker in an electric furnace, and heat gently to char(3).
- **c)** Ignite at 450 ºC ± 5 ºC to incinerate (4).
- **d)** After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
- **e)** Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass [5], and continue heating on the hot plate or sand bath to concentrate until nearly dried up.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5)[6] to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.

i) As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare the blank test solution.

Note **(3)** Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.

(4) Example of ignition period: 8 - 16 hours

(5) The watch glass can be removed.

(6) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 Do not conduct the procedures in **(4.1) b) - c)** in the case of fertilizers not containing organic matters.

Comment 2 The procedures in **(4.1)** are the same as in **(4.1)** in **4.9.1.b, 4.10.1.a, 4.10.1.b, 5.3.a, 5.3.b, 5.4.a, 5.4.b, 5.5.a, 5.5.d, 5.5.a** and **5.6.b**. Additionally, it also can be used as the sample solution in **4.2.1.a, 4.2.1.b, 4.3.1.a, 4.3.1.b,** and **4.5.1.a**.

Comment 3 The sample solution prepared in **(4.1.2)** in **4.2.1.a** can also be used.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 213.9 nm

b) Calibration curve preparation

1) Spray the zinc standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 213.9 nm.

2) Prepare a curve for the relationship between the zinc concentration and the indicated value of the zinc standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Subject the sample solution [7] to the same procedure as in **b) 1)** to read the indicated value.

2) Subject the blank test solution to the same procedure as in **b) 1)** to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the zinc content from the calibration curve, and calculate the zinc concentration in the analytical sample.

Note **(7)** If there is a possibility that the zinc concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount with hydrochloric acid (1+23).

Comment 4 The zinc concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1)** and **3)** to obtain the zinc content in the blank test solution.

Comment 5 Replicate testing was conducted using human waste sludge fertilizer, mixed sludge fertilizer and composted sludge fertilizer (3 samples); as a result, repeatability in the range of 510 mg/kg - 1,100 mg/kg total zinc (mean determined value) was 0.6 % - 1.9 % relative standard deviation.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.193 - 194, Yokendo, Tokyo (1988)

2) Kimie KATO, Masayuki YOSHIMOTO and Yuji SHIRAI: Systematization of Determination Methods of Major Components in Sludge Fertilizer, Compost and Organic Fertilizer, Research Report of Fertilizer, Vol.3, 2010. (107-116)

(5) Flow sheet for total zinc: Flow sheet for total zinc in fertilizers is shown below:

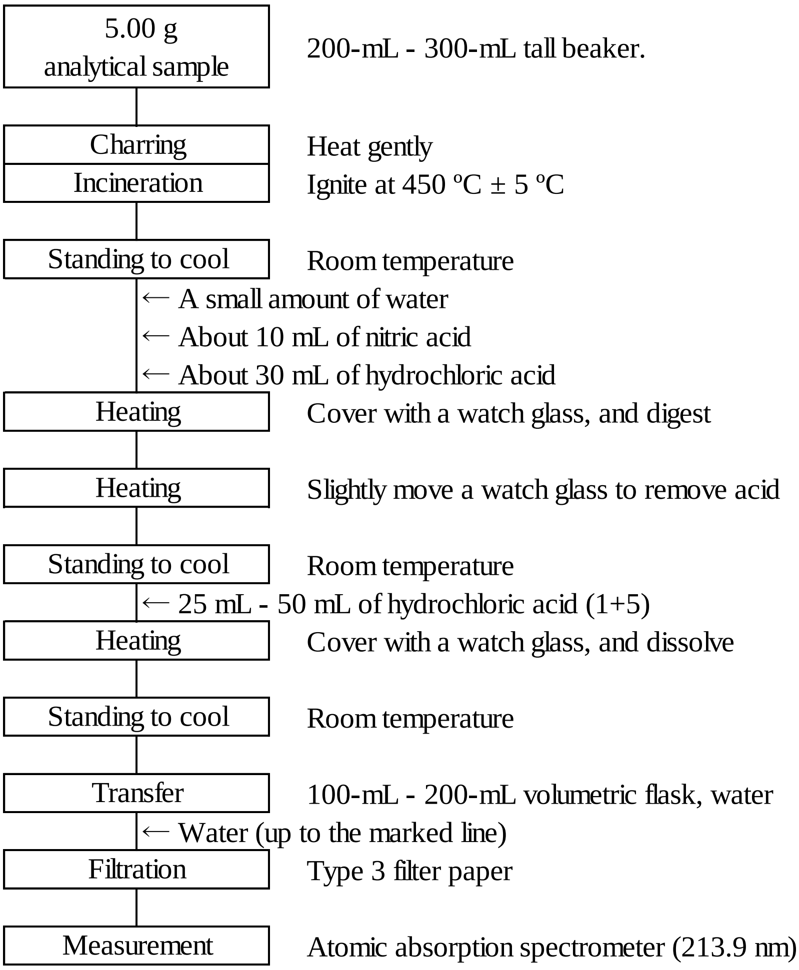


Figure Flow sheet for total zinc in fertilizers

4.9.1.b ICP Atomic Emission Spectrometry <Reference method>

(1) Summary

The test method is applicable to sludge fertilizers, etc.

Pretreat an analytical sample with incineration, nitric acid - hydrochloric acid (1+3), introduce it to ICP Atomic Emission Spectrometer ("ICP-AES") and measure the emission with zinc at a wavelength of 206.191 nm to quantify zinc.

(2) Reagents, etc.: Reagents and water are as shown below:

a) Water: Water of A3 specified in JIS K 0557.

b) Nitric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) Hydrochloric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) Zinc standard solution (0.1 mg/mL): A zinc standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.

e) Zinc standard solutions (2.5 µg/mL) (1):
Dilute a predetermined amount of zinc standard solution (0.1 mg/mL) with hydrochloric acid (1+23) to prepare a zinc standard solution (25 µg/mL).

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as shown below:

a) ICP Atomic Emission Spectrometer: An atomic emission spectrometer specified in JIS K 0116.

1) Gas: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) Electric furnace: An electric furnace that can keep the test temperature at ± 5 ºC

c) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh 5.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (2).

c) Ignite at 450 ºC ± 5 ºC to incinerate (3).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass (4), and continue heating on the hot plate or sand bath to concentrate until nearly dried up.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (5) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.

i) As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare the blank test solution.

Note **(2)** Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.

(3) Example of ignition period: 8 - 16 hours

(4) The watch glass can be removed.

(5) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 Do not conduct the procedures in **(4.1) b) - c)** in the case of fertilizers not containing organic matters.

Comment 2 The procedures in **(4.1)** are the same as in **(4.1)** in **4.9.1.a.**

(4.2) Measurement: Conduct measurement (Standard Addition Method) according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Atomic Emission Spectrometry used in measurement.

a) Measurement conditions for the ICP Atomic Emission Spectrometer: Set up the measurement conditions for the ICP Atomic Emission Spectrometer considering the following:

Analytical line wavelength: 206.191 nm

b) Calibration curve preparation and sample measurement.

1) Put 5mL of sample solution to three 10-mL volumetric flasks respectively.

2) Add 2mL and 4 mL (0.25 μg/mL) of zinc standard solution to volumetric flasks of **1)** above, then add hydrochloric acid (1+23) up to the marked line to make the sample solution of Standard Addition Method.

3) Add hydrochloric acid (1+23) to the marked line of the remaining volumetric flask of **1)** above to make the sample solution without standard solution.

4) Spray the sample solution of Standard Addition Method and the sample solution without standard solution into the induction plasma, and read the indicated value at a wavelength of 206.191 nm.

5) Transfer 5 mL of blank test solution to a 10-mL volumetric flask, conduct the same procedures as in **3) - 4)** to read the indicated value, and correct the indicated value obtained from the respective sample solutions.

6) Prepare a curve for the relationship between the added zinc concentration and the corrected indicated value of the sample solution for Standard Addition Method and the sample solution without standard solution.

7) Obtain the zinc content from the intercept of the calibration curve to calculate the concentration of zinc in the analytical sample.

Comment 3 The zinc concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1**) - **4)** and **6) - 7)** to obtain the zinc content in the blank test solution.

Comment 4 Simultaneous measurement of multiple elements by ICP-AES is available. In this case, mix a predetermined amount of copper standard solution (0.1 mg/mL), zinc standard solution (0.1 mg/mL), cadmium standard solution (0.1 mg/mL), nickel standard solution (0.1 mg/mL), chromium standard solution (0.1 mg/mL) and lead standard solution (0.1 mg/mL), dilute with hydrochloric acid (1 +23) to prepare mixture standard solution (Cu 25μg/mL, Zn 25 μg/mL, Cd 0.25μg/mL, Ni 2.5 μg/mL, Cr 2.5 μg/mL and Pb 2.5 μg/mL) (1). Use the mixture solution instead of zinc standard solution (25 μg/mL) in **(4.2) b) 2)**. After that, conduct the same procedures as in **(4.2) b)** to calculate the concentration of respective elements in the analytical sample. Note that the wavelength for measurement of respective elements is Cu 324.754 nm, Zn 206.191 nm, Cd 228.802 nm, Ni 231.604 nm, Cr 205.552 nm and Pb 220.351 nm.

In addition, the respective element concentrations for respective standard addition samples are shown in the below Table.

Table The additive amount in mixture standard solution
and the additive concentration of respective element in respective sample solution

	Additive amount (mL) in mixture standard solution	Cd (mg/L)	Pb (mg/L)	Ni (mg/L)	Cr (mg/L)	Cu (mg/L)	Zn (mg/L)
Sample solution without standard solution	0	0	0	0	0	0	0
Sample solution 1 of Standard Addition Method	2	0.05	0.5	0.5	0.5	5	5
Sample solution 2 of Standard Addition Method	4	0.1	1	1	1	10	10

Comment 5 For each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixture sludge fertilizer, calcined sludge fertilizer and composted sludge fertilizer, the repeatability obtained from triplicates measurement is 0.1 % - 2.3 % relative standard deviation.

Additionally, the minimum limit of quantification of the test method is about 8 mg/kg.

Reference

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI and Tetuya NOMURA: Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer, Vol.4, 2011. (36 - 48)

(5) Flow sheet for zinc: The flow sheet for zinc in fertilizers is shown below:

5.00 g analytical sample	200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	Ignite at 450 °C ± 5 °C
Standing to cool	Room temperature
	← A small amount of water
	← About 10 mL of nitric acid
	← About 30 mL of hydrochloric acid
Heating	Cover with a watch glass, and digest for 30 minutes
Heating	Slightly move a watch glass to remove acid
Standing to cool	Room temperature
	← 25 mL - 50 mL of hydrochloric acid (1+5)
Heating	Cover with a watch glass, and dissolve
Standing to cool	Room temperature
Transfer	100-mL - 200-mL volumetric flask, water
	← Water (up to the marked line)
Filtration	Type 3 filter paper
Aliquot 5 mL	10-mL volumetric flask, 3 flasks
	← 0, 2 and 4 mL of zinc standard solution (25 µg / mL)
	← Hydrochloric acid (1+23) (up to the marked line)
Measurement	ICP Atomic Emission Spectrometer (206.191nm)

Figure Flow sheet for zinc in fertilizers

4.9.2 Water-soluble zinc

4.9.2.a Flame atomic absorption spectrometry

(1) Summary
This test method is applicable to fertilizers that indicate zinc content as a response modifier. Extract by adding water to an analytical sample, spray in an acetylene-air flame and measure the atomic absorption with zinc at a wavelength of 213.9 nm to quantify water-soluble zinc.

(2) Reagents, etc.: Reagents and water are as shown below:

a) Water: Water of A3 specified in JIS K 0557.

b) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

c) Zinc standard solution (0.1 mg/mL): A zinc standard solution (0.1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.

d) Zinc standard solutions (0.5 µg - 5 µg/mL) for the calibration curve preparation (1): Transfer 2.5 mL - 25 mL of zinc standard solution (0.1 mg/mL) to 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

e) Blank test solution for the calibration curve preparation (1): Hydrochloric acid (1+23) used in the procedure in **d**).

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as follows:

a) Rotary shaker: A rotary shaker that can rotate upside down a 500-mL volumetric flask at 30 - 40 revolutions/min.

b) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (2) function.

1) **Light source**: A zinc hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)

2) **Gas:** Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

Note (2) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and transfer to a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make the sample solution.

Comment 1 In the procedure in **a)**, it is also allowed to weigh 2.50 g of the analytical sample and transfer to a 250-mL volumetric flask.

Comment 2 The procedure in **(4.1)** is the same as the procedure in **(4.1)** in **4.10.2.a**, **4.13.1.a** and **4.14.1.a**.

Comment 3 The sample solution prepared in **(4.1)** in **4.2.4.a** can also be used.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 213.9 nm

b) Calibration curve preparation

1) Spray the zinc standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 213.9 nm.

2) Prepare a curve for the relationship between the zinc concentration and the indicated value of the zinc standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.05 mg - 0.5 mg as Zn) to a 100-mL volumetric flask.

2) Add about 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Conduct similarly as in **b) 1)** to read the indicated value.

4) Obtain the zinc content from the calibration curve, and calculate the water-soluble zinc (W-Zn) in the analytical sample.

Comment 4 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for water-soluble zinc hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2012	Fluid mixed fertilizer	75	0.0586	0.0023	3.7

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:

RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.192 - 194, Yokendo, Tokyo (1988)

(5) Flow sheet for water-soluble zinc: The flow sheet for water-soluble zinc in fertilizers is shown below:

5.00 g analytical sample	500-mL volumetric flask.
← About 400 mL of water,	
Shaking to mix	Rotary shaker (30 - 40 revolutions/minutes), for 30 minutes
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined amount)	100-mL volumetric flasks
← 25 mL of hydrochloric acid (1+5)	
← Water (up to the marked line)	
Measurement	Atomic absorption spectrometer (213.9 nm)

Figure Flow sheet for water-soluble zinc in fertilizers.

4.10 Copper

4.10.1 Total copper

4.10.1.a Flame atomic absorption spectrometry

(1) Summary
This test method is applicable to fertilizers.
Pretreat an analytical sample with incineration and nitric acid-hydrochloric acid (1+3), and then spray in an acetylene-air flame, and measure the atomic absorption with copper at a wavelength of 324.8 nm to quantify total copper.

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Copper standard solution (0.1 mg/mL):** A copper standard solution (Cu 0.1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **e) Copper standard solutions (0.5 µg - 5 µg/mL) for the calibration curve preparation(1):** Transfer 2.5 mL - 25 mL of copper standard solution (0.1 mg/mL) to 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.
- **f) Blank test solution for the calibration curve preparation (1):** Hydrochloric acid (1+23) used in the procedure in **e**).

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as shown below:

- **a) Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121 with the background correction (2) function.
  - **1) Light source**: A copper hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)
  - **2) Gas:** Gas for heating by flame
    - (i) Fuel gas: acetylene
    - (ii) Auxiliary gas: Air sufficiently free of dust and moisture.
- **b) Electric furnace:** An electric furnace that can be adjusted to 450 ºC ± 5 ºC.
- **c) Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note (2) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

- **a)** Weigh 5.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.
- **b)** Put the tall beaker in an electric furnace, and heat gently to char(3).
- **c)** Ignite at 450 ºC ± 5 ºC to incinerate (4).
- **d)** After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
- **e)** Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass (5), and continue heating on the hot plate or sand bath to concentrate until nearly dried up.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5)(6) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer the solution to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.

i) As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare the blank test solution.

Note **(3)** Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.

(4) Example of ignition period: 8 - 16 hours

(5) The watch glass can be removed.

(6) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h)**, about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 Do not conduct the procedures in **(4.1) b)** - **c)** in the case of fertilizers not containing organic matters.

Comment 2 The procedures in **(4.1)** are the same as in **(4.1**) in **4.9.1.a**.

Comment 3 The sample solution prepared in **(4.1.2)** in **4.2.1.a** can also be used.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 324.8 nm

b) Calibration curve preparation

1) Spray the copper standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 324.8 nm.

2) Prepare a curve for the relationship between the copper concentration and the indicated value of the copper standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Subject the sample solution (7) to the same procedure as in **b) 1)** to read the indicated value.

2) Subject the blank test solution to the same procedure as in **b) 1)** to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the copper content from the calibration curve, and calculate the total copper in the analytical sample.

Note **(7)** If there is a possibility that the copper concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount with hydrochloric acid (1+23).

Comment 4 The copper concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1)** and **3)** to obtain the copper content in the blank test solution.

Comment 5 Replicate testing was conducted using human waste sludge fertilizer (2 samples), calcined sludge fertilizer (2 samples) and composted sludge fertilizer (2 samples); as a result, repeatability in the range of 210 mg/kg - 830 mg/kg total copper (mean determined value) was 0.6 % - 3.7 % relative standard deviation.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.254 - 255, Yokendo, Tokyo (1988)

2) Kimie KATO, Masayuki YOSHIMOTO and Yuji SHIRAI: Systematization of Determination Methods of Major Components in Sludge Fertilizer, Compost and Organic Fertilizer, Research Report of Fertilizer, Vol.3, 2010. (107 - 116)

(5) Flow sheet for total copper: The flow sheet for total copper in fertilizers is shown below:

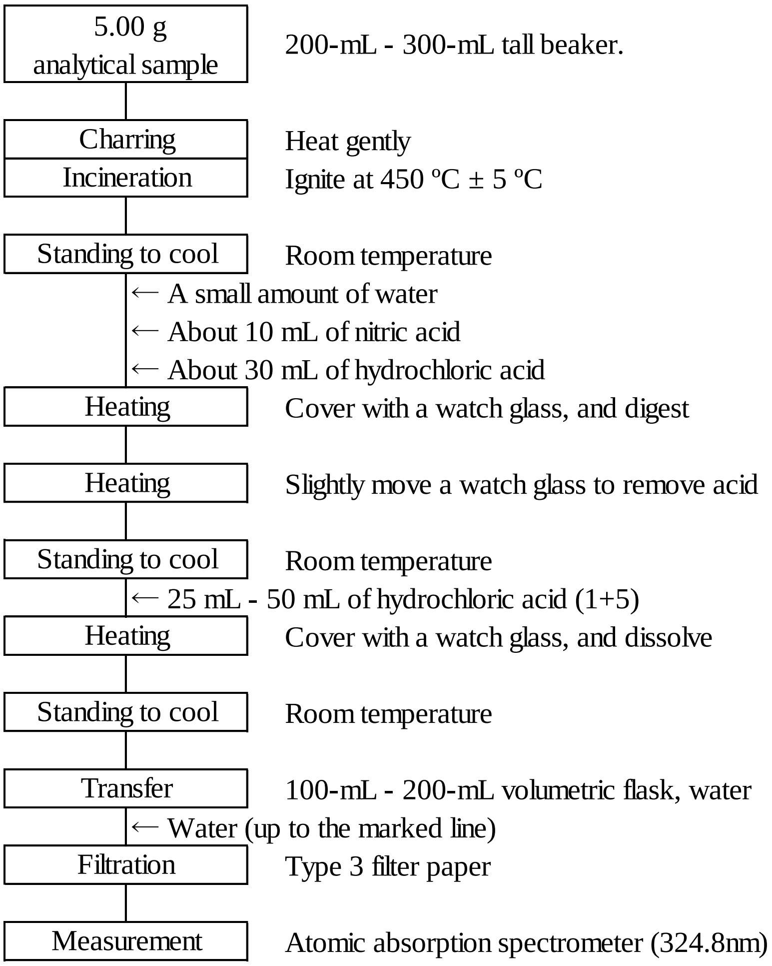


Figure Flow sheet for total copper in sludge fertilizers

4.10.1.b ICP Atomic Emission Spectrometry <Reference method>

(1) Summary

The test method is applicable to sludge fertilizers, etc.
Pretreat an analytical sample with incineration, nitric acid - hydrochloric acid (1+3), introduce it to ICP Atomic Emission Spectrometry ("ICP-AES") and measure the emission with copper at a wavelength of 324.754 nm to quantify copper.

(2) Reagents, etc.: Reagents and water are as shown below:

a) **Water:** Water of A3 specified in JIS K 0557.
b) **Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
c) **Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
d) **Copper standard solution (0.1 mg/mL):** Copper standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
e) **Copper standard solutions (2.5 µg/ mL)** (1)**:**
Dilute a predetermined amount of copper standard solution (0.1 mg/mL) with hydrochloric acid (1+23) to prepare copper standard solution (2.5 µg/mL).

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as shown below:

a) **ICP Atomic Emission Spectrometer:** An atomic emission spectrometer specified in JIS K 0116.
   1) **Gas:** Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity
b) **Electric furnace**: An electric furnace that can keep the test temperature at ± 5 ºC
c) **Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh 5.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.
b) Put the tall beaker in an electric furnace, and heat gently to char(2).
c) Ignite at 450 ºC ± 5 ºC to incinerate (3).
d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
f) Slightly move the watch glass (4), and continue heating on the hot plate or sand bath to concentrate until nearly dried up.
g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (5) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.
i) As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare the blank test solution.

Note **(2)** Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.

(3) Example of ignition period: 8 - 16 hours

(4) The watch glass can be removed.

(5) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 Do not conduct the procedures in **(4.1) b) - c)** in the case of fertilizers not containing organic matters.

Comment 2 The procedures in **(4.1)** are the same as in **(4.1)** in **4.9.1.a**.

(4.2) Measurement: Conduct measurement (Standard Addition Method) according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Atomic Emission Spectrometer used in measurement.

a) Measurement conditions for the ICP Atomic Emission Spectrometer: Set up the measurement conditions for the ICP Atomic Emission Spectrometer considering the following:

Analytical line wavelength: 324.754 nm

b) Calibration curve preparation and sample measurement.

1) Put 5mL of sample solution to three 10-mL volumetric flasks respectively.

2) Add 2mL and 4 mL (2.5 μg/mL) of copper standard solution to volumetric flasks of **1**) above, then add hydrochloric acid (1+23) to the marked line to make the sample solution of Standard Addition Method.

3) Add hydrochloric acid (1+23) to the marked line of the remaining volumetric flask of **1**) above to make the sample solution without standard solution.

4) Spray the sample solution of Standard Addition Method and the sample solution without standard solution into the induction plasma, and read the indicated value at a wavelength of 324.754 nm.

5) Transfer 5mL of the blank test solution to a 10-mL volumetric flask, conduct the same procedures as in **3) - 4)** to read the indicated value, and correct the indicated value obtained from the respective sample solutions.

6) Prepare a curve for the relationship between the added copper concentration and the corrected indicated value of the sample solution for Standard Addition Method and the sample solution without standard solution.

7) Obtain the copper content from the intercept of the calibration curve to calculate the concentration of copper in the analytical sample.

Comment 3 The copper concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1**) - **4)** and **6) - 7)** to obtain the copper content in the blank test solution.

Comment 4 Simultaneous measurement of multiple elements by ICP-AES is available. In this case, mix a predetermined amount of copper standard solution (0.1 mg/mL), zinc standard solution (0.1 mg/mL), cadmium standard solution (0.1 mg/mL), nickel standard solution (0.1 mg/mL), chromium standard solution (0.1 mg/mL) and lead standard solution (0.1 mg/mL), dilute with hydrochloric acid (1 +23) to prepare mixture standard solution (Cu 25μg/mL, Zn 25 μg/mL, Cd 0.25μg/mL, Ni 2.5 μg/mL, Cr 2.5 μg/mL and Pb 2.5 μg/mL) (1) Use the mixture solution instead of copper standard solution (25 μg/mL) in **(4.2) b) 2)**. After that, conduct the same procedures as in **(4.2) b)** to calculate the concentration of respective elements in the analytical sample. Note that the wavelength for measurement of respective elements is Cu 324.754 nm, Zn 206.191 nm, Cd 228.802 nm, Ni 231.604 nm, Cr 205.552 nm and Pb 220.351 nm.

In addition, the respective element concentrations for respective standard addition samples are shown in the below Table.

Table The additive amount in mixture standard solution
and the additive concentration of respective elements in respective sample solutions

	Additive amount (mL) in mixture standard solution	Cd (mg/L)	Pb (mg/L)	Ni (mg/L)	Cr (mg/L)	Cu (mg/L)	Zn (mg/L)
Sample solution without standard solution	0	0	0	0	0	0	0
Sample solution 1 of Standard Addition Method	2	0.05	0.5	0.5	0.5	5	5
Sample solution 2 of Standard Addition Method	4	0.1	1	1	1	10	10

Comment 5 For each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixture sludge fertilizer, calcined sludge fertilizer and composted sludge fertilizer, the repeatability obtained from triplicates measurement is 0.6 % - 1.8 % relative standard deviation. Additionally, the minimum limit of quantification of the test method is about 3 mg/kg.

Reference

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI and Tetuya NOMURA: Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer, Vol.4, 2011. (36 - 48)

(5) Flow sheet for copper: The flow sheet for copper in fertilizers is shown below:

5.00 g analytical sample	200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	Ignite at 450 ºC ± 5 ºC
Standing to cool	Room temperature
← A small amount of water	
← About 10 mL of nitric acid	
← About 30 mL of hydrochloric acid	
Heating	Cover with a watch glass, and digest for 30 minutes
Heating	Slightly move a watch glass to remove acid
Standing to cool	Room temperature
← 25 mL - 50 mL of hydrochloric acid (1+5)	
Heating	Cover with a watch glass, and dissolve
Standing to cool	Room temperature
Transfer	100-mL - 200-mL volumetric flask, water
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot 5 mL	10-mL volumetric flask, 3 flasks
← 0, 2 and 4 mL of copper standard solution (25 µg / mL)	
← Hydrochloric acid (1+23) (up to the marked line)	
Measurement	ICP Atomic Emission Spectrometer (324.754 nm)

Figure Flow sheet for copper in fertilizers

4.10.2 Water-soluble copper

4.10.2.a Flame atomic absorption spectrometry

(1) Summary
This test method is applicable to fertilizers that indicate copper content as a response modifier. Extract by adding water to an analytical sample, spray in an acetylene-air flame and measure the atomic absorption with copper at a wavelength of 324.8 nm to quantify water-soluble copper (W-Cu).

(2) Reagents, etc.: Reagents and water are as shown below:

a) Water: Water of A3 specified in JIS K 0557.
b) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
c) Copper standard solution (0.1 mg/mL): A copper standard solution (0.1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
d) Copper standard solutions (0.5 µg - 5 µg/mL) for the calibration curve preparation (1)**:** Transfer 2.5 mL - 25 mL of copper standard solution (0.1 mg/mL) to 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.
e) Blank test solution for the calibration curve preparation (1)**:** Hydrochloric acid (1+23) used in the procedure in **d**).

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as follows:

a) Rotary shaker: A rotary shaker that can rotate upside down a 500-mL volumetric flask at 30 - 40 revolutions/min.
b) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (2) function.
  1) Light source: A copper hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)
  2) Gas: Gas for heating by flame
    (i) Fuel gas: acetylene
    (ii) Auxiliary gas: Air sufficiently free of dust and moisture.

Note (2) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedures
(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and transfer to a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make the sample solution.

Comment 1 In the procedure in **a)**, it is also allowed to weigh 2.50 g of the analytical sample and put it into a 250-mL volumetric flask.
Comment 2 The procedure in **(4.1)** is the same as the procedure in **(4.1)** in **4.9.2.a**.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 324.8 nm

b) Calibration curve preparation

1) Spray the copper standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 324.8 nm.

2) Prepare a curve for the relationship between the copper concentration and the indicated value of the copper standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.05 mg - 0.5 mg as Cu) to a 100-mL volumetric flask.

2) Add about 25 mL of hydrochloric acid, and add water up to the marked line.

3) Conduct similarly as in **b) 1)** to read the indicated value.

4) Obtain the copper content from the calibration curve, and calculate the water-soluble copper (W-Cu).

Comment 3 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for water-soluble cupper hosted by Japan Ferttilizer Quality Assuarance Committee [1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2012	Fluid mixed fertilizer	76	0.0546	0.0014	2.5

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:

RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.254 - 255, Yokendo, Tokyo (1988)

(5) Flow sheet for water-soluble copper: The flow sheet for water-soluble copper in fertilizers is shown below:

5.00 g analytical sample — 500-mL volumetric flask.
← Water, about 400 mL
Shaking to mix — Rotary shaker (30 - 40 revolutions/min), for 30 minutes
← Water (up to the marked line)
Filtration — Type 3 filter paper
Aliquot (predetermined amount) — 100-mL volumetric flask
← 25 mL of hydrochloric acid (1+5)
← Water (up to the marked line)
Measurement — Atomic absorption spectrometer (324.8 nm)

Figure Flow sheet for water-soluble cupper in fertilizers.

4.11 Organic carbon and carbon-nitrogen ratio

4.11.1 Organic carbon

4.11.1.a Dichromate oxidation

(1) Summary

This test method is applicable to sludge fertilizers and compost, etc.

Add a potassium dichromate-sulfuric acid solution to an analytical sample and heat to oxidize organic carbon with potassium dichromate. Quantify unconsumed potassium dichromate by oxidation-reduction titration to obtain organic carbon (O-C). This test method is also referred to as the Method of Tyulin.

(2) Reagents: Reagents are as shown below:

a) **Sulfuric acid:** A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

b) **0.2 mol/L ammonium iron (II) sulfate solution** (1)**:** Weigh 80 g of ammonium iron (II) sulfate hexahydrate specified in JIS K 8979 into a 2,000-mL beaker, and add 1,000 mL of sulfuric acid (1+50) to dissolve.

Standardization: Grind potassium dichromate reference material for volumetric analysis specified in JIS K 8005 in an agate mortar to powder, heat at 150 ºC ± 2 ºC for 1 hour, let it stand to cool in a desiccator, and then transfer about 1 g to a weighing dish, and weigh the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 100-mL volumetric flask, and add water up to the marked line to make the potassium dichromate standard solution(1) (2). On each day to use 0.2 mol/L ammonium iron (II) sulfate solution, transfer 10 mL of the potassium dichromate standard solution to a 100-mL Erlenmeyer flask, add about 5 mL of sulfuric acid (1+2), and then conduct the procedures in (**4.2**) **b**) - **c**), and calculate the factor of 0.2 mol/L ammonium iron (II) sulfate solution by the following formula:

Factor (f) of 0.2 mol/L ammonium iron (II) sulfate solution

$= W \times (A/100) \times (6/294.18) \times (V_1/V_2) \times (1{,}000/V_3)\ /C$

$= (W \times A/V_3) \times (30/294.18)$

- W: Mass (g) of potassium dichromate weighed
- A: Purity (% (mass fraction)) of potassium dichromate
- V_1: Volume (10 mL) of potassium dichromate solution transferred
- V_2: Constant volume (100 mL) of potassium dichromate solution
- V_3: Volume (mL) of 0.2 mol/L ammonium iron (II) sulfate solution needed for titration
- C: Set concentration (0.2 mol/L) of 0.2 mol/L ammonium iron (II) sulfate solution

c) **Potassium dichromate-sulfuric acid solution** (1)**:** Weigh 40 g of potassium dichromate specified in JIS K 8517 to a 3,000-mL beaker. Add 1,000 mL of water to dissolve, and further add gradually 1,000 mL of sulfuric acid while cooling and mixing.

d) **N-Phenylanthranilic acid solution:** Dissolve 0.2 g of N-phenylanthranilic acid of no less than 98 % (mass fraction) in purity and 0.2 g of sodium carbonate specified in JIS K 8625 in a small amount of water, and add water to make 100 mL.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(2) This corresponds to the standard potassium dichromate solution (0.2 M (1/6 $K_2Cr_2O_7$) solution) in **7.1 B 1)** in the Official Methods of Analysis of Fertilizers (1992).

(3) Apparatus and instruments: Apparatus and instruments are as follows:

a) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

b) Sample digestion flask [3]**:** A 100-mL borosilicate glass volumetric flask 100 mL (180 mm total height, 13 mm mouth diameter)

Note (3) Distinguish the volumetric flask used in digestion as a sample digestion flask and do not use it for any other purposes.

(4) Test procedures

(4.1) Dichromate oxidation: Conduct oxidation as follows:

a) Weigh 0.05 g of an analytical sample to the order of 0.1 mg [4], and transfer to a sample digestion flask.

b) Add 25 mL of potassium dichromate-sulfuric acid solution.

c) Heat on a hot plate at 200 ºC until organic matters are completely digested.

d) After standing to cool, fill up with water to 100 mL to make the sample solution.

e) As a blank test, conduct the procedures in **b)** and **d)** using another sample digestion flask to prepare the blank test solution.

Note (4) Up to about 28 mg as organic carbon (O-C).

(5) Heat for no less than 1 hour after boiling

(4.2) Measurement: Conduct measurement as shown below.

a) Transfer 20 mL of the sample solution to a 100-mL Erlenmeyer flask.

b) Add 0.2 mol/L ammonium iron (II) sulfate solution drop-by-drop until the brown color of dichromate ion almost disappears from the sample solution.

c) Add about 0.25 mL of N-phenylanthranilic acid solution[6], and titrate with 0.2 mol/L ammonium iron (II) sulfate solution until the color of the solution changes from dark red-purple to blue-green.

d) Transfer 20 mL of the blank test solution to a 100-mL Erlenmeyer flask, and conduct the procedures in **b) - c)** to titrate.

e) Calculate the organic carbon (O-C) in the analytical sample by the following formula:

Organic carbon (% (mass fraction)) in the analytical sample

$$= (V_4 - V_5) \times C \times f \times (12.011/4)/W \times (100/1{,}000) \times (V_6/V_7)$$

$$= (V_4 - V_5) \times f \times (12.011/40)/W$$

V_4: Volume (mL) of 0.2 mol/L ammonium iron (II) sulfate solution needed for the titration of the blank test solution

V_5: Volume (mL) of 0.2 mol/L ammonium iron (II) sulfate solution needed for the titration of the sample solution

C: Set concentration (0.2 mol/L) of 0.2 mol/L ammonium iron (II) sulfate solution

f: Factor of 0.2 mol/L ammonium iron (II) sulfate solution

V_6: Predetermined volume (100 mL) of the sample solution and the blank test solution in **(4.1) d)**

V_7: Transferred volume (20 mL) of the sample solution and the blank test solution subjected to titration in **(4.2) a)** and **(4.2) d)**

W: Mass (g) of an analytical sample

Note (6) About 5 drops with a 1-mL - 2-mL Komagome pipette. Add the same volume to the sample solution and the blank test solution.

Comment 1 Sample an analytical sample from a test sample prepared in **2.2.3 Grinding (3) Procedure (3.1) b)** by grinding with a mill until it completely passes through a sieve of 500 µm aperture.

Comment 2 Replicate testing was conducted using composted sludge fertilizer (2 samples), human waste sludge fertilizer (1 sample), industrial sludge fertilizer (2 samples), compost (3 samples) and animal excreta (1 sample); as a result, the mean was in the range of 14.2 % - 50.7 % (mass fraction), and the standard deviation and relative standard deviation were 0.04 % - 0.6 % (mass fraction) and 0.1 % - 2.6 %, respectively.

Additionally, the minimum limit of quantification of this test method is about 1.5 % (mass fraction).

Reference

1) Yuji SHIRAI, Yuko SEKINE, and Toshiaki HIROI: Validation of Determination Method for Organic Form Carbon in Sludge Fertilizer and Compost, Research Report of Fertilizer, Vol.3, 2010. (117 - 122)

(5) Flow sheet for organic carbon: The flow sheet for organic carbon in sludge fertilizers and compost, etc. is shown below:

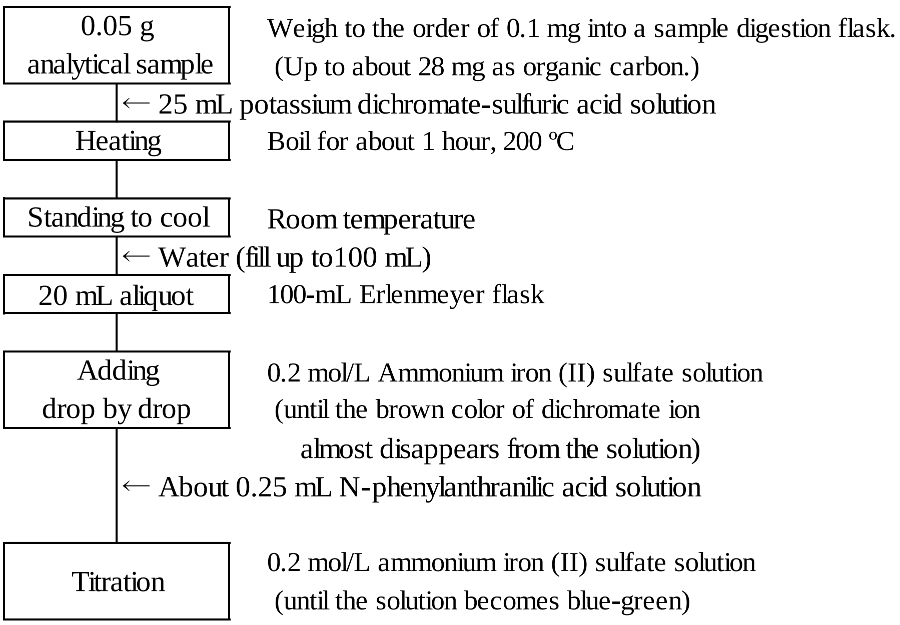


Figure Flow sheet for organic carbon in sludge fertilizers and compost, etc.

4.11.1.b Combustion method

(1) Summary

This test method is applicable to compost and sludge fertilizers.

Drop hydrochloric acid (1+3) to an analytical sample and evaporate inorganic carbon as carbon dioxide, then thermally decompose carbon compounds using a total nitrogen-total carbon analyzer by the combustion method to measure carbon dioxide gas with a thermal conductivity detector.

(2) Reagents: Reagents are as shown below:

a) Sea sand: Particle diameter 425 µm – 850 µm

b) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

Comment 1 Sea sand **(**particle diameter 425 µm – 850 µm) is commercially sold by Wako Pure Chemical Industries, Ltd. and YONETAMA YAKUHIN KOGYO Co., Ltd.

(3) Instruments: Instruments are as shown below:

a) Total nitrogen-total carbon analyzer by the combustion method: A total nitrogen-total carbon analyzer configured on the basis of the principle of the combustion method (modified Dumas' method).

1) Turn on the total nitrogen-total carbon analyzer by the combustion method (1), and adjust so that stable indicated values can be obtained.

(i) Combustion gas: Oxygen having purity no less than 99.99 % (volume percentage)

(ii) Carrier gas: Helium having purity no less than 99.99 % (volume percentage)

b) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

c) Drying apparatus: A drying apparatus that can adjust test temperature to ± 2 ºC.

Note (1) The setup of the program and the parameters of the analyzer are according to the specification and the operation method of the total nitrogen-total carbon analyzer by the combustion method used.

(4) Test procedures: Conduct measurement as shown below. However, confirm that there is no difference from the measured value of organic carbon obtained in advance according to **4.11.1.a** by using an analytical sample.

(4.1) Hydrochloric acid treatment

a) Weigh 0.05 g of an analytical sample to the order of 0.1 mg, and transfer to a container for combustion.

b) Cover the analytical sample with about 0.2 g of sea sand and moisten the analytical sample by dropping a few drops of water.

c) After dropping 0.5 mL - 0.7 mL of hydrochloric acid (1+3) little by little (2), drop about 0.3 mL of water (3) (4).

d) Heat a container for combustion on a hot plate at 100 ºC for 90 minutes to make it dry up.

e) Put the container for combustion into a drying apparatus and heat for 30 minutes (5).

f) After heating, let it stand to cool to make a test sample.

Note (2) The additive amount of hydrochloric acid (1+3) is merely a target. It is enough to make the whole analytical sample come into contact with hydrochloric acid. Let it stand for a short time in the case of producing bubbles.

(3) Shake calmly the container for combustion to make the analytical sample completely come into contact with hydrochloric acid.

(4) Let it stand for a short time in the case of producing bubbles when dropping hydrochloric acid (1+3).

(5) Remove hydrochloric acid completely

Comment 2 Sample an analytical sample from a test sample prepared in **2.2.3 Grinding (3) Procedure (3.1) b)** by grinding with a mill until it completely passes through a sieve of 500 µm aperture.

Comment 3 When it is confirmed that, for example, the volatilization of hydrogen chloride is not detected by using a test paper, etc. and hydrochloric is completely removed in the procedure **d)**, the procedure **e)** can be skipped.

(4.2) Measurement: Specific measurement procedures are according to the operation method of a total nitrogen-total carbon analyzer by the combustion method.

a) Measurement conditions for the total nitrogen-total carbon analyzer by the combustion method: Set up **t**he total nitrogen-total carbon analyzer considering the following:

Combustion temperature: No less than 870 ºC

b) Calibration curve preparation

1) Turn on the total nitrogen-total carbon analyzer by the combustion method (1), and adjust so that stable indicated values can be obtained.

2) Weigh a predetermined amount of the standard for calibration curves (6) to the order of 0.1 mg into a combustion vessel.

3) Insert the combustion vessel into the total nitrogen-total carbon analyzer by the combustion method, and read the indicated value.

4) Conduct the procedure in **3)** for another combustion vessel for a blank test, and read the indicated value.

5) Prepare a curve for the relationship between the carbon content and the indicated value of the standard for calibration curves and the blank test for calibration curves.

c) Sample measurement

1) Insert the combustion vessel containing the test sample to the total nitrogen-total carbon analyzer by the combustion method, and read the indicated value.

2) Obtain the carbon content from the calibration curve, and calculate organic carbon in the analytical sample.

Note (6) Standard for calibration curves: DL-Aspartic acid (purity no less than 99 % (mass fraction)), EDTA (purity no less than 99 % (mass fraction)), hippuric acid (purity no less than 98 % (mass fraction)) or other reagents having equivalent purity recommended by the total nitrogen-total carbon analyzer by the combustion method used.

Comment 4 Replicate testing was conducted using sewage sludge fertilizer (1 sample), human waste sludge fertilizer (1 samples), industrial sludge fertilizer (1 sample), calcined sludge fertilizer (1 sample) and composted sludge fertilizer (2 samples); as a result, the mean was in the range of 8.5 % - 46.0% (mass fraction), and the repeatability standard deviation and relative repeatability standard deviation were 0.1 % - 0.5% (mass fraction) and 0.2 % - 2.0%, respectively.

Additionally, the minimum limit of quantification of the test method is about 0.05 % (mass fraction).

Table 1 Results and statistical analysis results from a collaborative study for the organic carbon method

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Calcined sludge fertilizer	9	9.45	0.17	1.8	0.38	4.0
Industrial sludge fertilizer	8	15.13	0.20	1.3	0.42	2.8
Compost	9	20.50	0.76	3.7	0.94	4.6
Human waste sludge fertilizer	8	34.96	0.07	0.2	0.62	1.8
Composted sludge fertilizer	9	38.20	0.27	0.7	0.73	1.9

1) Number of laboratories used in analysis
2) Total mean (number of laboratories × 2 samples replicate analysis)
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Reference

1) Aiko YANO, Satono AKIMOTO, and Yuji SHIRAI: Determination of Organic Carbon in Sludge Fertilizer and Compost by Hydrochloric Acid-Treated Combustion Method, Research Report of Fertilizer, Vol.9, 2013. (9 - 19)

(5) Flow sheet for organic carbon: The flow sheet for organic carbon in compost and sludge fertilizers is shown below:

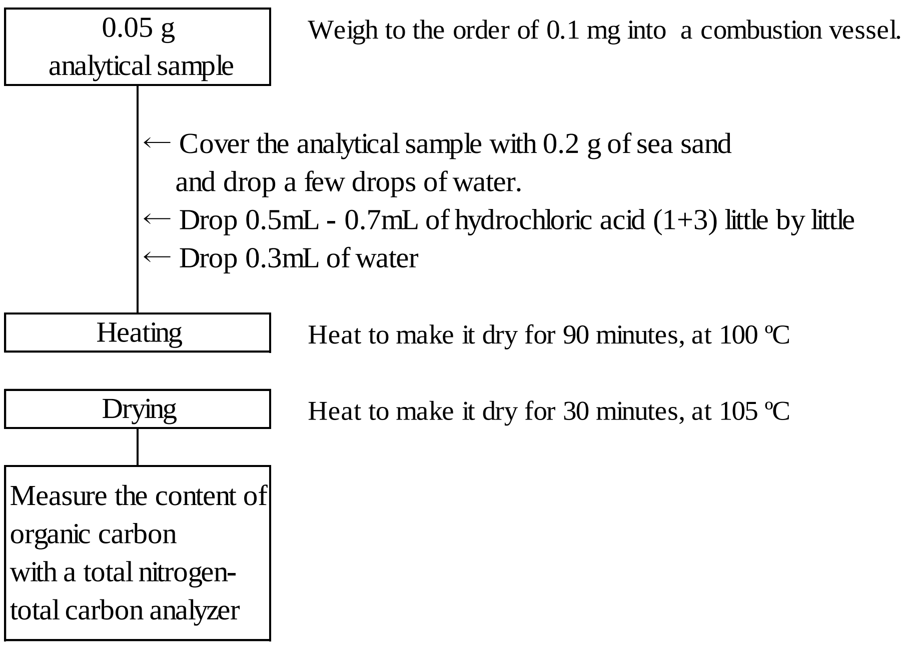
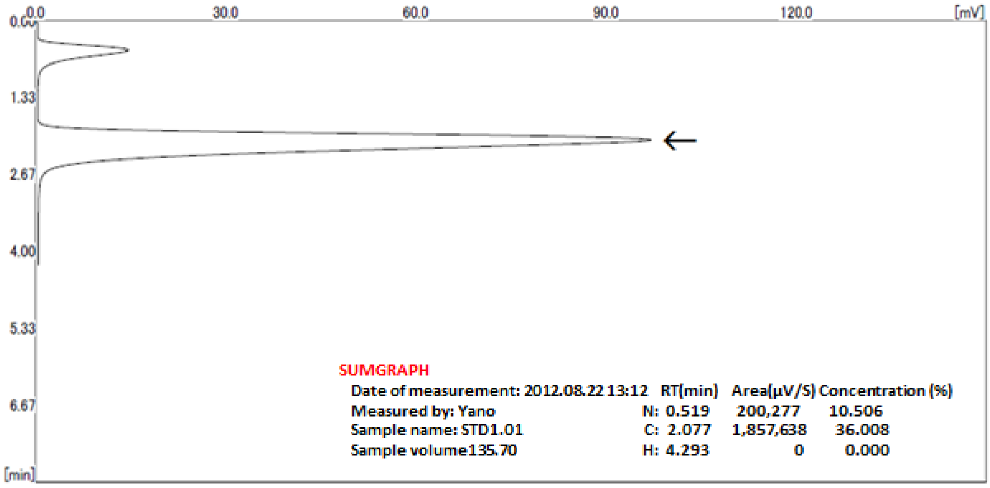
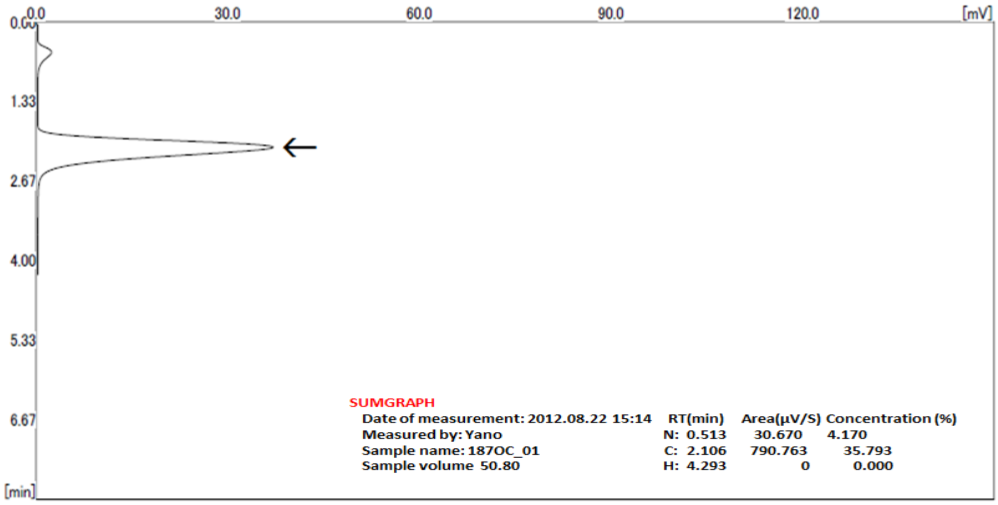


Figure Flow sheet for organic carbon by the combustion method

Reference: Chromatograms of a standard calibration curve and an analytical sample are shown below



1) Total carbon in a standard calibration curve (DL-Aspartic acid)



2) Total carbon in an analytical sample (sludge fertilizer)

Reference figures Chromatograms of organic carbon.

Measurement conditions for total nitrogen-total carbon analyzer by the combustion method

Combustion gas: Highly pure oxygen, purity no less than 99.99995 % (volume fraction), flow rate 200 mL/min

Carrier gas: Highly pure helium, purity no less than 99.9999 % (volume fraction), flow rate 80 mL/min

Separation column: A silica gel stainless column (Length: 1 m)

Detector: Thermal Conductivity Detector (TCD)

Measurement cycle: Purge time = 60 seconds, circulation combustion time = 300 seconds, measurement time = 270 seconds

Current value of Detector: 160 mA

Temperature conditions: Reaction furnace temperature: 870 ºC
Reduction furnace temperature: 600 ºC
Column oven temperature: 70 ºC
Detector temperature: 100 ºC

4.11.2 Carbon-nitrogen ratio

(1) Summary

Calculate carbon-nitrogen ratio (CN ratio) by dividing the organic carbon obtained in **4.11.1** by the total nitrogen obtained in **4.1.1**.

(2) Calculation of carbon-nitrogen ratio

a) Calculate the carbon-nitrogen ratio in an analytical sample by the following formula:

Carbon-nitrogen ratio in an analytical sample
= O-C/T-N

O-C: Organic carbon (% (mass fraction)) (1) in the analytical sample obtained in **4.11.1**

T-N: Total nitrogen (% (mass fraction)) (1) in the analytical sample obtained in **4.1.1**

Note (1) O-C and T-N use raw data without rounding numerical value

4.12 Sulfur

4.12.1 Total sulfur content

4.12.1.a Titration analysis <Reference method>

(1) Summary
This test method is applicable to fertilizers mainly containing ferrous sulfate among sulfur and its compound.
Dissolve an analytical sample in water and diluted sulfuric acid, add phosphate and then titrate with a standard potassium permanganate solution.

(2) Reagents, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557.

b) Sulfuric acid: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) Phosphate: A JIS Guaranteed Reagent specified in JIS K 90051 or a reagent of equivalent quality.

d) 0.02 mol/L potassium permanganate solution: Dissolve 3.16 g of potassium permanganate specified in JIS K 8247 in about 800 mL of water to boil and add water to make 1,000 mL and leave at rest 1 - 2 day (s). Further filter with a funnel type glass filter (G4) and store in a colored bottle. Or, a reagent of equivalent quality (volumetric analysis grade) that is commercially available.

Standardization: Dry sodium oxalate of reference materials for volumetric analysis specified in JIS K 8005 at 200 ºC for 1 hour and let it stand to cool in a sulfate desiccator, and then put about 0.3 g into a weighing dish to measure the mass to the order of 0.1 mg. Add about 250 mL of sulfuric acid (1+20) cooled down to 25 ºC - 30 ºC after boiling in advance and dissolve. Add about 40 mL of 0.02 mol/L potassium permanganate solution while gently shaking for about 1 minute. Heat up to 55 ºC - 60 ºC after the red color of potassium permanganate solution disappears. Titrate with 0.02 mol/L potassium permanganate solution while keeping the temperature and continue titrating until the color of solution becomes light red (1). Calculate the factor of 0.02 potassium permanganate solution by the following formula.

Factor (f) of 0.02 mol/L potassium permanganate solution
$= (W \times (A/100) \times ((2/5)/133.999) \times ((1{,}000/V_1)/C)$
$= W \times (A/V_1) \times 1.4925$

W: Mass (g) of sodium oxalate sampled
A: Purity (% (mass fraction)) of sodium oxalate sampled
V_1: Volume (mL) of 0.02 mol/L potassium permanganate needed for titration
C: 0.02 mol/L potassium permanganate solution (0.02 mol/L)

Note (1) The endpoint is reached when the color of solution keeps as it is for 30 seconds after coloring

(3) Instruments: Instruments are as follows:

a) Magnetic stirrer:

(4) Test procedures

(4.1) Measurement: Conduct measurement as shown below.

a) Weigh 0.5 g - 1 g of an analytical sample to the order of 0.1 mg, and transfer to a 200-mL tall beaker.

b) Add about 50 mL of water and about 15 mL of sulfuric acid (1+5) and shake with a magnetic stirrer to dissolve.

c) Immediately add about 1 mL of phosphate, and then titrate with 0.02 mol/L potassium permanganate solution until the color of solution becomes light red.

d) As a blank test, conduct the procedures in **b) - c)** using another sample digestion flask to titrate [(2)].

e) Calculate the total sulfur content in an analytical sample by the following formula.

Total sulfur content (% (mass fraction)) = $(5 \times 0.02 \times f \times (V_2 - V_3)/1{,}000 \times 80.064)/W \times 100$
$= (f \times (V_2 - V_3))/W \times 0.80064$

W: Mass (g) of an analytical sample sampled
V_2: Volume (mL) of 0.02 mol/L potassium permanganate solution needed for titration
V_3: Volume (mL) of 0.02 mol/L potassium permanganate solution needed for titration of a blank test
f: Factor of 0.02 mol/L potassium permanganate solution

Note (2) Titrate using a brown burette.

Comment 1 Replicate testing was conducted using ferrous sulfate materials (5 samples); as a result, the mean was in the range of 5.57 % - 44.74 % (mass fraction), and the standard deviation and relative standard deviation were 0.01 % - 0.04 % (mass fraction) and 0.02 % - 0.7 %, respectively.

References

1) Yasushi SUGIMURA and Shinjiro IZUKA: Method validation of Redox Titration for Determination of Sulfur content (as sulfur trioxide) in Fertilizers of Ferrous sulfate and its mixture materials, Research Report of Fertilizer, Vol.3, 2010. (25 - 29)

2) JIS K 8978: Iron (II) sulfate heptahydrate (Reagent) (2008)

(5) Flow sheet for total sulfur content: The flow sheet for total sulfur content in sludge fertilizers mainly containing ferrous sulfate is shown below.

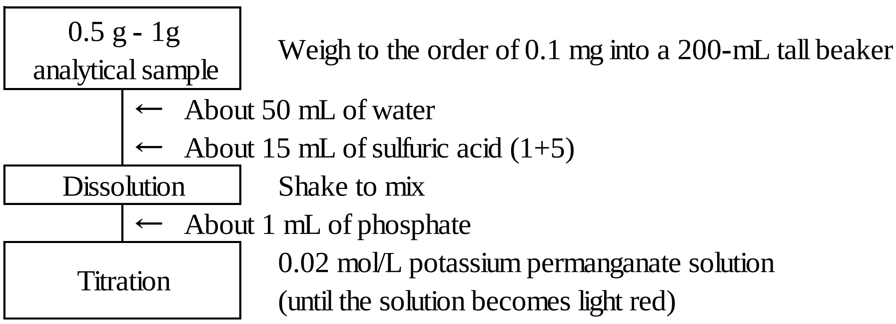


Figure Flow sheet for total sulfur content (raw material: ferrous sulfate)

4.12.1.b Barium chloride gravimetric analysis

(1) Summary

This test method is applicable to fertilizers mainly containing sulfur and sulfuric acid among sulfur and its compound.

Dissolve an analytical sample in a potassium hydroxide-ethanol solution and add hydrogen peroxide to oxidize, and then add thermal barium chloride to form the precipitate of barium sulfate and obtain total sulfur content from the mass.

(2) Reagents: Reagents are as shown below.

- **a) Potassium hydroxide/ethanol solution:** Dissolve 10 g of potassium hydroxide specified in JIS K 8574 in 50 mL of ethanol (95) specified in JIS K 8102, further add 50 mL of water.
- **b) Hydrogen peroxide:** A JIS Guaranteed Reagent (30 % (mass fraction)) specified in JIS K 8230 or a reagent of equivalent quality.
- **c) Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **d) Nitric acid:** A JIS Guaranteed Reagent (HNO_3 60 % (mass fraction)) specified in JIS K 8541 or a reagent of equivalent quality.
- **e) Barium chloride solution (100 g/L):** Dissolve 100 g of barium chloride dihydrate specified in JIS K 8155 in water to make 1,000 mL.
- **f) Silver nitrate solution (2 g/100mL):** Dissolve 2 g of silver nitrate specified in JIS K 8550 in water to make 100 mL.
- **g) Phenolphthalein solution (1 g/100 mL):** Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

(3) Apparatus and instruments: Apparatus and instruments are as follows:

- **a) Hot plate:** A hot plate whose surface temperature can be adjusted up to 250 ºC.
- **b) Water bath:** A water bath that can adjust test temperature to ± 2 ºC.
- **c) Crucible:** After heating porcelain crucible or platinum crucible in an electric furnace at 800 ºC in advance, let it stand to cool in a desiccator and measure the mass to the order of 1 mg.
- **d) Electric furnace:** An electric furnace that can keep the test temperature to ± 5 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows (1):

- **a)** Weigh 1 g - 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL tall beaker.
- **b)** Add about 50 mL of potassium hydroxide/ethanol solution, cover with a watch glass and heat on a hot plate to boil (2).
- **c)** After standing to cool, transfer to a 250-mL volumetric flask and add water up to the marked line.
- **d)** Filter with Type 3 filter paper (3) to make the sample solution.

Note **(1)** Omit extraction if fluid fertilizers are made from only sulfuric acid and all materials are dissolved

(2) Until sulfur content is dissolved. About 5 minutes when raw materials, etc. are not dissolved.

(3) Omit the procedures in **d)** when all materials are dissolved.

(4.2) Measurement: Conduct measurement as shown below.

a) Transfer a predetermined amount (the equivalents of about 30 mg - 170 mg as SO_3) of the sample solution to a 300-mL tall beaker. [(4)]

b) Add about 50 mL of water and about 5 mL of hydrogen peroxide and heat in a water bath at 80 ºC - 90 ºC for about 1 hour while sometimes shaking [(5)].

c) After standing cool, add 1 - 2 drop(s) of phenolphthalein solution (1 g/100 mL) [(6)], and add hydrochloric acid (2+1) until the color of solution disappears [(7)].

d) Further add hydrochloric acid (2+1), add water to make about 100 mL and boil on a hotplate for about 5 minutes.

e) Immediately add about 6 mL of thermal barium chloride solution (100 g/L) [(8)] while sometimes shaking in a water bath at 80 ºC - 90 ºC [(9)].

f) After standing to cool for a few minutes [(10)], add a few drops of thermal barium chloride solution (100 g/L) and check that new precipitate of barium sulfate is no longer formed.

g) Further add about 2 mL of thermal barium chloride solution (100 g/L) while shaking [(11)].

h) After heating in a water bath at 80 ºC - 90 ºC for about 2 hours, stop the heat source of water bath and let it stand to cool for no less than 4 hours[(5)].

i) Filter with filter paper (Type 5 - C), wash a container with water and move the whole precipitate to a filter paper.

j) Wash the precipitate and the filter paper (Type 5 - C) with water several times [(12)].

k) Put the precipitate together with the filter paper into a crucible.

l) Put the crucible into a drying apparatus and dry at about 120 ºC for 1 hour.

m) After standing to cool, put the crucible into an electric furnace and heat gently to be carbonized.

n) Ignite at about 800 ºC for 2 hours.

o) After ignition [(13)], move the crucible to a desiccator and let it stand to cool [(14)].

p) Measure the mass of crucible to the order of 0.1 mg.

q) Calculate the total sulfur content in an analytical sample by the following formula.

Total sulfur content (% (mass fraction)) = $(A \times 0.343)/(W \times V_2/V_1) \times 100$
$= 34.3 \times A \times V_1/(W \times V_2)$

A: Mass (g) of precipitate in **p)**
W: Mass (g) of an analytical sample
V_1: Constant volume (mL) of sample solution
V_2: Volume (mL) of sample solution transferred

Note **(4)** Weigh 1g - 5g of an analytical sample to the order of 0.1 mg if the fluid fertilizers of the analytical sample are made from only sulfuric acid and all materials are dissolved.

(5) It can be suspended after the procedures end.

(6) A pH meter can be used for neutralization.

(7) Omit the procedures in **c)** if fluid fertilizers are made from only sulfuric acid and all materials are dissolved.

(8) Heated up to 70 ºC - 80 ºC in a water bath in advance.

(9) Add drop-by-drop.

(10) Until precipitate settles.

(11) Add barium chloride solution slightly excessively to reduce the solubility of barium sulfate.

(12) Wash the precipitate until a white turbidity is no longer formed when about 5 mL of nitric acid (1+2) and about 1 mL of silver nitrate solution (2 g/100 mL) are added to about 20 mL of washing.

(13) To prevent a crucible from chipping, it is recommended to let it stand to cool gently in an electric furnace until the temperature of an electric furnace falls to no more than 200 ºC.

(14) Time to let it stand to cool in a desiccator should be constant. In the case of a porcelain crucible, it is about 45 - 60 minutes.

Comment 1 Replicate testing was conducted using sulfur and sulfur materials (6 samples); as a result, the mean was in the range of 3.28 %- 246.96 % (mass fraction), and the standard deviation and relative standard deviation were 0 % - 0.49 % (mass fraction) and 0 % - 0.30 %, respectively.
Additionally, table 1 shows results and analysis results from a collaborative study for test method validation.

Table 1 Results and analysis results from a collaborative study for total sulfur content with the barium chroride gravimetric analysis (analized as surfur (S))

Sample name	Number of laboratories[1)]	Mean[2)] (%)[8)]	Mean[3)] (%)[8)]	s_r[4)] (%)[8)]	RSD_r[5)] (%)	s_R[6)] (%)[8)]	RSD_R[7)] (%)
Surfur material a	8	8.32	3.33	0.02	0.7	0.05	1.4
Surfur material b	10	12.71	5.09	0.03	0.6	0.14	2.8
Surfur material c	9	247.6	99.17	0.24	0.2	1.39	1.4
Surfur material d	8	245.6	98.37	0.18	0.2	0.30	0.3
Surfur material e	8	1.41	0.564	0.002	0.4	0.003	0.6
Surfur material f	9	2.89	1.157	0.001	0.1	0.010	0.9

1) Number of laboratories used in analysis
2) Total mean as surfur trioxid (SO_3) (number of laboratories × number of repetition (2))
3) Total mean as surfur (S) obtained by dividing the total mean in 2) by 2.4969
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation
8) Mass fraction

References

1) JIS K 8088: Sulfur (Regent) (2010)
2) JIS M 8217: Iron ores - Methods for determination of sulfur content (1994)
3) Edited by KANTO CHEMICAL CO., INC.: Technique on Chemical Analysis of Regent - Practical Basic Technique and Knowledge, p.112 - 120 (2009)
4) Yasushi SUGIMURA: Validation of Gravimetric Analysis for Determination of Sulfur Content (as Sulfur Trioxide) in Sulfur and its Compounds as Fertilizers, Research Report of Fertilizer, Vol.4, 2011. (9 - 15)

(5) Flow sheet for total sulfur content: The flow sheet for total sulfur content in fertilizers mainly containing sulfur and sulfuric acid is shown below.

1 g -5 g analytical sample	Weigh to the order of 0.1 mg into a 200-mL tall beaker
←	About 50 mL of potassium hydroxide/ethanol solution
Heating	Cover with a watch glass and boil
Standing to cool	
Transfer	250-mL volumetric flask, water
←	Water (up to the marked line)
Filtration	Type 3 filter paper
Aliquot (predetermined	300-mL tall beaker
←	About 50 mL of water
←	About 5 mL of hydrogen peroxide
Heating	80 ºC - 90 ºC, 1 hour
Standing to cool	
←	1 - 2 drop(s) of phenolphthalein solution (1 g/100 mL)
Neutralization	Hydrochloric acid (2+1) (until the solution becomes transparent)
←	About 1mL of hydrochloric acid (2+1)
←	Water (the liquid volume to reach about 100 mL)
Heating	Boil, for 5 minutes
←	About 6 mL of thermal barium chloride solution (100 g/L), while shaking
Standing to cool	For several minutes
←	A few drops of thermal barium chloride solution (100 g/L) (Check that new precipitate is no longer formed)
←	About 2 mL of thermal barium chloride solution (100 g/L), while shaking
Heating	80 ºC - 90 ºC, 2 hour
Standing to cool	For no less than 4 hours, in a water bath whose heat source is stopped
Filtration	Type 5-C filter paper
Transfer	Type 5-C filter paper, water
←	Wash with water (until reaction of chloric matters disappears in filtrate)
Put	Crucible
Drying	Drying apparatus, about 120 ºC, 1 hour
Standing to cool	
Charring	Heat gently in an electric furnace
Incineration	800 ºC, 2 hours
Standing to cool	Desiccator
Measurement	Measure the mass to the order of 0.1 mg

Figure Flow sheet for total sulfur content in fertilizers

4.12.1.c Transmitted light analysis <Reference method>

(1) Summary

This test method is applicable to fertilizers mainly containing sulfur and sulfuric acid among sulfur and its compound.

Dissolve an analytical sample in a potassium hydroxide-ethanol solution and add hydrogen peroxide to oxidize, and then measure the absorbance from the intensity of transmitted light of suspension of barium sulfate formed by the reaction with barium chloride to obtain total sulfur content.

(2) Reagents: Reagents are as shown below.

- **a) Potassium hydroxide/ethanol solution:** Dissolve 10 g of potassium hydroxide specified in JIS K 8574 in 50 mL of ethanol (95) specified in JIS K 8102, further add 50 mL of water.
- **b) Hydrogen peroxide:** A JIS Guaranteed Reagent (H_2O_2 30 % (mass fraction)) specified in JIS K 8230 or a reagent of equivalent quality.
- **c) Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **d) Glycerin – ethanol solution (1+1):** Add 250 mL of ethanol (95) specified in JIS K 8102 to 250 mL of glycerin specified in JIS K 8295.
- **e) Sodium chloride solution:** Dilute 240 g of sodium chloride specified on JIS K 8150 in water containing 20 mL of hydrochloric acid specified in JIS K 8180, further add water to make 1,000 mL.
- **f) Barium chloride:** Sieve barium chloride dihydrate specified in JIS K 8155 to make barium chloride whose particle size is between 710 μm and 500 μm.
- **g) Sulfate standard solution (SO_3 2 mg/mL):** Heat potassium sulfate specified in JIS K 8962 until it becomes constant weight value at 800 ºC in advance, stand to cool it in a desiccator, and then transfer 4.3531 g to a weighing dosh. Disolve with a small amount of water, move it into a 1,000-mL of volumetric flask and add water up to the marked line.
- **h) Sulfate standard solution (SO_3 0.02 mg/mL – 0.1 mg/mL):** Transfer 2mL – 10 mL of sulfate standard solution (SO_3 2 mg/mL) to 200-mL volumetric flasks step by step and add water up to the marked line.
- **i) Phenolphthalein solution (1 g/100 mL):** Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

(3) Apparatus and instruments: Apparatus and instruments are as follows:

- **a) Hot plate:** A hot plate whose surface temperature can be adjusted up to 250 ºC.
- **b) Water bath:** A water bath that can adjust test temperature to ± 2 ºC.
- **c) Magnetic stirrer:**
- **d) Spectrophotometer:** A spectrophotometer specified in JIS K 0115

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows [1]:

- **a)** Weigh 1 g - 2 g of an analytical sample to the order of 1 mg, and put it in a 200-mL tall beaker.
- **b)** Add about 50 mL of potassium hydroxide/ethanol solution, cover with a watch glass and heat on a hot plate to boil [2].
- **c)** After standing to cool, transfer to a 250-mL volumetric flask and add water up to the marked line.
- **d)** Filter with Type 3 filter paper [3] to make the extract.

Note **(1)** Omit extraction if fluid fertilizers are made from only sulfuric acid and all materials are dissolved
(2) Until sulfur content is dissolved. About 5 minutes when raw materials, etc. are not dissolved.
(3) Omit the procedures in **d)** when all materials are dissolved.

(4.2) Oxidation: Conduct oxidation as shown below.

a) Transfer a predetermined amount (the equivalents of about 5 mg - 200 mg as SO_3) of the extract to a 300-mL tall beaker. (4)

b) Add about 50 mL of water and about 5 mL of hydrogen peroxide and heat in a water bath at 80 ºC - 90 ºC for about 1 hour while sometimes shaking (5).

c) After standing to cool, add 1 - 2 drop(s) of phenolphthalein solution (1 g/100 mL) (6), and add hydrochloric acid (2+1) until the color of solution disappears (7).

d) After standing to cool, transfer to a 200-mL volumetric flask and add water up to the marked line.

e) Filter with 0.3μm glass filter paper.

Note **(4)** Weigh 1g - 5g of an analytical sample to the order of 0.1 mg if the fluid fertilizers of the analytical sample are made from only sulfuric acid and all materials are dissolved.
(5) It can be suspended after the procedures end.
(6) A pH meter can be used for neutralization.
(7) Omit the procedures in **c)** if fluid fertilizers are made from only sulfuric acid and all materials are dissolved.

(4.3) Precipitate formation: Form precipitate as shown below.

a) Transfer 50 mL of filtrate to a 100-mL Erlenmeyer flask with screw cap.

b) Add about 10 mL of glycerin – ethanol solution (1+1) and about 5 mL of sodium chloride solution in the Erlenmeyer flask with screw cap.

c) Warm up on a water bath at 30ºC± 2 ºC.

d) After warming, add 0.30 g of barium chloride and stir with a magnetic stirrer for about 2 minutes.

e) Warm up on a water bath at 30ºC± 2 ºC for about 4 minutes.

f) After warming, stir with a magnetic stirrer for about 3 minutes to make the sample solution.

g) As a blank test, conduct the procedures in **a) - b)** and **e) – f)** using another 100-mL Erlenmeyer flask with screw cap to prepare the blank test solution.

(4.4) Measurement: Conduct measurement according to JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used in measurement.

a) **Measurement conditions for the spectrophotometer:** Set up the measurement conditions for the spectrophotometer considering the following:
Detection wavelength: 450 nm

b) **Preparation of calibration curve**

1) Transfer 50 mL of sulfate standard solution (SO_3 0.02 mg/mL – 0.1 mg/mL) to a 100-mL Erlenmeyer flasks with screw cap and conduct the procedure **(4.3 b) - f)** to make the SO3 1 mg/65 mL - 5 mg/65 mL sulfate standard solution for the calibration curve preparation.

2) Transfer 50 mL of water to another 100-mL Erlenmeyer flask with screw cap and conduct the same procedures as **1)** to make the blank test solution for the calibration curve preparation.
3) Measure absorbance at wavelength 450 nm of the sulfate standard solution for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control(8)(9).
4) Prepare a curve for the relationship between the sulfate concentration and the absorbance of the sulfate standard solutions for the calibration curve preparation.

c) Sample measurement

1) For the sample solution, conduct procedures similarly as in **b) 4)** to measure absorbance.
2) For the blank test solution, conduct procedures similarly as in **d) 1)** to measure absorbance, and correct the absorbance obtained for the sample solution.
3) Obtain the sulfate (SO_3) content from the calibration curve, and calculate the total sulfur content (SO_3) in the analytical sample.

Note **(8)** Measure right after stirring because barium sulfate easily precipitates.
(9) A spectrophotometer with automatic sample introducing device is preferable.

Comment 1 The range of calibration curve with linearity is SO_3 1 mg/65 mL - 5 mg/65 mL and the curve does not pass through the origin.

Comment 2 Replicate testing was conducted using sulfur and sulfuric acid materials (6 samples); as a result, the mean was in the range of 3.21 % - 248% (mass fraction), and the standard deviation and relative standard deviation were 0.01 % - 6% (mass fraction) and 0.2 % - 2.4%, respectively. As a result of the replicate testing using simple substance of sulfur fertilizer containing no materials, the constant value is 98.4 % - 99.4 % of the theoretical value of sulfur.

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References

1) JIS M 8001: General rule for test methods of reagents (2009)
2) JIS K 8088: Sulfur (Regent) (2010)
3) JAPAN Sewage Works Association: Sewage Sludge Analysis -2007-, p132 – 134 Tokyo (1964)
4) Edited by KANTO CHEMICAL CO., INC.: Technique on Chemical Analysis of Regent - Practical Basic Technique and Knowledge, p.131 - 135 (2009)
5) Yasushi SUGIMURA: Method Validation of Turbidimetry for Determination of Sulfur Content (as Sulfur Trioxide) in Fertilizers of Sulfur, Sulfuric acid and Its Mixture , Research Report of Fertilizer Vol.6, 2013, (20 - 26)

(5) Flow sheet for total sulfur content: The flow sheet for total sulfur content in fertilizers mainly containing sulfur and sulfuric acid is shown below:

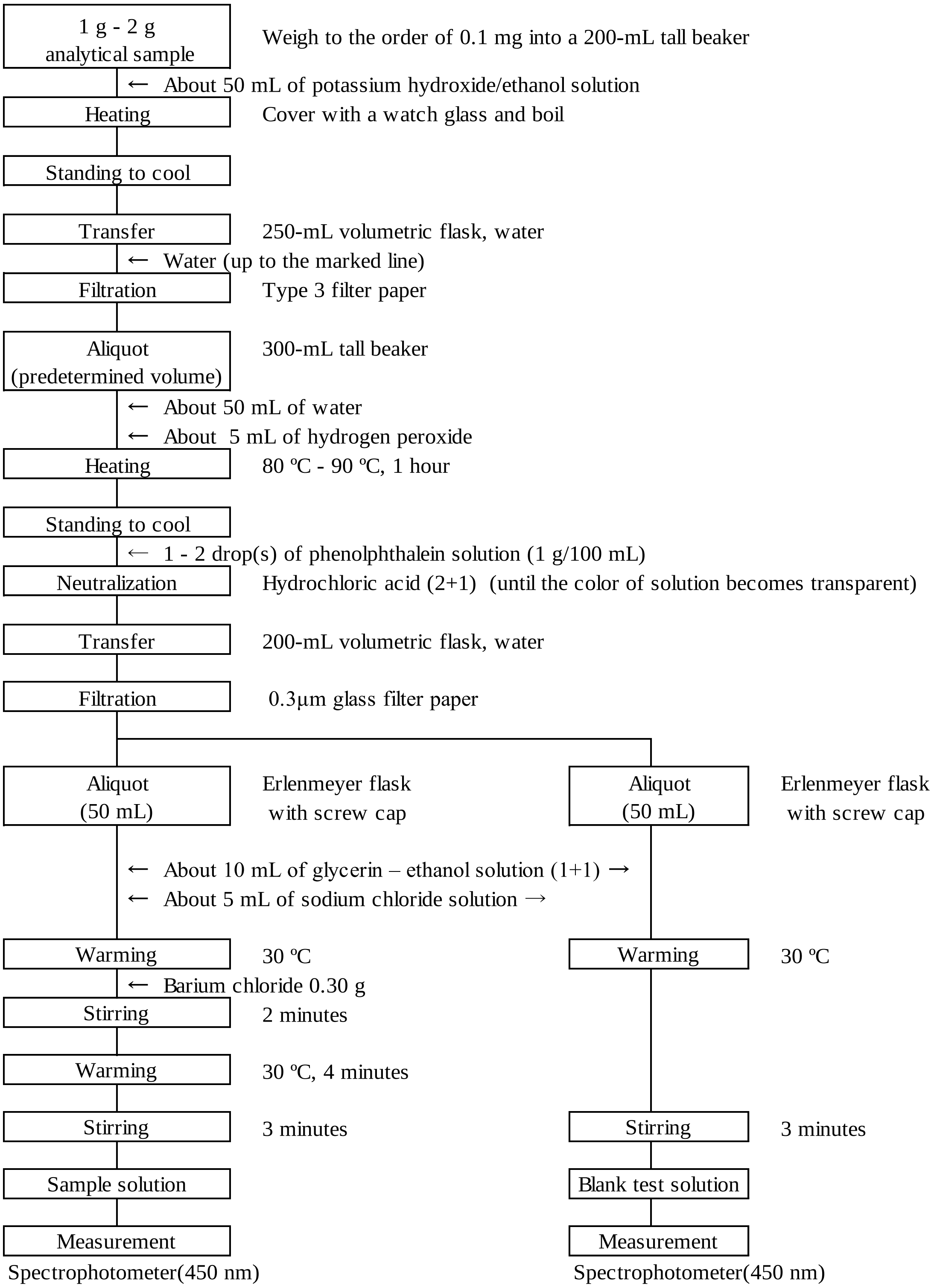


Figure Flow sheet for total sulfur content in fertilizers

4.13 Iron

4.13.1 Water-soluble iron

4.13.1.a Flame atomic absorption spectrometry

(1) Summary
This test method is applicable to fertilizers that indicate iron content as a response modifier. Extract by adding water to an analytical sample, spray in an acetylene-air flame and measure the atomic absorption with iron at a wavelength of 248.3 nm to quantify water-soluble iron (W-Fe).

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Iron standard solution (0.1 mg/mL):** Iron standard solution (Fe 0.1 mg/mL) for atomic absorption traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **d) Iron standard solutions (0.5 µg/ mL - 5 µg/ mL) for the calibration curve preparation** (1)**:** Transfer 2.5 mL - 25 mL of iron standard solution (0.1 mg/mL) to 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.
- **e) Blank test solution for the calibration curve preparation** (1)**:** Hydrochloric acid (1+23) used in the procedure in **d**).

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as follows:

- **a) Rotary shaker:** A rotary shaker that can rotate upside down a 500-mL volumetric flask at 30 - 40 revolutions/min.
- **b) Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121 with the background correction (2) function.
  - **1) Light source**: An iron hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)
  - **2) Gas:** Gas for heating by flame
    - (i) Fuel gas: acetylene
    - (ii) Auxiliary gas: Air sufficiently free of dust and moisture.

Note (2) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

- **a)** Weigh 5.00 g of an analytical sample and transfer to a 500-mL volumetric flask.
- **b)** Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.
- **c)** Add water up to the marked line.
- **d)** Filter with Type 3 filter paper to make the sample solution.

Comment 1 In the procedure in **a)**, it is also allowed to weigh 2.50 g of the analytical sample and put it into a 250-mL volumetric flask.

Comment 2 The procedure in **(4.1)** is the same as the procedure in **(4.1)** in **4.9.2.a**.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 248.3 nm

b) Calibration curve preparation

1) Spray the iron standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 248.3 nm.

2) Prepare a curve for the relationship between the iron concentration and the indicated value of the iron standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.05 mg - 0.5 mg as Fe) to a 100-mL volumetric flask.

2) Add about 25 mL of hydrochloric acid (1 + 5), and add water up to the marked line.

3) Conduct similarly as in **b) 1)** to read the indicated value.

4) Obtain the iron content from the calibration curve, and calculate the water-soluble iron (W-Fe) in the analytical sample.

Comment 3 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for water-soluble iron hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2012	Fluid mixed fertilizer	71	0.243	0.013	5.5

1) Proficiency testing and external quality control testing

2) Median (M) agrees with the mean in normal distribution.

3) Mass fraction

4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.

5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:

RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.252, Yokendo, Tokyo (1988)

(5) Flow sheet for water-soluble iron: The flow sheet for water-soluble iron in fertilizers is shown below:

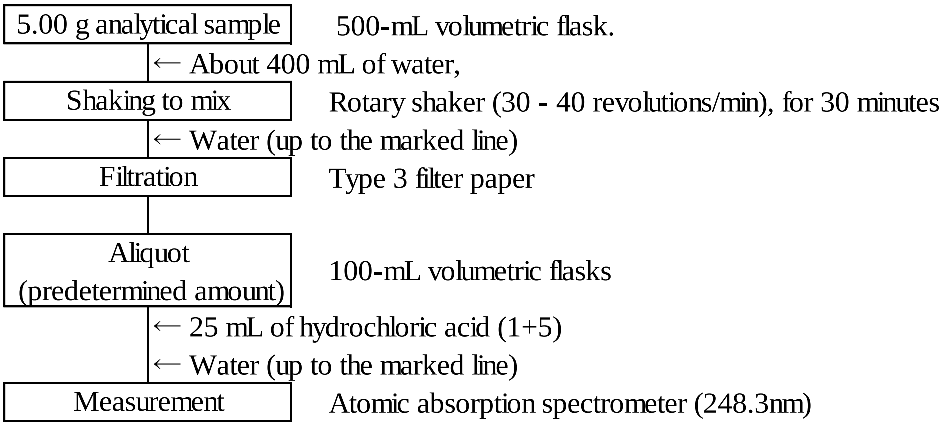


Figure Flow sheet for water-soluble iron in fertilizers.

4.14 Molybdenum

4.14.1 Water-soluble molybdenum

4.14.1.a Sodium thiocyanate absorptiometric analysis

(1) Summary

This test method is applicable to fertilizers that indicate molybdenum content as a response modifier.

Extract by adding water to an analytical sample, add sulfuric acid (1+1) and perchloric acid, further add a sodium thiocyanate solution and a tin (II) chloride solution, and measure the absorbance with thiocyanate complex formed by the reaction of reduced molybdenum (V) with thiocyanate ion to obtain water-soluble molybdenum (W-Mo).

(2) Reagents: Reagents are as shown below:

- **a) Sulfuric acid:** A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.
- **b) Perchloric acid:** A JIS Guaranteed Reagent specified in JIS K 8223 or a reagent of equivalent quality.
- **c) Iron (III) sulfate solution** (1)**:** Dissolve 5 g of iron (III) sulfate specified in JIS K 8981 in about 10 mL of sulfuric acid (1+1) and a proper amount of water, and further add water to make 100 mL.
- **d) Sodium thiocyanate solution** (1)**:** Dissolve 50 g of sodium thiocyanate specified in JIS K 9002 in water to make 500 mL.
- **e) Tin (II) chloride solution (1):** Dissolve 50 g of tin (II) chloride dihydrate specified in JIS K 8136 in 200 mL of hydrochloric acid (1+1) while heating, then add water to make 500 mL, add a small amount of granulated tin specified in JIS K 8580 and store in a colored bottle.
- **f) Molybdenum standard solution (1 mg/mL)** (1)**:** After leaving at rest molybdenum (VI) oxide (2) in a desiccator for about 24 hours to dry, put it to a 1,500 g weighing dish. Dissolve in a small amount of water, transfer to a 1,000 mL volumetric flask and add about 5 g of sodium hydroxide specified in JIS K 8576 to dissolve and add water up to the marked line.
- **g) Molybdenum standard solution (0.01 mg/mL):** Dilute a predetermined amount of molybdenum standard solution (1 mg/mL) precisely 100 times with water.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 99.5 % (mass fraction) in purity is commercially sold as molybdenum (VI) oxide.

Comment 1 Instead of a molybdenum standard solution (1 mg/mL) in **(2) f**, a chemical analysis grade molybdenum standard solution (1 mg/mL) traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act can be used.

(3) Instruments: Instruments are as follows:

- **a) Rotary shaker:** A rotary shaker that can rotate upside down a 500-mL volumetric flask at 30 - 40 revolutions/min.
- **b) Spectrophotometer:** A spectrophotometer specified in JIS K 0115.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

- **a)** Weigh 5.00 g of an analytical sample and transfer to a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make the sample solution.

Comment 2 In the procedure in **a)**, it is also allowed to weigh 2.50 g of the analytical sample and put it into a 250-mL volumetric flask.
Comment 3 The procedure in **(4.1)** is the same as the procedure in **(4.1)** in **4.9.2.a**.
Comment 4 When the sample solution in **d)** contains organic matters that affect the determination, put a predetermined amount of the sample solution to a 100-mL tall beaker, add a small amount of sulfuric acid and nitric acid to heat and digest the organic matters until white smoke of sulfuric acid evolves. After standing to cool, transfer the solution to a 100-mL volumetric flask and add water up to the marked line to filter. The filtrate is used as the sample solution in **(4.2) a)**.

(4.2) Coloring: Conduct coloring as shown below.
a) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 0.3 mg as Mo) to a 100-mL volumetric flask.
b) Add about 5 mL of sulfuric acid (1+1), about 5 mL of perchloric acid and about 2 mL of iron (III) sulfate solution.
c) Add about 16 mL of sodium thiocyanate solution and about 10 mL of tin (II) chloride solution successively while shaking to mix and further add water up to the marked line (3).

Note (3) When the solution becomes muddy, centrifuge after the procedure in **c)**. However if it is presumed that copper (I) thiocyanate caused the muddying, centrifuge after leaving at rest for 1 hour.

(4.3) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used in measurement.
a) Measurement conditions for the spectrophotometer**:** Set up the measurement conditions for the spectrophotometer considering the following:
Detection wavelength: 460 nm
b) Preparation of calibration curve
1) Transfer 1 mL - 30 mL of molybdenum standard solution (0.01 mg/mL) to 100-mL volumetric flasks step-by-step.
2) Conduct the same procedure as **(4.2 b) - c)** to make the 0.01 mg/100 mL - 0.3 mg/100 mL molybdenum standard solution for the calibration curve preparation.
3) Conduct the same procedures as **2)** for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.
4) Measure absorbance at wavelength 460 nm of the molybdenum standard solution for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control.
5) Prepare a curve for the relationship between the molybdenum concentration and the absorbance of the molybdenum standard solutions for the calibration curve preparation.
c) Measurement of a sample
1) Measure the absorbance of the solution in **(4.2) c)** by conducting the same procedure as **b) 4)**.

2) Obtain the molybdenum (Mo) content from the calibration curve and calculate the water-soluble molybdenum (W-Mo) in the analytical sample.

Comment 5 Table 1 shows results from a simultaneous analysis with the same samples (proficiency testing and external quality control testing) hosted by the Japan Fertilizer Quality Assurance Committee analyzed by the robust method.

Table 1 Results and analysis results from simultaneous analysis with the same samples for water-soluble molybdenum hosted by the Japan Fertilizer Quality Assurance Committee[1)]

Year	Sample	Number of laboratories	Median M[2)] (%)[3)]	NIQR[4)] (%)[3)]	RSD_{rob}[5)] (%)
2012	Fluid mixed fertilizer	32	0.212	0.009	4.2

1) Proficiency testing and external quality control testing
2) Median (M) agrees with the mean in normal distribution.
3) Mass fraction
4) Robust standard deviation (NIQR) agrees with standard deviation in normal distribution.
5) RSD_{rob} stands for relative standard deviation obtained from the robust method by the formula shown below:
RSD_{rob} = (NIQR/M) × 100

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.281 - 283, Yokendo, Tokyo (1988)

(5) Flow sheet for water-soluble molybdenum: The flow sheet for water-soluble molybdenum in fertilizers is shown below:

5.00 g analytical sample	500-mL volumetric flask.
← About 400 mL of water	
Shaking to mix	Rotary shaker (30 - 40 revolutions/min), for 30 minutes
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined	100-mL volumetric flask
← About 5 mL of sulfuric acid (1+1)	
← About 5 mL of perchloric acid	
← About 2 mL of iron (III) sulfate solution	
←16 mL of sodium thiocyanate solution (while shaking to mix)	
←About 10 mL of tin (II) chloride solution (while shaking to mix)	
← Water (up to the marked line)	
Leaving at rest	Until the red color of iron disappears
Measurement	Spectrophotometer (460 nm)

Figure Flow sheet for water-soluble molybdenum in fertilizers.

5. Harmful components

5.1 Mercury

5.1.a Cold vapor atomic absorption spectrometry

(1) Summary

The test method is applicable to fertilizers.
Pretreat an analytical sample with nitric acid- perchloric acid, and then reduce mercury (II) in the solution with tin (II) chloride. Aerate this solution, and measure the atomic absorption for generated mercury vapor at a wavelength of 253.7 nm to quantify mercury.

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Perchloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Sulfuric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **e) Tin (II) chloride solution:** To 10 g of tin (II) chloride dihydrate (1) specified in JIS K 8136, add 60 mL of sulfuric acid (1+20), and stir while heating to dissolve. After standing to cool, add water to make 100 mL.
- **f) L-cystein solution:** To 10 mg of L-cystein ($HSCH_2CH(NH_2)COOH$) of no less than 98.0 % in purity, add 100 mL of water and 2 mL of nitric acid to dissolve, and further add water to make 1,000 mL. Store in a refrigerator, and do not use after 6 months after preparation.
- **g) Tri-*n*-butyl phosphate (2):** A reagent of no less than 98 % in purity.
- **h) Mercury standard solution (1 mg/mL):** Mercury standard solution (1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **i) Mercury standard solution (10 µg/mL):** Dilute a predetermined amount of mercury standard solution (1 mg/mL) with L-cystein solution to prepare mercury standard solution (10 µg/mL). Store in a refrigerator, and do not use after 6 months after preparation.
- **j) Mercury standard solution (0.1 µg/mL):** Dilute a predetermined amount of mercury standard solution (10 µg/mL) with L-cystein solution to prepare mercury standard solution (0.1 µg/mL). Store in a refrigerator, and do not use after 1 month after preparation.

Note (1) Use a reagent with low mercury content, such as for mercury analysis or for harmful metal analysis.

(2) This is used as an antifoaming agent.

(3) Apparatus and instruments Apparatus and instruments are as follows:

- **a) Atomic absorption spectrometer or mercury atomic absorption spectrometer**
- **b) Light source**: A mercury hollow cathode lamp or a mercury lamp.
- **c) Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 180 ºC - 200 ºC.
- **d) Sample digestion flask (3):** A 100-mL borosilicate glass volumetric flask (180 mm total height, 13 mm mouth diameter)

Note (3) Distinguish the volumetric flask used in digestion as a sample digestion flask and do not use it for any other purposes.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh 1.00 g of an analytical sample, and transfer to a sample digestion flask.

b) Add about 10 mL of nitric acid, and heat on a hot plate or sand bath for a short time. (4)

c) After standing to cool, add about 10 mL of perchloric acid, and digest by heating on a hot plate or sand bath at 180 ºC - 200 ºC for about 30 minutes - 1 hour(5).

d) After standing to cool, fill up with water to 100 mL to make the sample solution.

e) As a blank test, conduct the procedures in **b)** - **d)** using another sample digestion flask to prepare the blank test solution.

Note (4) If it foams vigorously, leave at rest overnight.

(5) The sample solution and the blank test solution should be stored when they are cooled after the procedure in **(4.1) c)**. After filling up the sample solution and the blank test solution with water, immediately conduct the procedure in **(4.2)**.

(4.2) Measurement: Conduct measurement by cold vapor atomic absorption spectrometry specified in JIS K 0121. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used. An example of measurement using a mercury atomic absorption spectrometer is shown below:

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 253.7 nm

b) Calibration curve preparation

1) Transfer 1 mL - 20 mL of mercury standard solution (0.1 µg/mL) to 100-mL volumetric flasks step-by-step, and add water up to the marked line. Transfer 5 mL of these solutions to respective reduction vessels, add 1 drop of tri-*n*-butyl phosphate (6), to make mercury standard solutions for the calibration curve preparation.

2) Add 5 mL of water to another reduction vessel, and add 1 drop of tri-*n*-butyl phosphate (6), to make the blank test solution for the calibration curve preparation.

3) Connect the reduction vessel to the mercury atomic absorption spectrometer, and introduce sulfuric acid (1+1) and tin (II) chloride solution, and circulate air.

4) Read the indicated value at a wavelength of 253.7 nm.

5) Prepare a curve for the relationship between the mercury content (µg) and the indicated value of the mercury standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer 5 mL of a sample solution to respective reduction vessels, add 1 drop of tri-*n*-butyl phosphate (6), and conduct similarly as in **b) 3) - 4)** to read the indicated value.

2) Transfer 5 mL of the blank test solution to a reduction vessel, add 1 drop of tri-*n*-butyl phosphate (6), and conduct similarly as in **b) 2) - 4)** to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the mercury content (µg) from the calibration curve, and calculate the mercury concentration in the analytical sample.

Note (6) It is not required to add tri-*n*-butyl phosphate if not needed.

Comment 1 The mercury concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1)** and **3)** and obtaining the mercury content in the blank test solution.

Comment 2 A recovery testing was conducted using industrial sludge fertilizer (1 sample), composted sludge fertilizer (3 samples) and human waste sludge fertilizer (1 sample); as a result, the mean recovery at the concentration level of 2 mg/kg and 0.2 mg/kg was 98.7 % - 101.6 % and 100.7 % - 105.4 %, respectively, and the repeatability was 0.3 % - 1.4 % and 1.4 % - 2.5 % relative standard deviation, respectively. Also, a recovery testing was conducted using soybean meal and powdered soybean meal, rape seed meal and powdered rape seed meal, and compound fertilizer (2 samples) and blended fertilizer; as a result, the mean recovery at the concentration level of 40 mg/kg and 0.5 mg/kg was 98.5 % - 101.5 % and 100.4 % - 103.3 %, respectively, and the repeatability was 0.2 % - 2.1 % and 0.8 % - 2.8 % relative standard deviation, respectively.

In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 1.

Additionally, the minimum limit of quantification of the test method is about 0.01 mg/kg.

Table 1 Results and analysis results from a collaborative study for mercury test method validation

Sample name	Number of laboratories[1]	Mean[2] (mg/kg)	RSD_r[3] (%)	RSD_R[4] (%)
Human waste sludge fertilizer A	11	0.651	5.3	11.6
Human waste sludge fertilizer B	11	1.10	6.3	10.2
Composted sludge fertilizer A	11	0.489	6.8	10.2
Composted sludge fertilizer B	11	0.822	8.1	13.1
Composted sludge fertilizer C	9	0.182	10.6	10.6

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories x number of samples (2))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Fumihiro ABE, Takeshi HASHIMOTO and Yasushi SUGIMURA: Determination of mercury in sludge fertilizer - Improved decomposition method -, Research Report of Fertilizer Vol.1 2008. (60 - 66)

2) Fumihiro ABE, Takeshi HASHIMOTO and Norio HIKICHI: Determination of mercury in sludge fertilizer - Collaborative Test Results -, Research Report of Fertilizer Vol.1 2008. (67 - 73)

3) Akira SHIMIZU, Kaori OKADA, Takeshi HASHIMOTO, Yasuto IDE and Toshiaki HIROI: Determination of Mercury in Fertilizer- Expanding the scope of application of improved method -, Research Report of Fertilizer Vol.2 2009. (12 - 17)

(5) Flow sheet for mercury: The flow sheet for mercury in fertilizers is shown below:

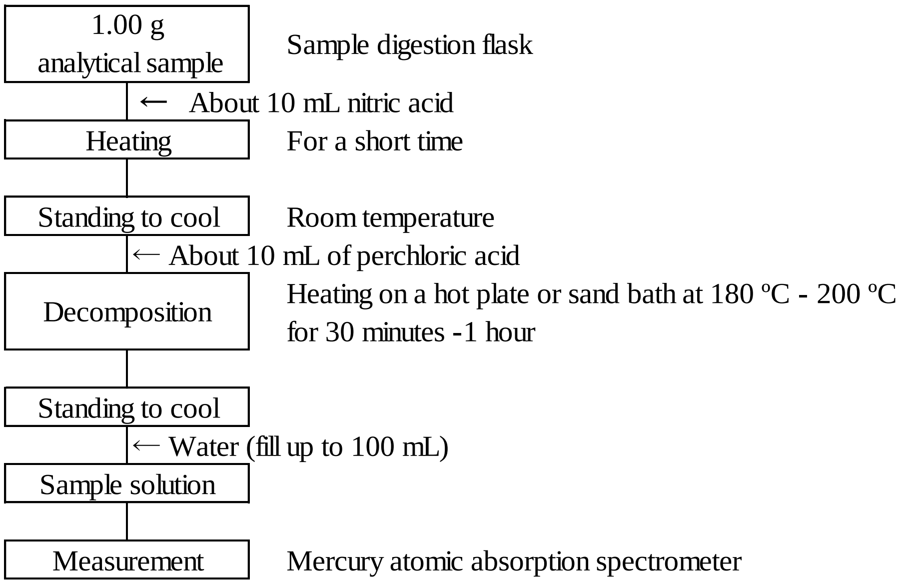


Figure Flow sheet for mercury in fertilizers

5.2 Arsenic

5.2.a Hydride generation atomic absorption spectrometry

(1) Summary

The test method is applicable to fertilizers.

Pretreat an analytical sample with nitric acid-sulfuric acid-perchloric acid, and then generate arsenic hydride by the addition of sodium tetrahydroborate in the acidic condition with hydrochloric acid, introduce it with argon gas to a heated absorption cell, and measure the atomic absorption with arsenic at a wavelength of 193.7 nm to quantify arsenic.

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Sulfuric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Perchloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **e) Hydrochloric acid:** Hydrochloric acid for arsenic analysis specified in JIS K 8180, or of harmful metal analysis grade, microanalysis grade or equivalents.
- **f) Potassium iodide solution** (1)**:** Dissolve 20 g of potassium iodide specified in JIS K 8913 in water to make 100 mL.
- **g) Sodium hydroxide:** A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.
- **h) Sodium tetrahydroborate solution** (1)**:** Dissolve 10 g of sodium tetrahydroborate ($NaBH_4$) for atomic absorption spectrometry in sodium hydroxide solution (4 g/L) to make 1 L.
- **i) Arsenic standard solution (0.1 mg/mL):** Arsenic standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **j) Arsenic standard solution (1 µg/mL):** Dilute a predetermined amount of arsenic standard stock solution (0.1 mg/mL) accurately with hydrochloric acid (1+100) to prepare arsenic standard solution (1 µg/mL). Store in a refrigerator, and do not use after 6 months after preparation.
- **k) Arsenic standard solution (0.1 µg/mL):** Dilute a predetermined amount of arsenic standard solution (1 µg/mL) with hydrochloric acid (1+100) to prepare arsenic standard solution (0.1 µg/mL)). Store in a refrigerator, and do not use after 1 month after preparation.

Note (1) The concentrations of potassium iodide solution and sodium tetrahydroborate solution vary depending on the instrument used.

(3) Instruments: Instruments are as shown below:

- **a) Atomic absorption spectrometer:** To an atomic absorption spectrometer specified in JIS K 0121, connect a hydride generator and parts shown below. Also, an atomic absorption spectrometer with a built-in hydride generator can be used.
  - **1) Light source**: An arsenic hollow cathode lamp or an arsenic high-intensity discharge lamp.
  - **2) Atomizer**: Heated absorption cell (2)
  - **3) Gas:** Gases for heating the heated absorption cell.
    - (i) Fuel gas: acetylene
    - (ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Hydride generator:** A batch-type or continuous-type hydride generator specified in JIS K 0121. For continuous hydride generators, there is a method to introduce potassium iodide solution on-line in addition to a sample solution, hydrochloric acid, and sodium tetrahydroborate solution.

1) **Argon:** Argon of grade 2 specified in JIS K 1105 or equivalents.

c) **Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 350 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to no less than 300 ºC.

Note **(2)** For cell heating, there are a method of electric heating and a method of flame heating.

(4) Test procedures

(4.1) **Extraction:** Conduct extraction as follows:

a) Weigh 1.00 g - 2.00 g of an analytical sample, and transfer to a 200-mL - 300-mL tall beaker.

b) Add about 10 mL of nitric acid and about 5 mL of sulfuric acid, cover the tall beaker with a watch glass, and leave at rest overnight.

c) Heat mildly on a hot plate or sand bath at 170 ºC - 220 ºC for no less than 30 minutes. After bubbles cease to form, set the temperature of the hot plate or sand bath to no less than 300 ºC, and heat until nitroxide (yellow-brown smoke) is no longer generated(3)(4).

d) After standing to cool, add about 5 mL of perchloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or sand bath at no less than 300 ºC for 2 - 3 hours to digest(5).

f) Slightly move the watch glass(6), and keep on heating on the hot plate or sand bath to concentrate until the liquid volume becomes no more than 2 mL(7).

g) After standing to cool, add about 5 mL of hydrochloric acid (1+10) and about 20 mL of water, cover the tall beaker with a watch glass and heat mildly to dissolve.

h) After standing to cool, transfer to a 100-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.

i) As a blank test, conduct the procedures in b) - h) using another tall beaker to prepare the blank test solution.

Note **(3)** Carbonization (degradation) of organic matters by sulfuric acid begins by heating when nitric acid no longer remains. In this state, As5+ may be reduced to As3+ and evaporate; therefore stop heating immediately after the end of the generation of nitroxide (yellow-brown smoke).

(4) Oxidation of organic matters by perchloric acid progresses extremely rapidly and explosively. For that reason, add perchloric acid after fully degrading organic matters with nitric acid to avoid danger.

(5) When the white smoke of perchloric acid is generated, if the solution is colored such as black-brown or brown, stop heating immediately, and after standing to cool, add nitric acid, and heat again to degrade remaining organic matters.

(6) The watch glass can be removed.

(7) The generation of arsenic hydride is inhibited by the presence of nitric acid; therefore remove nitric acid by sufficiently generating the white smoke of sulfuric acid.

Comment 1 The procedures in **(4.1)** are the same as in **(4.1)** in **5.2.b** and **5.5.c**. However, the sampling amount of the analytical sample in **(4.1) a**) in **5.5.c** is 1.00 g.

Comment 2 When the analytical sample solidifies in the procedure in **(4.1) b)**, moisten the analytical sample with a small amount of water as necessary in advance.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used. Two examples of measurement procedures with a continuous hydride generator are shown below.

(4.2.1) Measurement (A): Leaving at rest after adding potassium iodide solution.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 193.7 nm

b) Calibration curve preparation

1) Transfer 2.5 mL - 10 mL of arsenic standard solution (0.1 µg/mL) to 50-mL volumetric flasks step-by-step.

2) Add 5 mL of hydrochloric acid and 5 mL of potassium iodide solution, leave at rest for about 15 minutes, and then add water up to the marked line, to make 5 ng/mL -20 ng/mL arsenic standard solutions for the calibration curve preparation.

3) Conduct the procedure in **2)** for another 50-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) While letting argon flow, introduce hydrochloric acid (1+1) and sodium tetrahydroborate solution, such as arsenic standard solutions for the calibration curve preparation, to the hydride generator to generate arsenic hydride.

5) Separate arsenic hydride and liquid waste, and then introduce the gas containing arsenic hydride to a heated absorption cell, and read the indicated value at a wavelength of 193.7 nm.

6) Prepare a curve for the relationship between the arsenic concentration and the indicated value of the arsenic standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution to a 50-mL volumetric flask, and conduct procedures similarly as in **b) 2)** and **b) 4) - 5)** to read the indicated value.

2) Transfer a predetermined amount of the blank test solution to a 50-mL volumetric flask, and conduct procedures similarly as in **b) 2)** and **b) 4) - 5)** to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the arsenic content from the calibration curve, and calculate the arsenic concentration in the analytical sample.

(4.2.2) Measurement (B): On-line introduction of potassium iodide solution.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 193.7 nm

b) Calibration curve preparation

1) Transfer 5 mL - 25 mL of arsenic standard solution (0.1 µg/mL) to 50-mL volumetric flasks step-by-step, add water up to the marked line, to make the 10 ng/mL - 50 ng/mL arsenic standard solution for the calibration curve preparation. Use water as the blank test solution for the calibration curve preparation.

2) While letting argon flow, introduce arsenic standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation, respectively, and further introduce potassium iodide solution, hydrochloric acid (1+1) and sodium tetrahydroborate solution to the hydride generator to generate arsenic hydride.

3) Separate arsenic hydride generated and liquid waste, and then introduce the gas containing arsenic hydride to a heated absorption cell, and read the indicated value at a wavelength of 193.7 nm.

4) Prepare a curve for the relationship between the arsenic concentration and the indicated value of the arsenic standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer a predetermined amount of the sample solution to a 50-mL volumetric flask, add water up to the marked line, and conduct similarly as in **b) 2)-3)** to read the indicated value.

2) Transfer a predetermined amount of the blank test solution to a 50-mL volumetric flask, add water up to the marked line, and conduct similarly as in **b) 2)-3)** to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the arsenic content from the calibration curve, and calculate the arsenic concentration in the analytical sample.

Comment 3 The coexistence of iron, nickel, and cobalt at over 5, 10, 80 fold amount of arsenic, respectively, inhibits the generation of arsenic hydride. However, the inhibition of arsenic hydride generation even in the coexistence of iron at 1,000 fold amount can be removed by adding or introducing potassium iodide solution.

Comment 4 The arsenic concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1)** and **3)** and obtaining the arsenic content in the blank test solution.

Comment 5 A recovery testing was conducted using industrial sludge fertilizer, composted sludge fertilizer (3 samples) and human waste sludge fertilizer; as a result, the recovery at the concentration level of 50 mg/kg and 5 mg/kg was 94.6 % - 100.6 % and 99.9 % - 103.3 %, respectively, and the repeatability was 0.3 % - 5.1 % and 0.4 % - 4.0 % relative standard deviation, respectively. Also, a recovery testing was conducted using processed slug phosphate fertilizer, soybean meal, rape seed meal, compound fertilizer and potassium-magnesium-sulfate; as a result, the recovery at the concentration level of 50 mg/kg and 5 mg/kg was 98.5 % - 109.8 % and 103.5 % - 107.9 %, respectively, and the repeatability was 0.4 % - 6.5 % and 0.5 % - 4.2 % relative standard deviation, respectively.

In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 1.

Additionally, the minimum limit of quantification of the test method is about 0.1 mg/kg.

Table 1 Results and analysis results from a collaborative study for arsenic test method validation

Sample name	Number of laboratories[1)]	Mean[2)] (mg/kg)	RSD_r[3)] (%)	RSD_R[4)] (%)
Sewage sludge fertilizer	11	6.42	3.5	10.7
Human waste sludge fertilizer	10	4.62	4.9	7.0
Industrial sludge fertilizer	12	0.632	5.7	19.7
Calcined sludge fertilizer	12	5.08	4.1	9.5
Composted sludge fertilizer	10	1.23	6.1	11.4

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories x number of samples (2))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Naoki ASAO, Yukie ISHIDA, Shinjiro IZUKA and Masakazu SAIKI: Determination of Arsenic in Sludge Fertilizer - Improved Decomposition Method - Research Report of Fertilizer Vol.1 2008, (74 - 81)

2) Naoki ASAO, Shinjiro IZUKA and Norio HIKICHI: Determination of Arsenic in Sludge Fertilizer - Collaborative Test Results - Research Report of Fertilizer Vol.1 2008, (82 - 89)

3) Yasushi SUGIMURA, Naoki ASAO and Shinjiro IZUKA: Determination of Arsenic in Fertilizer- Expanding the scope of application of improved method -, Research Report of Fertilizer Vol.2 2009. (18 - 24)

(5) Flow sheet for arsenic: The flow sheet for arsenic in fertilizers is shown below:

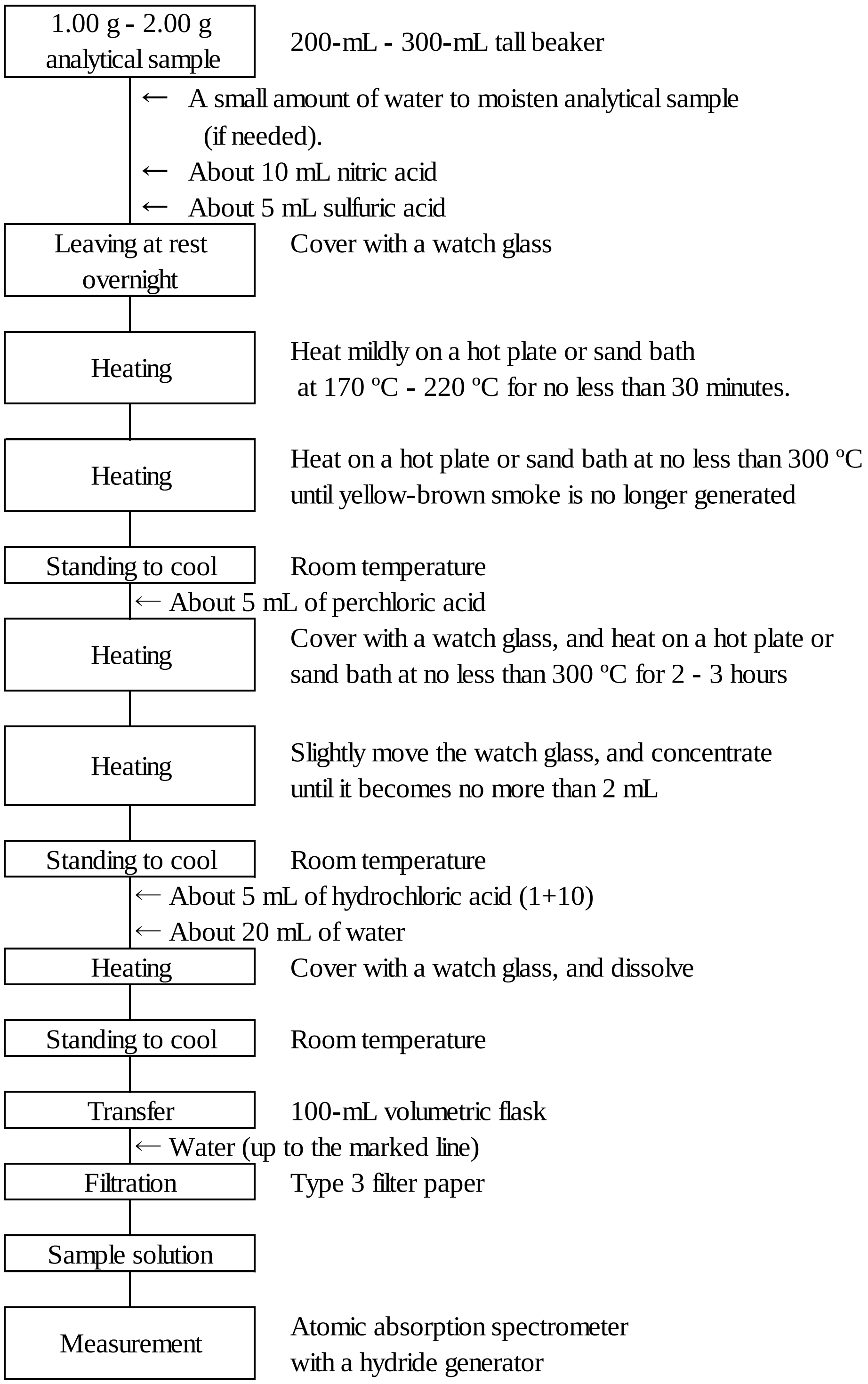


Figure Flow sheet for arsenic in fertilizers

5.2.b Silver diethyl dithiocarbamate absorptiometric analysis

(1) Summary

This test method is applicable to fertilizers other than sulfur and its compound.

Pretreat an analytical sample with nitric acid - sulfuric acid - perchloric acid, and then put the predetermined volume into an arsenic hydride generation bottle, and generate arsenic hydride by adding potassium iodide solution, tin chloride solution and zinc successively in the acidic condition with hydrochloric acid to react with silver diethyl dithiocarbamate in pyridine. Measure the absorbance with silver diethyl dithiocarbamate solution, the coloring liquid, at a wavelength of 510 nm to quantify arsenic.

(2) Reagents, etc.: Reagents and water are as shown below:

a) **Water:** Water of A3 specified in JIS K 0557.

b) **Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Sulfuric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) **Perchloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

e) **Hydrochloric acid:** Hydrochloric acid for arsenic analysis specified in JIS K 8180, or of harmful metal analysis grade, microanalysis grade or equivalents.

f) **Potassium iodide solution:** Dissolve 20 g of potassium iodide specified in JIS K 8913 in water to make 100 mL.

g) **Tin (II) chloride solution:** Dissolve 15 g of tin (II) chloride dihydrate specified in JIS K 8136 in 100 mL of hydrochloric acid (1+1), add a small amount of granular tin specified in JIS K 8580 then store in a colored bottle.

h) **Ascorbic acid:** A JIS Guaranteed Reagent specified in JIS K 9502 or a reagent of equivalent quality.

i) **Zinc:** A reagent of arsenic analysis grade specified in JIS K 8012 or equivalents. (1 mm - 1.5 mm particle diameter)

j) **Lead acetate glass wool:** Glass wool air-dried after moisturizing with 100 mL of the solution, where 10 g of lead acetate (II) trihydrate specified in JIS K 8374 is dissolved in water.

k) **Silver diethyldithiocarbamate solution:** Dissolve 0.5 g of silver N, N-diethyl dithiocarbamate specified in JIS K 9512 in 100 ml of pyridine specified in JIS K 8777, then store in cool and dark place

l) **Arsenic standard solution (0.1 mg/mL):** Arsenic standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.

m) **Arsenic standard solution (1 µg/mL):** Dilute a predetermined amount of arsenic standard solution (0.1 mg/mL) accurately with water to prepare arsenic standard solution (1 µg/mL).

(3) Instruments: Instruments are as shown below:

a) **Arsenic hydride generator:** An arsenic hydride generator specified in 61.1 in JIS K 0102 or equivalents.

b) **Spectrophotometer:** A spectrophotometer specified in JIS K 0115.

c) **Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 350 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to no less than 300 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

- **a)** Weigh 1.00 g - 2.00 g of an analytical sample, and transfer to a 200-mL - 300-mL tall beaker.
- **b)** Add about 10 mL of nitric acid and about 5 mL of sulfuric acid, cover the tall beaker with a watch glass, and leave at rest overnight.
- **c)** Heat mildly on a hot plate or sand bath at 170 ºC - 220 ºC for no less than 30 minutes. After bubbles cease to form, set the temperature of the hot plate or sand bath to no less than 300 ºC, and heat until nitroxide (yellow-brown smoke) is no longer generated(1) (2).
- **d)** After standing to cool, add about 5 mL of perchloric acid.
- **e)** Cover the tall beaker with a watch glass, and heat on a hot plate or sand bath at no less than 300 ºC for 2 - 3 hours to digest(3).
- **f)** Slightly move the watch glass(4), and keep on heating on the hot plate or sand bath to concentrate until the liquid volume becomes no more than 2 mL(5).
- **g)** After standing to cool, add about 5 mL of hydrochloric acid (1+10) and about 20 mL of water, cover the tall beaker with a watch glass and heat mildly to dissolve.
- **h)** After standing to cool, transfer to a 100-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.
- **i)** As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare the blank test solution.

Note (1) Carbonization (degradation) of organic matters by sulfuric acid begins by heating when nitric acid no longer remains. In this state, As5+ may be reduced to As3+ and evaporate; therefore stop heating immediately after the end of the generation of nitroxide (yellow-brown smoke).

(2) Oxidation of organic matters by perchloric acid progresses extremely rapidly and explosively. For that reason, add perchloric acid after fully degrading organic matters with nitric acid to avoid danger.

(3) When the white smoke of perchloric acid evolves, if the solution is colored such as black-brown or brown, stop heating immediately, and after standing to cool, add nitric acid, and heat again to degrade remaining organic matters.

(4) The watch glass can be removed.

(5) The generation of arsenic hydride is inhibited by the presence of nitric acid; therefore remove nitric acid by sufficiently generating the white smoke of sulfuric acid.

Comment 1 The procedures in **(4.1)** are the same as in **(4.1)** in **5.2.a**.

Comment 2 When the analytical sample solidifies in the procedure in **(4.1) b)**, moisten the analytical sample with a small amount of water as necessary in advance.

(4.2) Reaction: Conduct reaction as shown below.

- **a)** Put a predetermined amount (the equivalents of 1 µg - 20 µg of As and liquid volume is no more than 40 mL) of the sample solution into an arsenic hydride generation bottle.
- **b)** Add water to make about 40 mL of liquid volume.
- **c)** Add hydrochloric acid to make it equivalent to 10 mL of hydrochloric acid.
- **d)** Add about 2 mL of potassium iodide solution, shake and leave at rest for a few minutes.
- **e)** Add about 1 mL of tin (II) chloride solution, shake and leave at rest for about 10 minutes (6).
- **f)** Connect arsenic hydride generation bottle, glass tube lightly stuffed with lead acetate glass wool in advance and 5 mL of silver diethyl dithiocarbamate solution (7), and quickly put 2.5g of zinc into an arsenic hydride generation bottle.

g) Leave at rest at room temperature (15ºC - 25ºC) for about 45 minutes, and allow generated arsenic hydride to absorb into silver diethyl dithiocarbamate solution and color.

h) Transfer a predetermined amount of blank test solution into the arsenic hydride generation bottle, conduct procedures similarly as in **b) - g)** to allow generated arsenic hydride to absorb into silver diethyl dithiocarbamate solution and color.

Note (6) When a large amount of iron is contained, add 1 g of ascorbic acid and 2mL of tin (II) chloride solution and shake to mix to leave at rest for about 10 minutes instead of the procedure in **e).**

(7) Apply a small amount of silicone grease, etc. to the connecting parts of an arsenic hydride generation bottle, glass tube and arsenic hydride absorption tube to maintain their airtightness.

(4.3) Measurement: Conduct measurement according to JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used.

a) Measurement conditions for the spectrophotometer: Set up the measurement conditions for the spectrophotometer considering the following:
Detection wavelength: 510 nm

b) Calibration curve preparation

1) Transfer 2.5 mL - 20 mL of arsenic standard solution (0.1 µg/mL) to arsenic hydride generation bottles step-by-step.

2) Conduct procedures similarly as in **(4.2) b) - g**) and allow them to react.

3) For another arsenic hydride generation bottle, the silver diethyl dithiocarbamate solution prepared similarly as in the procedure in **2)** is used as the blank test solution for the calibration curve preparation.

4) Measure the absorbance at wavelength 510 nm of the silver diethyl dithiocarbamate solution of arsenic standard solution for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control.

5) Prepare a curve for the relationship between the arsenic concentration and the indicated value of the arsenic standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) For silver diethyl dithiocarbamate solution of **(4.2) g)**, conduct procedures similarly as in **b) 4)** to measure absorbance.

2) For silver diethyl dithiocarbamate solution of **(4.2) h)**, conduct procedures similarly as in **b) 4)** to measure absorbance, and correct the absorbance obtained for the sample solution.

3) Obtain the arsenic content from the calibration curve, and calculate the arsenic concentration in the analytical sample.

Comment 3 The arsenic concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1)** and **3)** to obtain the arsenic content in the blank test solution.

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.270 - 273, Yokendo, Tokyo (1988)

(5) Flow sheet for arsenic: The flow sheet for arsenic in fertilizers is shown below:

1.00 g -2.00 g analytical sample	200-mL - 300-mL tall beaker
← A small amount of water to moisten analytical sample (if needed).	
← About 10 mL nitric acid, about 5 mL sulfuric acid	
Leaving at rest overnight	Cover with a watch glass
Heating	Heat mildly on a hot plate or sand bath at 170 °C - 220 °C for no less than 30 minutes.
Heating	Heat on a hot plate or sand bath at no less than 300 °C until yellow-brown smoke is no longer generated
Standing to cool	Room temperature
← About 5 mL of perchloric acid	
Heating	Cover with a watch glass, and heat on a hot plate or sand bath at no less than 300 °C for 2 - 3 hours
Heating	Slightly move the watch glass, and concentrate until it becomes no more than 2 mL
Standing to cool	Room temperature
← About 5 mL of hydrochloric acid (1+10), about 20 mL of water	
Heating	Cover with a watch glass, and dissolve
Standing to cool	Room temperature
Transfer	100-mL volumetric flask
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Sample solution	
Aliquot (predetermined volume)	Transfer into an arsenic hydride generation bottle
← Water (until liquid volume becomes about 40 mL)	
← Hydrochloric acid (until hydrochloric acid becomes equivalent to 10 mL)	
← About 2 mL of potassium iodide solution	
Shaking to mix	
Leaving at rest	For a few minutes
←About 1 mL of tin (II) chloride solution	
Shaking to mix	
Leaving at rest	For about 10 minutes
←2.5 g of zinc	
Generation of arsenic hydride	Room temperature (15 °C - 25 °C), about 45 minutes
Absorption	5 mL of silver diethyldithiocarbamate solution
Measurement	Wavelength 510 nm

Figure Flow sheet for arsenic in fertilizers

5.3 Cadmium

5.3.a Flame atomic absorption spectrometry

(1) Summary

The test method is applicable to fertilizers.

Pretreat an analytical sample with incineration and nitric acid-hydrochloric acid (1+3), spray into an acetylene-air flame, and measure the atomic absorption with cadmium at a wavelength of 228.8 nm to quantify cadmium.

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Cadmium standard solution (0.1 mg/mL):** Cadmium standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **e) Cadmium standard solution (10 µg/mL):** Transfer 10 mL of cadmium standard solution (0.1 mg/mL) to a 100-mL volumetric flask, and add hydrochloric acid (1+23) up to the marked line.
- **f) Cadmium standard solutions (0.05 µg/mL - 0.5 µg/mL) for the calibration curve preparation** (1) (2)**:** Transfer 2.5 mL - 25 mL of cadmium standard solution (10 µg/mL) to 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.
- **g) Blank test solution for the calibration curve preparation** (1) (2)**:** Hydrochloric acid (1+23) used in the procedures in **e**) and **f**).

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) Store at room temperature, and do not use after 6 months after preparation.

(3) Instruments: Instruments are as shown below:

- **a) Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121 with the background correction (3) function.
  - **1) Light source**: A cadmium hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)
  - **2) Gas:** Gas for heating by flame
    - (i) Fuel gas: acetylene
    - (ii) Auxiliary gas: Air sufficiently free of dust and moisture.
- **b) Electric furnace:** An electric furnace that can be adjusted to 450 ºC ± 5 ºC.
- **c) Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note (3) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

- **a)** Weigh 5.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.
- **b)** Put the tall beaker in an electric furnace, and heat gently to char(4).
- **c)** Ignite at 450 ºC ± 5 ºC to incinerate (5).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
f) Slightly move the watch glass (6), and continue heating on the hot plate or sand bath to concentrate until nearly dried up.
g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (7) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer the solution to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.
i) As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare the blank test solution.

Note **(4)** Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.
(5) Example of ignition period: 8 - 16 hours
(6) The watch glass can be removed.
(7) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 Do not conduct the procedures in **(4.1) b)-c)** in the case of fertilizers not containing organic matters.
Comment 2 The procedures in **(4.1)** are the same as in **(4.1)** in **4.9.1.a**.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 228.8 nm

b) Calibration curve preparation
1) Spray the cadmium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 228.8 nm.
2) Prepare a curve for the relationship between the cadmium concentration and the indicated value of the cadmium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement
1) Subject the sample solution (8) to the same procedure as in **b) 1)** to read the indicated value.
2) Subject the blank test solution to the same procedure as in **b) 1)** to read the indicated value, and correct the indicated value obtained for the sample solution.
3) Obtain the cadmium content from the calibration curve, and calculate the cadmium concentration in the analytical sample.

Note **(8)** If there is a possibility that the cadmium concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount with hydrochloric acid (1+23).

Comment 3 The cadmium concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1**) and **3**) to obtain the cadmium content in the blank test solution.

Comment 4 A recovery testing was conducted using industrial sludge fertilizer and composted sludge fertilizer (5 samples); as a result, the recovery at the concentration level of 5 mg/kg and 0.5 mg/kg was 97.5 % - 99.2 % and 96.7 % - 99.7 %, respectively, and the repeatability was 0.3 % - 1.9 % and 1.0 - 2.4 % relative standard deviation, respectively.

In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 1.

Additionally, the minimum limit of quantification of the test method is about 0.1 mg/kg.

Table 1 Results and analysis results from a collaborative study for cadmium test method validation

Sample name	Number of laboratories[1)]	Mean[2)] (mg/kg)	RSD_r[3)] (%)	RSD_R[4)] (%)
Sewage sludge fertilizer a	10	1.50	5.5	6.4
Sewage sludge fertilizer b	10	3.35	1.2	4.2
Composted sludge fertilizer a	10	1.96	1.0	4.4
Composted sludge fertilizer b	11	3.81	1.9	3.2
Composted sludge fertilizer c	10	1.80	3.5	4.9

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories x number of samples (2))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Yoshinari SAKAKIBARA, Manabu MATSUZAKI and Tadao AMANO: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Improved Decomposition Method - Research Report of Fertilizer Vol.1 2008. (41 - 49)

2) Yoshinari SAKAKIBARA and Manabu MATSUZAKI: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Collaborative Test Results - Research Report of Fertilizer Vol.1 2008. (50 - 59)

3) Hisanori ARAYA and Yoshimi TAKEBA: Validation of atomic absorption spectrometry for determination of cadmium, lead, nickel and chromium in calcined sludge fertilizer - Research Report of Fertilizer Vo3. 2010. (30 - 42)

(5) Flow sheet for cadmium: Flow sheet for cadmium in fertilizers is shown below:

5.00 g analytical sample	200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	Ignite at 450 °C ± 5 °C
Standing to cool	Room temperature
← A small amount of water	
← About 10 mL of nitric acid	
← About 30 mL of hydrochloric acid	
Heating	Cover with a watch glass, and digest
Heating	Slightly move a watch glass to remove acid
Standing to cool	Room temperature
← About 25 mL - 50 mL of hydrochloric acid (1+5)	
Heating	Cover with a watch glass, and dissolve
Standing to cool	Room temperature
Transfer	100-mL - 200-mL volumetric flask, water
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Measurement	Atomic absorption spectrometer (228.8 nm)

Figure Flow sheet for cadmium in fertilizers

5.3.b ICP Atomic Emission Spectrometry <Reference method>

(1) Summary

The test method is applicable to sludge fertilizers.

Pretreat an analytical sample with incineration and nitric acid-hydrochloric acid (1+3), and then introduce it to an ICP Atomic Emission Spectrometer ("ICP-AES") and measure the emission with cadmium at a wavelength of 228.802 nm to quantify cadmium.

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Cadmium standard solution (0.1 mg/mL):** Cadmium standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **e) Cadmium standard solutions (0.25μg/mL)** (1) (2)**:** Dilute a predetermined amount of cadmium standard solution (0.1 mg/mL) with hydrochloric acid (1+23) to prepare cadmium standard solution (0.25 μg/mL)

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) Store at room temperature, and do not use after 6 months after preparation.

(3) Instruments: Instruments are as shown below:

- **a) ICP Atomic Emission Spectrometer:** An Atomic Emission Spectrometer specified in JIS K 0116.
  - **1) Gas:** Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity
- **b) Electric furnace:** An electric furnace that can keep the test temperature at ± 5 ºC
- **c) Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

- **a)** Weigh 5.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.
- **b)** Put the tall beaker in an electric furnace, and heat gently to char(3).
- **c)** Ignite at 450 ºC ± 5 ºC to incinerate (4).
- **d)** After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
- **e)** Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
- **f)** Slightly move the watch glass (5), and continue heating on the hot plate or sand bath to concentrate until nearly dried up.
- **g)** After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (5) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
- **h)** After standing to cool, transfer the solution to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.
- **i)** As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare the blank test solution.

Note (3) Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.

(4) Example of ignition period: 8 - 16 hours

(5) The watch glass can be removed.

(6) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 Do not conduct the procedures in **(4.1) b) - c)** in the case of fertilizers not containing organic matters.

Comment 2 The procedures in **(4.1)** are the same as in **(4.1)** in **4.9.1.a**.

(4.2) Measurement: Conduct measurement (Standard Addition Method) according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Atomic Emission Spectrometry used in measurement.

a) Measurement conditions for the ICP Atomic Emission Spectrometer: Set up the measurement conditions for the ICP Atomic Emission Spectrometer considering the following:

Analytical line wavelength: 228.802 nm

b) Calibration curve preparation and Sample measurement.

1) Put 5mL of sample solution into three 10-mL volumetric flasks respectively.

2) Add 2mL and 4 mL of cadmium standard solution (0.25 μg/mL) to volumetric flasks of **1)** above, and further add hydrochloric acid (1+23) to the marked line to make the sample solutions of Standard Addition Method.

3) Add hydrochloric acid (1+23) up to the marked line of the remaining volumetric flask of **1)** above to make the sample solution without the standard solution.

4) Spray the sample solution of Standard Addition Method and the sample solution without the standard solution into the induction plasma, and read the indicated value at a wavelength of 228.802 nm.

5) Put 5 mL of blank test solution into a 10-mL volumetric flask, conduct the same procedures as in **3) - 4)** to read the indicated value, and correct the indicated value obtained from the respective sample solutions.

6) Prepare a curve for the relationship between the added cadmium concentration and the corrected indicated value of the sample solution for Standard Addition Method and sample solution without standard solution.

7) Obtain the cadmium content from the intercept of the calibration curve to calculate the concentration of cadmium in the analytical sample

Comment 3 The cadmium concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1**) **- 4)** and **6) - 7)** to obtain the cadmium content in the blank test solution.

Comment 4 Simultaneous measurement of multiple elements by ICP-AES is possible. In this case, mix a predetermined amount of copper standard solution (0.1 mg/mL), zinc standard solution (0.1 mg/mL), cadmium standard solution (0.1 mg/mL), nickel standard solution (0.1 mg/mL), chromium standard solution (0.1 mg/mL) and lead standard solution (0.1 mg/mL), dilute with hydrochloric acid (1 +23) to prepare mixture standard solution (Cu 25μg/mL, Zn 25 μg/mL, Cd 0.25μg/mL, Ni 2.5 μg/mL, Cr 2.5 μg/mL and Pb 2.5 μg/mL) (1) (2). Use the mixture solution instead of cadmium standard solution (0.25 μg/mL) in **(4.2) b) 2)**. After that, conduct the same procedures as in **(4.2) b)** to calculate the concentration of respective elements in the analytical sample. Note that the wavelength for measurement of respective elements is Cu 324.754 nm, Zn 206.191 nm, Cd 228.802 nm, Ni 231.604 nm, Cr 205.552 nm and Pb 220.351 nm.

In addition, the respective element concentrations for respective standard addition samples are shown in the below Table.

Table The additive amount of mixed standard solution
and the additive concentration of respective elements for respective sample solutions

	Additive amount (mL) of mixed standard solution	Cd (mg/L)	Pb (mg/L)	Ni (mg/L)	Cr (mg/L)	Cu (mg/L)	Zn (mg/L)
Sample solution without standard solution	0	0	0	0	0	0	0
Sample solution 1 by Standard Addition Method	2	0.05	0.5	0.5	0.5	5.0	5.0
Sample solution 2 by Standard Addition Method	4	0.1	1.0	1.0	1.0	10	10

Comment 5 For each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixed sludge fertilizer, calcined sludge fertilizer and composted sludge fertilizer, the repeatability obtained from triplicates measurement is 0.8 % - 4.1 % relative standard deviation.

Additionally, the minimum limit of quantification of the test method is about 0.2 mg/kg.

Reference

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI, Tetsuya NOMURA : Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer Vol.4 2011. (36 - 48)

(5) Flow sheet for cadmium test method: The flow sheet for cadmium test method in fertilizers is shown below:

5.00 g analytical sample	200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	Ignite at 450 °C ± 5 °C
Standing to cool	Room temperature
← A small amount of water	
← About 10 mL of nitric acid	
← About 30 mL of hydrochloric acid	
Heating	Cover with a watch glass, and digest
Heating	Slightly move a watch glass to remove acid
Standing to cool	Room temperature
← 25 mL - 50 mL of hydrochloric acid (1+5)	
Heating	Cover with a watch glass, and dissolve
Standing to cool	Room temperature
Transfer	100-mL - 200-mL volumetric flask, water
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot 5 mL	10-mL volumetric flask, 3 flasks
← 0, 2 and 4 mL of cadmium standard solution (0.25 µg / mL)	
← Hydrochloric acid (1+23) (up to the marked line)	
Measurement	ICP Atomic Emission Spectrometer (228.802 nm)

Figure　Flow sheet for cadmium in fertilizers

5.4 Nickel

5.4.a Flame atomic absorption spectrometry

(1) Summary

The test method is applicable to fertilizers.

Pretreat an analytical sample with incineration and nitric acid-hydrochloric acid (1+3), spray into an acetylene-air flame, and measure the atomic absorption with nickel at a wavelength of 232.0 nm to quantify nickel.

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Nickel standard solution (0.1 mg/mL):** Nickel standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **e) Nickel standard solutions (0.5 µg/mL - 5 µg/mL) for the calibration curve preparation** (1) (2)**:** Transfer 2.5 mL - 25 mL of nickel standard solution (0.1 mg/mL) to 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.
- **f) Blank test solution for the calibration curve preparation** (1) (2)**:** Hydrochloric acid (1+23) used in the procedure in **e).**

Note (1) This is an example of preparation; prepare an amount as appropriate.
(2) Store at room temperature, and do not use after 6 months after preparation.

(3) Instruments: Instruments are as shown below:

- **a) Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121 with the background correction (3) function.
  - **1) Light source**: A nickel hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)
  - **2) Gas:** Gas for heating by flame
    - (i) Fuel gas: acetylene
    - (ii) Auxiliary gas: Air sufficiently free of dust and moisture.
- **b) Electric furnace:** An electric furnace that can be adjusted to 450 ºC ± 5 ºC.
- **c) Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note (3) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

- **a)** Weigh 5.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.
- **b)** Put the tall beaker in an electric furnace, and heat gently to char(4).
- **c)** Ignite at 450 ºC ± 5 ºC to incinerate (5).
- **d)** After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
- **e)** Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass [6], and continue heating on the hot plate or sand bath to concentrate until nearly dried up.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) [7] to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer the solution to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.

i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare the blank test solution.

Note **(4)** Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.

(5) Example of ignition period: 8 - 16 hours

(6) The watch glass can be removed.

(7) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h)**, about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 Do not conduct the procedures in **(4.1) b) - c)** in the case of fertilizers not containing organic matters.

Comment 2 The procedures in **(4.1)** are the same as in **(4.1)** in **4.9.1.a**.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 232.0 nm

b) Calibration curve preparation

1) Spray the nickel standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 232.0 nm.

2) Prepare a curve for the relationship between the nickel concentration and the indicated value of the nickel standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Subject the sample solution [8] to the same procedure as in **b**) **1**) to read the indicated value.

2) Subject the blank test solution to the same procedure as in **b**) **1**) to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the nickel content from the calibration curve, and calculate the nickel concentration in the analytical sample.

Note **(8)** If there is a possibility that the nickel concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount with hydrochloric acid (1+23).

Comment 3 The nickel concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1**) and **3**) to obtain the nickel content in the blank test solution.

Comment 4 A recovery testing was conducted using industrial sludge fertilizer and composted sludge fertilizer (5 samples); as a result, the recovery at the concentration level of 300 mg/kg and 30 mg/kg was 98.5 % - 100.3 % and 97.1 % - 99.9 %, respectively, and the repeatability was 1.3 % - 3.3 % and 2.1 % - 9.4 % relative standard deviation, respectively.

In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 1.

Additionally, the minimum limit of quantification of the test method is about 1 mg/kg.

Table 1 Results and analysis results from a collaborative study for nickel test method validation

Sample name	Number of laboratories[1)]	Mean[2)] (mg/kg)	RSD_r[3)] (%)	RSD_R[4)] (%)
Sewage sludge fertilizer a	11	56.9	1.1	4.6
Sewage sludge fertilizer b	11	21.8	2.2	3.9
Composted sludge fertilizer a	11	28.9	1.3	6.4
Composted sludge fertilizer b	11	28.5	1.8	4.4
Composted sludge fertilizer c	12	58.3	1.6	4.4

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories x number of samples (2))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Yoshinari SAKAKIBARA, Manabu MATSUZAKI and Tadao AMANO: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Improved Decomposition Method - Research Report of Fertilizer Vol.1 2008. (41 - 49)

2) Yoshinari SAKAKIBARA and Manabu MATSUZAKI: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Collaborative Test Results - Research Report of Fertilizer Vol.1 2008. (50 - 59)

3) Hisanori ARAYA and Yoshimi TAKEBA: Validation of Atomic Absorption Spectrometry for Determination of Cadmium, Lead, Nickel and Chromium in Calcined Sludge Fertilizer – Using Decomposition Method for Inorganic Fertilizer. Research Report of Fertilizer, Vo3, 2010 (30 - 42)

(5) Flow sheet for nickel: The flow sheet for nickel in fertilizers is shown below:

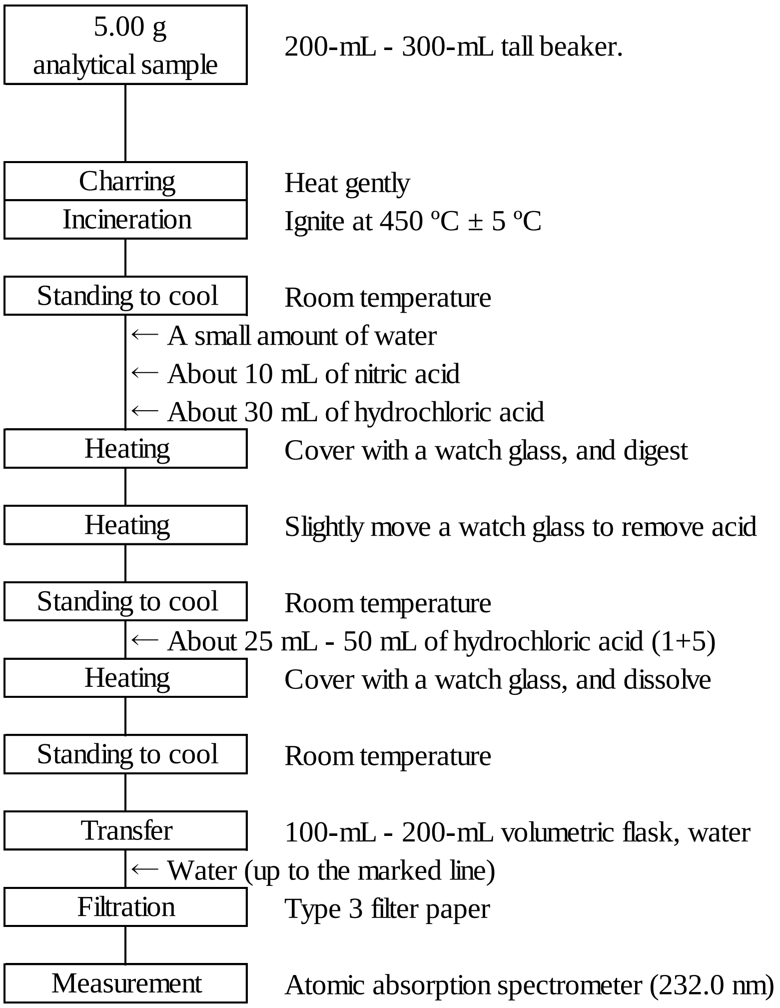


Figure Flow sheet for nickel in fertilizers

5.4.b ICP Atomic Emission Spectrometry <Reference method>

(1) Summary

The test method is applicable to sludge fertilizers.

Pretreat an analytical sample with incineration and nitric acid-hydrochloric acid (1+3), and then introduce it to an ICP Atomic Emission Spectrometer ("ICP-AES") and measure the emission with nickel at a wavelength of 231.604 nm to quantify nickel.

(2) Reagents, etc.: Reagents and water are as shown below:

a) Water: Water of A3 specified in JIS K 0557.

b) Nitric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) Hydrochloric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) Nickel standard solution (0.1 mg/mL): Nickel standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.

e) Nickel standard solutions (2.5 µg/mL) (1) (2)**:**
Dilute a predetermined amount of nickel standard solution (0.1 mg/mL) with hydrochloric acid (1+23) to prepare nickel standard solution (2.5 µg/mL)

Note (1) This is an example of preparation; prepare an amount as appropriate.
(2) Store at room temperature, and do not use after 6 months after preparation.

(3) Instruments: Instruments are as shown below:

a) ICP Atomic Emission Spectrometer: An Atomic Emission Spectrometer specified in JIS K 0116.

1) Gas: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) Electric furnace: An electric furnace that can keep the test temperature at ± 5 ºC

c) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh 5.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char(3).

c) Ignite at 450 ºC ± 5 ºC to incinerate (4).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass (5), and continue heating on the hot plate or sand bath to concentrate until nearly dried up.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (6) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer the solution to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.

i) As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare the blank test solution.

Note **(3)** Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.
(4) Example of ignition period: 8 - 16 hours
(5) The watch glass can be removed.
(6) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 Do not conduct the procedures in **(4.1) b) - c)** in the case of fertilizers not containing organic matters.
Comment 2 The procedures in **(4.1)** are the same as in **(4.1)** in **4.9.1.a**.

(4.2) Measurement: Conduct measurement (Standard Addition Method) according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Atomic Emission Spectrometer used in measurement.

a) Measurement conditions for the ICP Atomic Emission Spectrometer: Set up the measurement conditions for the ICP Atomic Emission Spectrometer considering the following:
Analytical line wavelength: 231.604 nm

b) Calibration curve preparation and Sample measurement.

1) Put 5 mL of sample solution to three 10-mL volumetric flasks respectively.
2) Add 2 mL and 4 mL (0.25 μg/mL) of nickel standard solution to volumetric flasks of **1)** above, then add hydrochloric acid (1+23) to the marked line to make the sample solution of Standard Addition Method.
3) Add hydrochloric acid (1+23) to the marked line of the remaining volumetric flask of **1)** above to make the sample solution without the standard solution.
4) Spray the sample solution of Standard Addition Method and sample solution without standard solution into the induction plasma, and read the indicated value at a wavelength of 231.604 nm.
5) Add 5 mL of blank test solution to a 10-mL volumetric flask, conduct the same procedures as in **3) - 4)** to read the indicated value, and correct the indicated value obtained from the respective sample solutions.
6) Prepare a curve for the relationship between the added nickel concentration and the corrected indicated value of the sample solution for Standard Addition Method and the sample solution without standard solution.
7) Obtain the nickel content from the intercept of the calibration curve to calculate the concentration of nickel in the analytical sample.

Comment 3 The nickel concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1**) - **4)** and **6) - 7)** to obtain the nickel content in the blank test solution.
Comment 4 Simultaneous measurement of multiple elements by ICP-AES is possible. In this case, mix a predetermined amount of copper standard solution (0.1 mg/mL), zinc standard solution (0.1 mg/mL), cadmium standard solution (0.1 mg/mL), nickel standard solution (0.1 mg/mL), chromium standard solution (0.1 mg/mL) and lead standard solution (0.1 mg/mL), dilute with hydrochloric acid (1 +23) to prepare mixture standard solution (Cu 25 μg/mL, Zn 25 μg/mL, Cd 0.25 μg/mL, Ni 2.5 μg/mL, Cr 2.5 μg/mL and Pb 2.5 μg/mL) (1) (2). Use the mixture solution instead of nickel standard solution (2.5

μg/mL) in **(4.2) b) 2)**. After that, conduct the same procedures as in **(4.2) b)** to calculate the concentration of respective elements in the analytical sample. Note that the wavelength for measurement of respective elements is Cu 324.754 nm, Zn 206.191 nm, Cd 228.802 nm, Ni 231.604 nm, Cr 205.552 nm and Pb 220.351 nm. In addition, the respective element concentrations for respective standard addition samples are shown in the below Table.

Table The additive amount of mixed standard solution
and the additive concentration of respective elements for respective sample solutions

	Additive amount (mL) of mixed standard solution	Cd (mg/L)	Pb (mg/L)	Ni (mg/L)	Cr (mg/L)	Cu (mg/L)	Zn (mg/L)
Sample solution without standard solution	0	0	0	0	0	0	0
Sample solution 1 by Standard Addition Method	2	0.05	0.5	0.5	0.5	5.0	5.0
Sample solution 2 by Standard Addition Method	4	0.1	1.0	1.0	1.0	10	10

Comment 5 For each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixed sludge fertilizer, calcined sludge fertilizer and composted sludge fertilizer, the repeatability obtained from triplicates measurement is 1.0 % - 2.6 % relative standard deviation.

Additionally, the minimum limit of quantification of the test method is about 8 mg/kg.

Reference

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI, Tetsuya NOMURA: Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer Vol.4 2011. (36 - 48)

(5) Flow sheet for nickel: The flow sheet for nickel in fertilizers is shown below:

5.00 g analytical sample	200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	Ignite at 450 °C ± 5 °C
Standing to cool	Room temperature
	← A small amount of water
	← About 10 mL of nitric acid
	← About 30 mL of hydrochloric acid
Heating	Cover with a watch glass, and digest
Heating	Slightly move a watch glass to remove acid
Standing to cool	Room temperature
	← 25 mL - 50 mL of hydrochloric acid (1+5)
Heating	Cover with a watch glass, and dissolve
Standing to cool	Room temperature
Transfer	100-mL - 200-mL volumetric flask, water
	← Water (up to the marked line)
Filtration	Type 3 filter paper
Aliquot 5 mL	10-mL volumetric flask, 3 flasks
	← 0, 2 and 4 mL of nickel standard solution (0.25 µg / mL)
	← Hydrochloric acid (1+23) (up to the marked line)
Measurement	ICP Atomic Emission Spectrometer (231.604 nm)

Figure Flow sheet for nickel in fertilizers

5.5 Chromium

5.5.a Flame atomic absorption spectrometry (Fertilizers containing organic matters)

(1) Summary

The test method is applicable to fertilizers containing organic matters.

Pretreat an analytical sample with incineration and nitric acid-hydrochloric acid (1+3), spray into an acetylene-air flame, and measure the atomic absorption with chromium at a wavelength of 357.9 nm or 359.3 nm to quantify chromium.

(2) Reagents, etc.: Reagents and water are as shown below:

a) **Water:** Water of A3 specified in JIS K 0557.

b) **Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) **Interference suppressor solution** (1)**:** Dissolve 100 g of potassium disulfate specified in JIS K 8783 in water to make 1,000 mL.

e) **Chromium standard solution (0.1 mg/mL):** Chromium standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.

f) **Chromium standard solutions (0.5 µg/mL - 5 µg/mL) for the calibration curve preparation**(1) (2)**:** Transfer 2.5 mL - 25 mL of chromium standard solution (0.1 mg/mL) to 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution(3), and further add hydrochloric acid (1+23) to the marked line.

g) **Blank test solution for the calibration curve preparation** (1) (2)**:** Transfer about 50 mL of interference suppressor solution (3) to a 500-mL volumetric flask, and add hydrochloric acid (1+23) used in the procedure in **f**) to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(2) Store at room temperature, and do not use after 6 months after preparation.

(3) Add interference suppressor solution that is 1/10 volume of the volume to be prepared.

(3) Instruments: Instruments are as shown below:

a) **Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121 with the background correction (4) function.

1) **Light source**: A chromium hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)

2) **Gas:** Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Electric furnace:** An electric furnace that can be adjusted to 450 ºC ± 5 ºC.

c) **Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note **(4)** There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh 5.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.
b) Put the tall beaker in an electric furnace, and heat gently to char(5).
c) Ignite at 450 ºC ± 5 ºC to incinerate (6).
d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
f) Slightly move the watch glass (7), and continue heating on the hot plate or sand bath to concentrate until nearly dried up.
g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5)(8) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer the solution to a 100-mL - 200-mL volumetric flask with water, and add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.
i) As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare the blank test solution.

Note **(5)** Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.
(6) Example of ignition period: 8 - 16 hours
(7) The watch glass can be removed.
(8) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h)**, about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 The procedures in **(4.1)** are the same as in **(4.1)** in **4.9.1.a**.

(4.2) **Measurement:** Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer:** Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 357.9 nm or 359.3 nm (9)

b) **Calibration curve preparation**
1) Spray the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame(10), and read the indicated value at a wavelength of 357.9 nm or 359.3 nm(9).
2) Prepare a curve for the relationship between the chromium concentration and the indicated value of the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**
1) Transfer 25 mL (11) of the sample solution to a 100-mL volumetric flask.
2) Add about 10 mL of interference suppressor solution (3), and add hydrochloric acid (1+23) to the marked line.
3) Subject to the same procedure as in **b) 1)** to read the indicated value.
4) Subject the blank test solution to the same procedure as in **1) - 2)** and **b) 1)** to read the indicated value, and correct the indicated value obtained for the sample solution.
5) Obtain the chromium content from the calibration curve, and calculate the chromium concentration in the analytical sample.

Note **(9)** When background correction is conducted by the Zeeman method, 359.3 nm is recommended as the analytical line wavelength.

(10) Use low-fuel acetylene-air flame. Acetylene-nitrous oxide flame can also be used.

(11) If there is a possibility that the chromium concentration in the sample solution will exceed the maximum limit of the calibration curve, decrease the amount to be transferred.

Comment 2 In an acetylene-air flame, sensitivity is enhanced in high-fuel flame, but interference by coexisting substances such as iron and nickel will also be enhanced.
In an acetylene-nitrous oxide flame, such interference hardly affects the result.

Comment 3 The chromium concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1)** - **3)** and **5)** to obtain the chromium content in the blank test solution.

Comment 4 A recovery testing was conducted using industrial sludge fertilizer and composted sludge fertilizer (5 samples); as a result, the recovery at the concentration level of 500 mg/kg and 50 mg/kg was 97.5 % - 100.0 % and 95.9 % - 101.9 %, respectively, and the repeatability was 0.6 % - 2.7 % and 1.0 % - 6.7 % relative standard deviation, respectively.
In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 1.
Additionally, the minimum limit of quantification of the test method is about 1 mg/kg.

Table 1 Results and analysis results from a collaborative study for chromium test method validation

Sample name	Number of laboratories[1)]	Mean[2)] (mg/kg)	RSD_r[3)] (%)	RSD_R[4)] (%)
Sewage sludge fertilizer a	12	33.6	5.3	15.6
Sewage sludge fertilizer b	12	26.3	4.9	18.7
Composted sludge fertilizer a	11	41.3	2.1	11.0
Composted sludge fertilizer b	12	30.2	5.5	13.8
Composted sludge fertilizer c	12	85.0	6.4	12.5

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories x number of samples (3))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Yoshinari SAKAKIBARA, Manabu MATSUZAKI and Tadao AMANO: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Improved Decomposition Method - Research Report of Fertilizer Vol.1 2008. (41 - 49)

2) Yoshinari SAKAKIBARA and Manabu MATSUZAKI: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Collaborative Test Results - Research Report of Fertilizer Vol.1 2008. (50 - 59)

3) Yoshinari SAKAKIBARA, Chie INOUE: Validation of determination methods for chromium in sludge fertilizer - Evaluation of measurement procedure, Research Report of Fertilizer Vol.2 2009. (130 - 136)

(5) Flow sheet for chromium: The flow sheet for chromium in fertilizers containing organic matters is shown below:

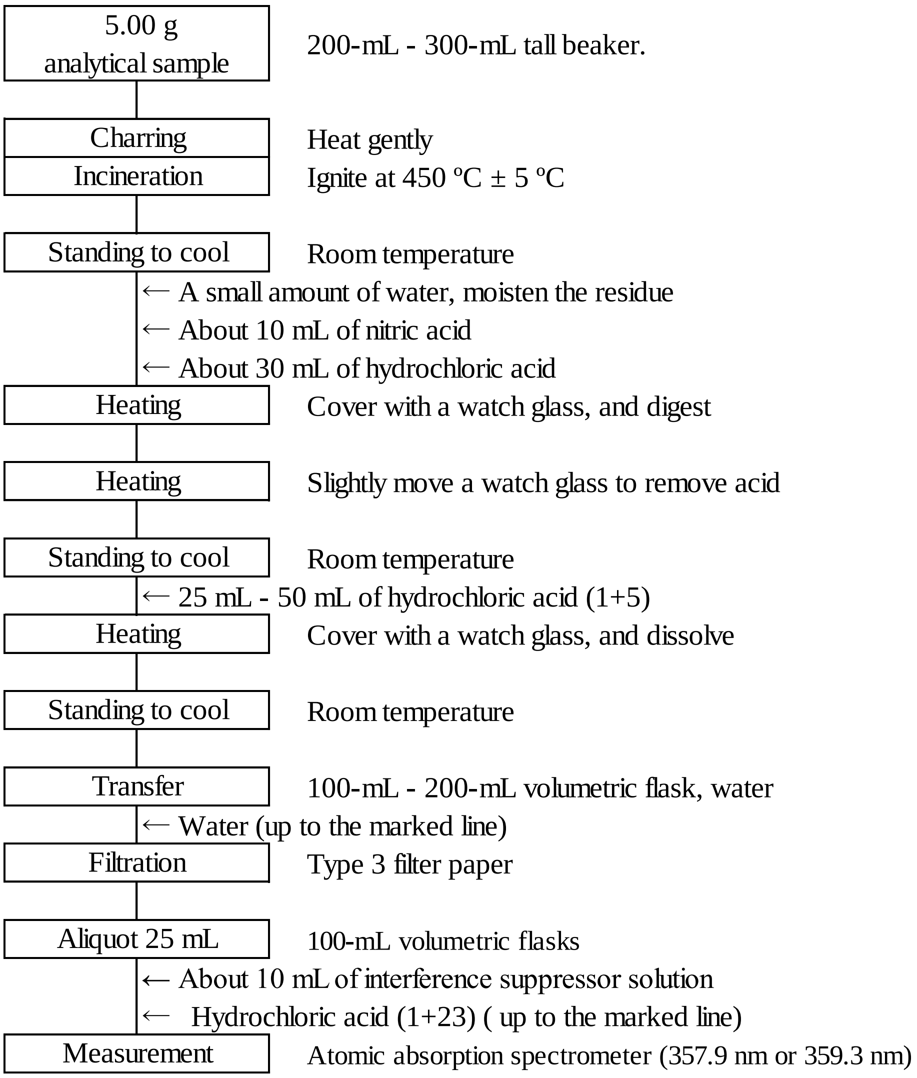


Figure Flow sheet for chromium in fertilizers containing organic matters

5.5.b Flame atomic absorption spectrometry (Fertilizers not containing organic matters)

(1) Summary

The test method is applicable to fertilizers not containing organic matters.

Pretreat an analytical sample with phosphate - nitric acid - sulfuric acid, and then spray into an acetylene-air flame, and measure the atomic absorption with chromium at a wavelength of 357.9 nm or 359.3 nm to quantify chromium.

(2) Reagents, etc.: Reagents and water are as shown below:

a) Water: Water of A3 specified in JIS K 0557.

b) Phosphate: A JIS Guaranteed Reagent specified in JIS K 9005 or a reagent of equivalent quality.

c) Nitric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) Sulfuric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

e) Hydrochloric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

f) Interference suppressor solution (1)**:** Dissolve 100 g of potassium disulfate specified in JIS K 8783 in water to make 1,000 mL.

g) Chromium standard solution (0.1 mg/mL): Chromium standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.

h) Chromium standard solutions (0.5 μg/mL - 5 μg/mL) for the calibration curve preparation(1) (2)**:** Transfer 2.5 mL - 25 mL of chromium standard solution (0.1 mg/mL) to 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution(3), and further add hydrochloric acid (1+23) to the marked line.

i) Blank test solution for the calibration curve preparation (1) (2)**:** Transfer about 50 mL of interference suppressor solution (3) to a 500-mL volumetric flask, and add hydrochloric acid (1+23) used in the procedure in **h**) to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) Store at room temperature, and do not use after 6 months after preparation.

(3) Add interference suppressor solution that is 1/10 volume of the volume to be prepared.

(3) Instruments: Instruments are as shown below:

a) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (4) function.

1) Light source: A chromium hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)

2) Gas: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 350 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to about 300 ºC.

Note (4) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh 1.00 g of an analytical sample, and put it in a 200-mL tall beaker.

b) Moisten the analytical sample with a small amount of water, and add about 5mL - 10 mL of phosphate and about 10 mL of nitric acid.

c) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath for 10 minutes to digest.

d) Slightly move the watch glass (5), and continue heating on the hot plate or sand bath to vaporize nitric acid.

e) After standing to cool, add 5 mL - 10 mL of nitric acid, cover the tall beaker with the watch glass, and heat on the hot plate or sand bath to digest. (6)

f) After standing to cool, add 5 mL of sulfuric acid, cover the tall beaker with the watch glass and heat on the hot plate or sand bath until white smoke of sulfuric acid evolves (7).

g) After standing to cool, add gradually 15 mL – 20 mL of water, cover the tall beaker with the watch glass, and heat gently (8).

h) After standing to cool, transfer the solution to a 100-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.

i) As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare the blank test solution.

Note **(5)** The watch glass can be removed.
(6) Heat sufficiently to digest.
(7) For about 30 minutes at 300 ºC.
(8) The solution may be scattered by the sudden addition of water and heating.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer:** Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 357.9 nm or 359.3 nm (9)

b) **Calibration curve preparation**

1) Spray the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame(10), and read the indicated value at a wavelength of 357.9 nm or 359.3 nm(9).

2) Prepare a curve for the relationship between the chromium concentration and the indicated value of the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Transfer 25 mL (11) of the sample solution to a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add hydrochloric acid (1+23) up to the marked line.

3) Subject to the same procedure as in **b) 1)** to read the indicated value.

4) Subject the blank test solution to the same procedure as in **1) - 2)** and **b) 1)** to read the indicated value, and correct the indicated value obtained for the sample solution.

5) Obtain the chromium content from the calibration curve, and calculate the chromium concentration in the analytical sample.

Note **(9)** When background correction is conducted by the Zeeman method, 359.3 nm is recommended as the analytical line wavelength.

(10) Use low-fuel acetylene-air flame. Acetylene-nitrous oxide flame can also be used.

(11) If there is a possibility that the chromium concentration in the sample solution will exceed the maximum limit of the calibration curve, decrease the amount to be transferred.

Comment 1 In an acetylene-air flame, sensitivity is enhanced in high-fuel flame, but interference by coexisting substances such as iron and nickel will also be enhanced.

In an acetylene-nitrous oxide flame, such interference hardly affects the result.

Comment 2 A replicate testing was conducted using calcined sludge fertilizer (5 samples); as a result, in the range of 71.0 mg/kg - 113.1 mg/kg measurement value, the repeatability was 2.1 % - 3.8 % relative standard deviation.

Additionally, the minimum limit of quantification of the test method is about 1 mg/kg.

Reference

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.213 - 216, Yokendo, Tokyo (1988)

(5) Flow sheet for chromium test method: The flow sheet for test method of chromium in inorganic fertilizers and composted sludge fertilizers, etc.

1.00 g analytical sample	200-mL tall beaker.
← A small amount of water, moisten the analytical sample	
← About 5 mL - 10 mL of phosphate	
← About 10 mL of nitric acid	
Heating	Cover with a watch glass, and digest for 10 minutes
Heating	Slightly move the watch glass, vaporize nitric acid
Standing to cool	Room temperature
← 5 mL - 10 mL of nitric acid	
Heating	Cover with a watch glass, and digest
Standing to cool	Room temperature
← About 5 mL of nitric acid	
Heating	Cover with watch glass, until white smoke evolves
Standing to cool	Room temperature
← About 15 mL – 20 mL of water	
Heating	Cover with a watch glass, gently
Standing to cool	Room temperature
Transfer	100-mL volumetric flask, water
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot 25 mL	100-mL volumetric flask
← About 10 mL of interference suppressor solution	
← Hydrochloric acid (1+23) (up to the marked line)	
Measurement	Atomic absorption spectrometer (357.9 nm or 359.3 nm)

Figure Flow sheet for chromium in inorganic fertilizers

5.5.c Flame atomic absorption spectrometry (Calcined sludge fertilizer, etc.)

(1) Summary

The test method is applicable to calcined sludge fertilizers, etc.
Pretreat an analytical sample with nitric acid - sulfuric acid - perchloric acid, and then spray into an acetylene-air flame, and measure the atomic absorption with chromium at a wavelength of 357.9 nm or 359.3 nm to quantify chromium.

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Sulfuric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Perchloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **e) Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **f) Interference suppressor solution (1):** Dissolve 100 g of potassium disulfate specified in JIS K 8783 in water to make 1,000 mL.
- **g) Chromium standard solution (0.1 mg/mL):** Chromium standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **h) Chromium standard solution (0.01 mg/mL) (1):** Put 10 mL of chromium standard solution (0.1 mg/mL) to 100-mL volumetric flasks and add hydrochloric acid (1+23) up to the marked line.
- **i) Chromium standard solutions (0.05 µg/mL - 5 µg/mL) for the calibration curve preparation (1) (2):** Transfer 2.5 mL - 25 mL of chromium standard solution (0.01 mg/mL) to 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (3), and further add hydrochloric acid (1+23) up to the marked line.
- **j) Blank test solution for the calibration curve preparation (1) (2):** Transfer about 50 mL of interference suppressor solution (3) to a 500-mL volumetric flask, and add hydrochloric acid (1+23) used in the procedure in **h**) up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.
(2) Store at room temperature, and do not use after 6 months after preparation.
(3) Add interference suppressor solution that is 1/10 volume of the volume to be prepared.

(3) Instruments: Instruments are as shown below:

- **a) Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121 with the background correction (4) function.
  - **1) Light source**: A chromium hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)
  - **2) Gas:** Gas for heating by flame
    - (i) Fuel gas: acetylene
    - (ii) Auxiliary gas: Air sufficiently free of dust and moisture.
- **b) Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 350 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to no less than 300 ºC.

Note **(4)** There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh 1.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.

b) Add about 10 mL of nitric acid and about 5 mL of sulfuric acid, cover the tall beaker with a watch glass, and leave at rest overnight.

c) Heat mildly on a hot plate or sand bath at 170 ºC - 220 ºC for no less than 30 minutes. After bubbles cease to form, set the temperature of the hot plate or sand bath to no less than 300 ºC (5), and heat until nitrogen oxides (yellow-brown smoke) no longer evolve(6).

d) After standing to cool, add about 5 mL of perchloric acid.

e) Cover the tall beaker with a watch glass, and heat on the hot plate or sand bath at no less than 300 ºC for 2 - 3 hours to digest(7).

f) Slightly move the watch glass(8), and keep on heating on the hot plate or sand bath to concentrate until the liquid volume becomes no more than 2 mL(7).

g) After standing to cool, add about 5 mL of hydrochloric acid (1+10) and about 20 mL of water, cover the tall beaker with a watch glass and heat mildly to dissolve.

h) After standing to cool, transfer to a 100-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.

i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare the blank test solution.

Note **(5)** If there is vigorous bumping, increase the temperature gradually.

(6) Oxidation of organic matters with perchloric acid progresses extremely rapidly and explosively. For that reason, add perchloric acid after fully degrading organic matters with nitric acid to avoid danger.

(7) When the white smoke of perchloric acid is generated, if the solution is colored such as black-brown or brown, stop heating immediately, and after standing to cool, add nitric acid, and heat again to degrade remaining organic matters.

(**8)** If there is no possibility of bumping, the watch glass can be removed.

Comment 1 The procedures in **(4.1)** are the same as in **(4.1)** in **5.2.a**.

Comment 2 When the analytical sample solidifies in the procedure in **(4.1) b)**, moisten the analytical sample with a small amount of water as necessary in advance.

Comment 3 In some cases, about 10 minutes heating in procedure in **(4.1) g)** is required**.**

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 357.9 nm or 359.3 nm (9)

b) Calibration curve preparation

1) Spray the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame(10), and read the indicated value at a wavelength of 357.9 nm or 359.3 nm(9).

2) Prepare a curve for the relationship between the chromium concentration and the indicated value of the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Transfer 25 mL of the sample solution to a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add hydrochloric acid (1+17) up to the marked line.

3) Subject to the same procedure as in **b) 1)** to read the indicated value.

4) Subject the blank test solution to the same procedure as in **1) - 2)** and **b) 1)** to read the indicated value, and correct the indicated value obtained for the sample solution.

5) Obtain the chromium content from the calibration curve, and calculate the chromium concentration in the analytical sample.

Note (9) When background correction is conducted by the Zeeman method, 359.3 nm is recommended as the analytical line wavelength.

(10) Acetylene-nitrous oxide flame can also be used.

Comment 3 In an acetylene-air flame, sensitivity is enhanced in high-fuel flame, but interference by coexisting substances such as iron and nickel will also be enhanced.

In an acetylene-nitrous oxide flame, such interference hardly affects the result.

Comment 4 The chromium concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1) - 3)** and **5)** to obtain the chromium content in the blank test solution.

Comment 5 A replicate testing was conducted using calcined sludge fertilizer (5 samples); as a result, in the range of 82.4 mg/kg - 123.6 mg/kg mean, standard deviation and relative standard deviation are 0 mg/kg - 3.4 mg/kg and 0 % - 2.8 %, respectively. In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 1.

Additionally, the minimum limit of quantification of the test method is about 6 mg/kg.

Table 1 Results and analysis results from a collaborative study for chromium test method validation

Sample name	Number of laboratories[1]	Mean[2] (mg/kg)	RSD_r[3] (%)	RSD_R[4] (%)
Calcined sludge fertilizer 1	10	107	5.0	9.7
Calcined sludge fertilizer 2	9	136	3.4	3.6
Calcined sludge fertilizer 3	9	182	1.1	2.6
Calcined sludge fertilizer 4	9	213	1.1	3.9
Calcined sludge fertilizer 5	9	117	1.8	4.0

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories x number of samples (2))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

Reference

1) Hisanori ARAYA, Yoshimi TAKEBA and Toshiaki HIROI: Evaluation of Digest Method for Determination of Chromium in Calcined Sludge Fertilizer by Atomic Absorption Spectrometry - Research Report of Fertilizer Vol.4, 2011. (23 - 29).

2) Hisanori ARAYA, Yasuharu KIMURA and Yoshimi TAKEBA: Evaluation of Digest Method for Determination of Chromium in Calcined Sludge Fertilizer by Atomic Absorption Spectrometry: A Collaborative Study Research Report of Fertilizer Vol.5, 2012. (41 - 47).

(5) Flow sheet for chromium test method: The flow sheet for test method of chromium in calcined sludge fertilizer

1.00 g analytical sample	200-mL - 300-mL tall beaker.
	← A small amount of water, moisten the analytical sample (as necessary)
	← About10 mL of nitric acid
	← About 5 mL of sulfuric acid
Leaving at rest overnight	Cover with a watch glass
Heating	Heat gently on a hot plate or a sand bath at 170 °C - 220 °C for no less than 30 minutes
Heating	Heat on a hot plate or sand bath at no less than 300 °C, until yellow-brown smoke no longer evolves.
Standing to cool	Room temperature
	← About 5 mL of perchloric acid
Heating	Cover with a watch glass, digest on a hot plate or a sand bath at 300 °C for 2-3 hours
Heating	Slightly move the watch glass, concentrate until it becomes no more than 2mL
Standing to cool	Room temperature
	← About 5 mL of nitric acid
	← About 20 mL of water
Heating	Cover with watch glass, dissolve
Standing to cool	Room temperature
Transfer	100-mL volumetric flask, water
	← Water (up to the marked line)
Filtration	Type 3 filter paper
Sample solution	
Aliquot 25 mL	100-mL volumetric flask
	← About 10 mL of interference suppressor solution
	← Hydrochloric acid (1+17) (up to the marked line)
Measurement	Atomic absorption spectrometer (357.9 nm or 359.3 nm)

Figure Flow sheet for chromium in calcined sludge fertilizers, etc.

5.5.d ICP Atomic Emission Spectrometry <Reference method>

(1) Summary

The test method is applicable to sludge fertilizers, etc. (except for calcined sludge fertilizer) Pretreat an analytical sample with incineration, nitric acid - hydrochloric acid (1+3), and then introduce it to an ICP Atomic Emission Spectrometer ("ICP-AES") and measure the emission with chromium at a wavelength of 205.552 nm to quantify chromium.

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Chromium standard solution (0.1 mg/mL):** Chromium standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **e) Chromium standard solutions (2.5 µg/mL)** (1) (2)**:**
  Dilute a predetermined amount of chromium standard solution (0.1 mg/mL) with hydrochloric acid (1+23) to prepare chromium standard solution (2.5 µg/mL)

Note (1) This is an example of preparation; prepare an amount as appropriate.
(2) Store at room temperature, and do not use after 6 months after preparation.

(3) Instruments: Instruments are as shown below:

- **a) ICP Atomic Emission Spectrometer:** An Atomic emission spectrometer specified in JIS K 0116.
  - **1) Gas:** Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity
- **b) Electric furnace:** An electric furnace that can keep the test temperature at ± 5 ºC
- **c) Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

- **a)** Weigh 5.00 g of an analytical sample, and put it in a 200- mL - 300-mL tall beaker.
- **b)** Put the tall beaker in an electric furnace, and heat gently to char.(3)
- **c)** Ignite at 450 ºC ± 5 ºC to incinerate (4).
- **d)** After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
- **e)** Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
- **f)** Slightly move the watch glass (5), and continue heating on the hot plate or sand bath to concentrate until nearly dried up.
- **g)** After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5)(6) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
- **h)** After standing to cool, transfer the solution to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.
- **i)** As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare the blank test solution.

Note **(3)** Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.
(4) Example of ignition period: 8 - 16 hours
(5) The watch glass can be removed.
(6) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 The procedures in **(4.1)** are the same as in **(4.1)** in **4.9.1.a**.

(4.2) Measurement: Conduct measurement (Standard Addition Method) according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Atomic Emission Spectrometer used in measurement.

a) Measurement conditions for the ICP Atomic Emission Spectrometer: Set up the measurement conditions for the ICP Atomic Emission Spectrometer considering the following:
Analytical line wavelength: 205.552 nm

b) Calibration curve preparation and Sample measurement.

1) Put 5 mL of sample solution into three 10-mL volumetric flasks respectively.
2) Add 2 mL and 4 mL of chromium standard solution (0.25 μg/mL) to volumetric flasks of **1)** above, then add hydrochloric acid (1+23) to the marked line to make the sample solutions of Standard Addition Method.
3) Add hydrochloric acid (1+23) to the marked line of the remaining volumetric flask of **1)** above to make the sample solution without standard solution.
4) Spray the sample solution of Standard Addition Method and sample solution without standard solution into the induction plasma, and read the indicated value at a wavelength of 205.552 nm.
5) Add 5 mL of blank test solution to a 10-mL volumetric flask, conduct the same procedures as in **3) - 4)** to read the indicated value, and correct the indicated value obtained from the respective sample solutions.
6) Prepare a curve for the relationship between the added chromium concentration and the corrected indicated value of the sample solution for Standard Addition Method and the sample solution without standard solution.
7) Obtain the chromium content from the intercept of the calibration curve to calculate the concentration of chromium in the analytical sample.

Comment 2 The chromium concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1**) - **4)** and **6) - 7)** to obtain the chromium content in the blank test solution.

Comment 3 Simultaneous measurement of multiple elements by ICP-AES is possible. In this case, mix a predetermined amount of copper standard solution (0.1 mg/mL), zinc standard solution (0.1 mg/mL), cadmium standard solution (0.1 mg/mL), nickel standard solution (0.1 mg/mL), chromium standard solution (0.1 mg/mL) and lead standard solution (0.1 mg/mL), dilute with hydrochloric acid (1 +23) to prepare mixture standard solution (Cu 25 μg/mL, Zn 25 μg/mL, Cd 0.25 μg/mL, Ni 2.5 μg/mL, Cr 2.5 μg/mL and Pb 2.5 μg/mL) (1) (2). Use the mixture solution instead of chromium standard solution (2.5 μg/mL) in **(4.2) b) 2)**. After that, conduct the same procedures as in **(4.2) b)** to calculate the concentration of respective elements in the analytical sample. Note that the wavelength for measurement of respective elements is

Cu 324.754 nm, Zn 206.191 nm, Cd 228.802 nm, Ni 231.604 nm, Cr 205.552 nm and Pb 220.351 nm.

In addition, the respective element concentrations for respective standard addition samples are shown in the below Table.

Table The additive amount of mixed standard solution
and the additive concentration of respective elements for respective sample solutions

	Additive amount (mL) of mixed standard solution	Cd (mg/L)	Pb (mg/L)	Ni (mg/L)	Cr (mg/L)	Cu (mg/L)	Zn (mg/L)
Sample solution without standard solution	0	0	0	0	0	0	0
Sample solution 1 by Standard Addition Method	2	0.05	0.5	0.5	0.5	5.0	5.0
Sample solution 2 by Standard Addition Method	4	0.1	1.0	1.0	1.0	10	10

Comment 4 For each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixed sludge fertilizer, calcined sludge fertilizer and composted sludge fertilizer, the repeatability obtained from triplicates measurement is 0.9 % - 2.5 % relative standard deviation.

Additionally, the minimum limit of quantification of the test method is about 4 mg/kg.

Reference

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI, Tetsuya NOMURA : Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer Vol.4 2011. (36 - 48)

(5) Flow sheet for chromium: The flow sheet for chromium in fertilizers is shown below:

5.00 g analytical sample	200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	Ignite at 450 °C ± 5 °C
Standing to cool	Room temperature
	← A small amount of water, moisten the residue
	← About 10 mL of nitric acid
	← About 30 mL of hydrochloric acid
Heating	Cover with a watch glass, and digest for 30 minutes
Heating	Slightly move a watch glass to remove acid
Standing to cool	Room temperature
	← 25 mL - 50 mL of hydrochloric acid (1+5)
Heating	Cover with a watch glass, and dissolve
Standing to cool	Room temperature
Transfer	100-mL - 200-mL volumetric flask, water
	← Water (up to the marked line)
Filtration	Type 3 filter paper
Aliquot 5 mL	10-mL volumetric flask, 3 flasks
	← 0, 2 and 4 mL of chromium standard solution (2.5 μg / mL) respectively
	← Hydrochloric acid (1+23) (up to the marked line)
Measurement	ICP Atomic Emission Spectrometer (205.552 nm)

Figure Flow sheet for chromium in fertilizers

5.6 Lead

5.6.a Flame atomic absorption spectrometry

(1) Summary

The test method is applicable to fertilizers.

Pretreat an analytical sample with incineration and nitric acid-hydrochloric acid (1+3), spray into an acetylene-air flame, and measure the atomic absorption with lead at a wavelength of 217.0 nm or 283.3 nm to quantify lead.

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Lead standard solution (0.1 mg/mL):** Lead standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **e) Lead standard solutions (0.5 μg/mL - 5 μg/mL) for the calibration curve preparation** (1) (2)**:** Transfer 2.5 mL - 25 mL of lead standard solution (0.1 mg/mL) to 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.
- **f) Blank test solution for the calibration curve preparation** (1) (2)**:** Hydrochloric acid (1+23) used in the procedure in **e**).

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(2) Store at room temperature, and do not use after 6 months after preparation.

(3) Instruments: Instruments are as shown below:

- **a) Flame atomic absorption spectrometer:** An atomic absorption spectrometer specified in JIS K 0121 with the background correction (3) function.
  - **1) Light source**: A lead hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)
  - **2) Gas:** Gas for heating by flame
    - (i) Fuel gas: acetylene
    - (ii) Auxiliary gas: Air sufficiently free of dust and moisture.
- **b) Electric furnace:** An electric furnace that can be adjusted to 450 ºC ± 5 ºC.
- **c) Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note **(3)** There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

- **a)** Weigh 5.00 g of an analytical sample, and put it in a 200-mL- 300-mL tall beaker.
- **b)** Put the tall beaker in an electric furnace, and heat gently to char (4).
- **c)** Ignite at 450 ºC±5 ºC to incinerate (5).
- **d)** After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
- **e)** Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass [(6)], and continue heating on the hot plate or sand bath to concentrate until nearly dried up.

g) After standing to cool, add 25 mL -50 mL of hydrochloric acid (1+5) [(7)] to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer the solution to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.

i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare the blank test solution.

Note **(4)** Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.

(5) Example of ignition period: 8 - 16 hours

(6) The watch glass can be removed.

(7) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h)**, about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 Do not conduct the procedures in **(4.1) b) - c)** in the case of fertilizers not containing organic matters.

Comment 2 The procedures in **(4.1)** are the same as in **(4.1)** in **4.9.1.a**.

(4.2) Measurement: Conduct measurement according to JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:

Analytical line wavelength: 217.0 nm or 283.3 nm

b) Calibration curve preparation

1) Spray the lead standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 217.0 nm or 283.3 nm.

2) Prepare a curve for the relationship between the lead concentration and the indicated value of the lead standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Subject the sample solution [(8)] to the same procedure as in **b) 1)** to read the indicated value.

2) Subject the blank test solution to the same procedure as in **b) 1)** to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the lead content from the calibration curve, and calculate the lead concentration in the analytical sample.

Note **(8)** If there is a possibility that the lead concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount with hydrochloric acid (1+23).

Comment 3 The lead concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1**) and **3**) to obtain the lead content in the blank test solution.

Comment 4 A recovery testing was conducted using industrial sludge fertilizer and composted sludge fertilizer (5 samples); as a result, the recovery at the concentration level of 100 mg/kg and 10 mg/kg was 99.1 % - 100.6 % and 97.5 % - 99.6 %, respectively, and the repeatability was 0.3 % - 1.4 % and 0.4 % - 3.0 % relative standard deviation, respectively.

In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 1.

Additionally, the minimum limit of quantification of the test method is about 1 mg/kg.

Table 1 Results and analysis results from a collaborative study for lead test method validation

Sample name	Number of laboratories[1)]	Mean[2)] (mg/kg)	RSD_r[3)] (%)	RSD_R[4)] (%)
Sewage sludge fertilizer a	10	25.2	4.6	3.9
Sewage sludge fertilizer b	11	29.4	3.7	4.3
Composted sludge fertilizer a	10	18.6	3.2	5.0
Composted sludge fertilizer b	10	22.2	1.8	7.0
Composted sludge fertilizer c	11	86.8	1.3	4.0

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories x number of samples (2))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Yoshinari SAKAKIBARA, Manabu MATSUZAKI and Tadao AMANO: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Improved Decomposition Method - Research Report of Fertilizer Vol.1 2008. (41 - 49)

2) Yoshinari SAKAKIBARA and Manabu MATSUZAKI: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Collaborative Test Results - Research Report of Fertilizer Vol.1 2008. (50 - 59)

3) Hisanori ARAYA and Yoshimi TAKEBA: Determination of Cadmium, Lead, Nickel and Chromium in Calcined Sludge Fertilizer - Using Decomposition Method for Inorganic Fertilizer- Research Report of Fertilizer Vol.3 2010. (30 - 42)

(5) Flow sheet for lead: The flow sheet for lead in fertilizers is shown below:

5.00 g analytical sample	200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	Ignite at 450 °C ± 5 °C
Standing to cool	Room temperature
	← A small amount of water, moisten the residue
	← About 10 mL of nitric acid
	← About 30 mL of hydrochloric acid
Heating	Cover with a watch glass, and digest
Heating	Slightly move a watch glass to remove acid
Standing to cool	Room temperature
	← About 25 mL - 50 mL of hydrochloric acid (1+5)
Heating	Cover with a watch glass, and dissolve
Standing to cool	Room temperature
Transfer	100-mL - 200-mL volumetric flask, water
	← Water (up to the marked line)
Filtration	Type 3 filter paper
Measurement	Atomic absorption spectrometer (217.0 nm or 283.3 nm)

Figure Flow sheet for lead in fertilizers

5.6.b ICP Atomic Emission Spectrometry <Reference method>

(1) Summary

The test method is applicable to sludge fertilizers.

Pretreat an analytical sample with incineration and nitric acid-hydrochloric acid (1+3), introduce it to an ICP Atomic Emission Spectrometer (“ICP-AES”) and measure the emission with lead at a wavelength of 220.351 nm to quantify lead.

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Nitric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Hydrochloric acid:** A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Lead standard solution (0.1 mg/mL):** Lead standard solution (0.1 mg/mL) for atomic absorption spectrometry traceable to a specified reference material (national measurement standard) according to Article 134 of the Measurement Act.
- **e) Lead standard solutions (2.5 µg/mL)** (1) (2)**:**
  Dilute a predetermined amount of lead standard solution (0.1 mg/mL) with hydrochloric acid (1+23) to prepare lead standard solution (2.5 µg/mL).

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) Store at room temperature, and do not use after 6 months after preparation.

(3) Instruments: Instruments are as shown below:

- **a) ICP Atomic Emission Spectrometer:** An atomic emission spectrometer specified in JIS K 0116.
  - **1) Gas:** Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity
- **b) Electric furnace:** An electric furnace that can keep the test temperature at ± 5 ºC
- **c) Hot plate or sand bath:** A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

- **a)** Weigh 5.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.
- **b)** Put the tall beaker in an electric furnace, and heat gently to char.(3)
- **c)** Ignite at 450 ºC ± 5 ºC to incinerate (4).
- **d)** After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
- **e)** Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
- **f)** Slightly move the watch glass (5), and continue heating on the hot plate or sand bath to concentrate until nearly dried up.
- **g)** After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (6) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
- **h)** After standing to cool, transfer the solution to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make the sample solution.
- **i)** As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare the blank test solution.

Note (3) Example of the charring procedure: Heat at about 250 ºC until smoke is no longer formed.
(4) Example of ignition period: 8 - 16 hours
(5) The watch glass can be removed.
(6) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 1 Do not conduct procedure **(4.1) b) - c)** in the case of fertilizers not contain organic matter

Comment 2 The procedures in **(4.1)** are the same as in **(4.1)** in **4.9.1.a**.

(4.2) Measurement: Conduct measurement (Standard Addition Method) according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Atomic Emission Spectrometer used in measurement.

a) Measurement conditions for the ICP Atomic Emission Spectrometer: Set up the measurement conditions for the ICP Atomic Emission Spectrometer considering the following:
Analytical line wavelength: 220.351 nm

b) Calibration curve preparation and Sample measurement.

1) Put 5 mL of sample solution into three 10-mL volumetric flasks respectively.
2) Add 2 mL and 4 mL of lead standard (0.25 μg/mL) solution to volumetric flasks of **1)** above, then add hydrochloric acid (1+23) to the marked line to make the sample solution of Standard Addition Method.
3) Add hydrochloric acid (1+23) to the marked line of the remaining volumetric flask of **1)** above, and use this solution as the sample solution without standard solution.
4) Spray the sample solution of Standard Addition Method and sample solution without standard solution into the induction plasma, and read the indicated value at a wavelength of 220.351 nm.
5) Add 5 mL of blank test solution to a 10-mL volumetric flask, conduct the same procedures as in **3) - 4)** to read the indicated value, and correct the indicated value obtained from the respective sample solutions.
6) Prepare a curve for the relationship between the added lead concentration and the corrected indicated value of the sample solutions of Standard Addition Method and the sample solution without standard solution.
7) Obtain the lead content from the intercept of the calibration curve, and calculate the lead concentration in the analytical sample.

Comment 3 The lead concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **1**) - **4)** and **6) -7)** to obtain the lead content in the blank test solution.

Comment 4 Simultaneous measurement of multiple elements by ICP-AES is possible. In this case, mix a predetermined amount of copper standard solution (0.1 mg/mL), zinc standard solution (0.1 mg/mL), cadmium standard solution (0.1 mg/mL), nickel standard solution (0.1 mg/mL), chromium standard solution (0.1 mg/mL) and lead standard solution (0.1 mg/mL), dilute with hydrochloric acid (1 +23) to prepare mixture standard solution (Cu 25 μg/mL, Zn 25 μg/mL, Cd 0.25 μg/mL, Ni 2.5 μg/mL, Cr 2.5 μg/mL and Pb 2.5 μg/mL) (1) (2). Use the mixture solution instead of lead standard solution (2.5

μg/mL) in **(4.2) b) 2)**. After that, conduct the same procedures as in **(4.2) b)** to calculate the concentration of respective elements in the analytical sample. Note that the wavelength for measurement of respective elements is Cu 324.754 nm, Zn 206.191 nm, Cd 228.802 nm, Ni 231.604 nm, Cr 205.552 nm and Pb 220.351 nm In addition, the respective element concentrations for respective standard addition samples are shown in the below Table.

Table The additive amount of mixed standard solution
and the additive concentration of respective elements for respective sample solutions

	Additive amount (mL) of mixed standard solution	Cd (mg/L)	Pb (mg/L)	Ni (mg/L)	Cr (mg/L)	Cu (mg/L)	Zn (mg/L)
Sample solution without standard solution	0	0	0	0	0	0	0
Sample solution 1 by Standard Addition Method	2	0.05	0.5	0.5	0.5	5.0	5.0
Sample solution 2 by Standard Addition Method	4	0.1	1.0	1.0	1.0	10	10

Comment 5 For each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixed sludge fertilizer, calcined sludge fertilizer and composted sludge fertilizer, the repeatability obtained from triplicates measurement is 0.9 % - 3.3 % relative standard deviation.

Additionally, the minimum limit of quantification of the test method is about 5 mg/kg.

Reference

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI, Tetsuya NOMURA : Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer Vol.4 2011. (36 - 48)

(5) Flow sheet for lead: The flow sheet for lead in fertilizers is shown below:

Standing to cool — Room temperature

← A small amount of water

← About 10 mL of nitric acid

← About 30 mL of hydrochloric acid

Heating — Cover with a watch glass, and digest

Heating — Slightly move a watch glass to remove acid

Standing to cool — Room temperature

← 25 mL - 50 mL of hydrochloric acid (1+5)

Heating — Cover with a watch glass, and dissolve

Standing to cool — Room temperature

Transfer — 100-mL - 200-mL volumetric flask, water

← Water (up to the marked line)

Filtration — Type 3 filter paper

Aliquot 5 mL — 10-mL volumetric flask, 3 flasks

← 0, 2 and 4 mL of lead standard solution (2.5 µg / mL) respectively

← Hydrochloric acid (1+23) (up to the marked line)

Measurement — ICP Atomic Emission Spectrometer (220.351 nm)

Figure Flow sheet for lead in fertilizers

5.7 Sulfamic acid (amidosulfuric acid)

5.7.a Ion Chromatography <Reference method>

(1) Summary

The test method is applicable to ammonium sulfate.

Add water to an analytical sample to extract sulfamic acid, introduce it to an Ion Chromatograph (IC) or a High Performance Liquid Chromatograph (HPLC) to isolate it with an ion exchange column, then measure the sulfamic acid with an electric conductivity detector to quantify sulfamic acid.

Sulfamic acid and ammonium thiocyanate can be simultaneously quantified by using this method. (Refer to Comment 4).

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557 or equivalent quality
- **b) Phthalic acid:** A reagent of no less than 98 % (mass fraction) in purity.
- **c) *p*-hydroxybenzoic acid**: A reagent of no less than 95 % (mass fraction) in purity.
- **d) 1-sodium octane sulfonate:** A reagent of no less than 98 % (mass fraction) in purity.
- **e) 1-sodium hexane sulfonate:** A reagent of no less than 98 % (mass fraction) in purity.
- **f) Boric acid:** A JIS Guaranteed Reagent specified in JIS K 8863 or a reagent of equivalent quality.
- **g) Elute** (1) (2)**:** Weigh 0.083 g of phthalic acid, 0.552 g of *p*-hydroxybenzoic acid, 0.195 g of 1-sodium octane sulfonate, 0.376 g of 1-sodium hexane sulfonate and 6.183 g of boric acid to a 1-L volumetric flask, add about 500 mL of water to dissolve and add water up to the marked line. Filter with membrane type filter (pore size: no more than 0.5 μm) made of hydrophilic PTFE
- **h) Sulfamic acid standard solution (1,000 mg/L)** (1)**:** Put 0.1 g of sulfamic acid**,** reference material for volumetric analysis ($HOSO_2NH_2$: dried for 48 hours in a silica gel desiccator), in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.
- **i) Sulfamic acid standard solution (10 mg/L)** (1)**:** At the time of usage, put 10 mL of sulfamic acid standard solution (1,000 mg/L) to a 100-mL volumetric flask and add water up to the marked line.
- **j) Sulfamic acid standard solution for the calibration curve preparation (0.3 mg/L - 3 mg/L):** At the time of usage, put 3 mL - 30 mL of sulfamic acid standard solution (10 mg/L) to 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) The concentration of prepared solutions is phthalic acid 0.5 mmol/L, *p*-hydroxybenzoic acid 4.0 mmol/L, 1-sodium octane sulfonate 0.9 mmol/L, 1-sodium hexane sulfonate 2.0 mmol/L and boric acid 100 mmol/L.

(3) Apparatus and instruments: Apparatus and instruments are as shown below:

- **a) Ion Chromatograph (IC) or High Performance Liquid Chromatograph (HPLC):** IC specified in JIS K 0127 or HPLC specified in JIS K 0124 that satisfies following requirements.
  - **1) Column:** A 4-mm inner diameter 100-mm long stainless steel column tube filled with hydrophilic methacrylate-gel, to which 5-μm particle diameter class 4 ammonium group chemically bonds (3).
  - **2) Column bath:** A column bath whose temperature can be adjusted to 55 ºC - 60 ºC.
  - **3) Detection unit:** An electric conductivity detector

b) Membrane filters: Pore size is no more than 0.5 μm, made of hydrophilic PTFE

Note (3) A column is commercially sold under the name of Shodex IC NI-424, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, stopple the volumetric flask, and shake to dissolve, and then add water up to the marked line.

c) Transfer a predetermined amount of the solution, and dilute 12.5 times exactly with water.

d) Filter with a membrane filter (pore size: no more than 0.5 μm) to make the sample solution.

(4.2) Measurement: Conduct measurement according to JIS K 0127 or JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the Ion Chromatograph (IC) or the High Performance Liquid Chromatograph (HPLC) used in measurement.

a) Measurement conditions for the Ion Chromatograph (IC) or High Performance Liquid Chromatograph (HPLC): Set up the measurement conditions considering the following example of measurement conditions.

1) Column: A hydrophilic methacrylate-gel column (4-mm inner diameter, 100-mm long, 5-μm particle diameter) to which class 4 ammonium group chemically bonds.

2) Column bath temperature: 58 °C

3) Elute: Prepared by the procedures in **(2) g)**

4) Flow: 1 mL/min

5) Injection volume: 20 μL

6) Detection unit: An electric conductivity detector

b) Calibration curve preparation

1) Inject 20 μL of respective standard solutions for the calibration curve preparation to an IC or HPLC, and record the chromatogram of electric conductivity to obtain peak area.

2) Prepare a curve for the relationship between the concentration and the peak area of electric conductivity of respective standard solutions for the calibration curve preparation.
Prepare a calibration curve when the sample is measured.

Comment 1 In the measurement of sample solution, there is a possibility that the recovery rate becomes lower than actual due to the influence of matrix if the concentration is calculated with peak height. Therefore prepare a relationship line with peak area when calibration curve is prepared.

c) Sample measurement

1) Subject 20 μL of sample solution to the same procedure as in **b) 1)**

2) Obtain the sulfamic acid content from calibration curve by peak area to calculate the concentration of sulfamic acid in the analytical sample.

Comment 2 Calculate the concentration by the peak area similarly as the calibration curve preparation to prevent the influence of matrix.

Comment 3 Note that it takes time to stabilize the baseline due to the usage of the ion-pairing reagent in the elute. It is recommended to take about 120 minutes for stabilization time before starting measurement.

Comment 4 It is possible for the simultaneous measurement of sulfamic acid and ammonium thiocyanate in this testing method. In that case, mix a predetermined amount of sulfamic acid standard solution (1,000 mg/L) and ammonium thiocyanate standard solution (1,000 mg/L), dilute with water to prepare mixture standard solution (10 mg/L) [1] and use it instead of respective standard solutions (10 mg/L) in **(2) i)**. After that, conduct the same procedure in **(4.2) b)** to calculate the respective concentrations of materials subjected to measurement in the analytical sample.

Comment 5 A recovery testing of ammonium sulfate (3 brands) was conducted. As a result, the average recovery rate at additive level of 0.25 % (mass fraction) and 0.075 % (mass fraction) was 94.4 % - 103.5 %, and the repeatability was 0.7 % - 2.3 % relative standard deviation.
Additionally, the minimum limit of quantification of the test method is about 0.04 % (mass fraction).

(5) Flow sheet for testing method: The flow sheet for sulfamic acid in ammonium sulfate is shown below:

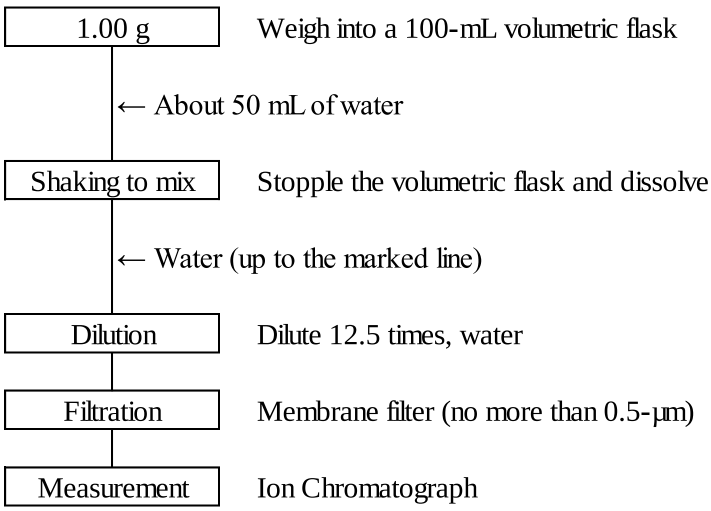
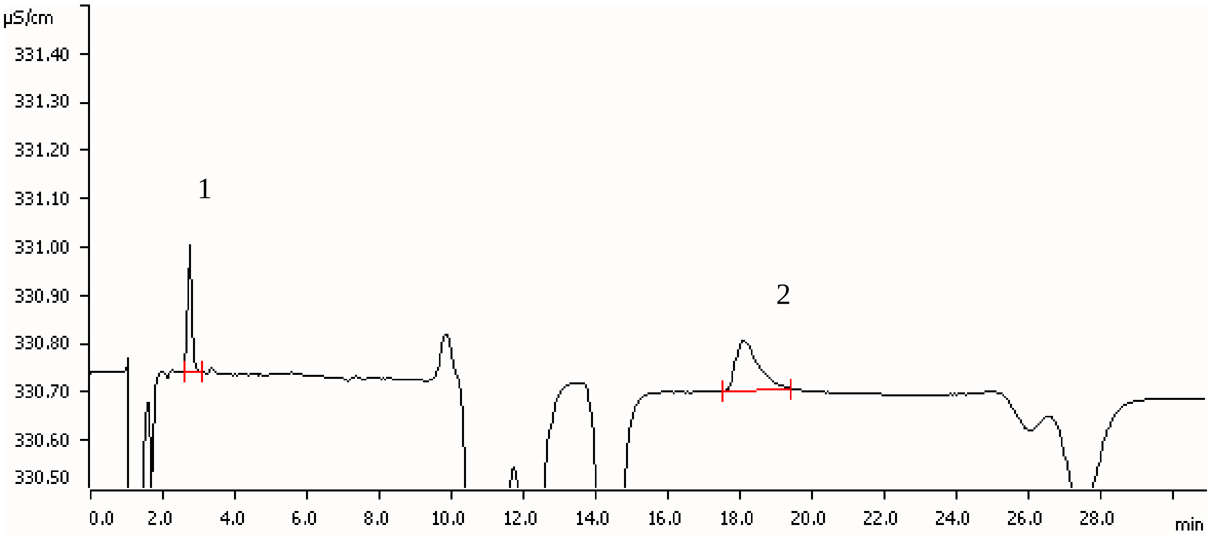


Figure Flow sheet for sulfamic acid in ammonium sulfate

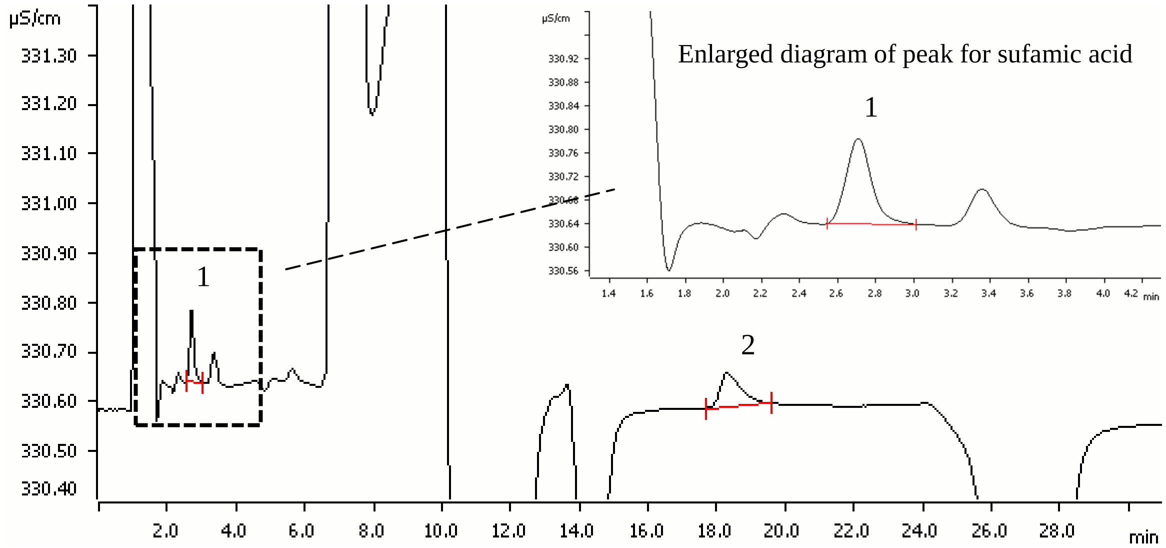
Reference

1) Toshiaki HIROI and Yuji SHIRAI: Simultaneous Determination of Sulfamic Acid and Ammonium Thiocyanate in Ammonium Sulfate by Nonsuppressed Ion Chromatography, Research Report of Fertilizer,Vol.5, 2012, (1 - 23)

Reference: The IC chromatogram of sulfamic acid and thiocyanic acid of the standard solution for the calibration curve preparation and sample solution (ammonium sulfate) are shown below.



(A) Mixture standard solution (the equivalents of 60 ng as sulfamic acid and ammonium thiocyanate (3 mg/L, 20 µL), respectively)



(B) Sample solution (the equivalents of 0.25 % (mass fraction) as sulfamic acid and ammonium thiocyanate added in ammonium sulfate (2,500 µg/g), respectively)

Reference diagram: IC chromatogram of sulfamic acid and ammonium thiocyanate.
(Peak: 1. Sulfamic acid, 2. Ammonium thiocyanate)

IC measurement conditions

Column: Shodex IC NI-424 (4.6-mm inner diameter, 100-mm long, 5µm-particle diameter)

Other conditions are according to the example of measurement conditions in **(4.2) a)**

5.8 Sulfurized cyanide (ammonium thiocyanate)

5.8.a Ion Chromatography <Reference method>

(1) Summary

The test method is applicable to ammonium sulfate.

Add water to an analytical sample to extract ammonium thiocyanate, introduce it to an Ion Chromatograph (IC) or a High Performance Liquid Chromatograph (HPLC) to isolate it with an ion exchange column, and then measure the thiocyanic acid with an electric conductivity detector to quantify ammonium thiocyanate.

Sulfamic acid and ammonium thiocyanate can be simultaneously quantified by using this method. (Refer to Comment 4).

(2) Reagents, etc.: Reagents and water are as shown below:

a) **Water:** Water of A3 specified in JIS K 0557 or equivalent quality

b) **Phthalic acid:** A reagent of no less than 98 % (mass fraction) in purity.

c) ***p*-hydroxybenzoic acid**: A reagent of no less than 95 % (mass fraction) in purity.

d) **1-sodium octane sulfonate:** A reagent of no less than 98 % (mass fraction) in purity.

e) **1-sodium hexane sulfonate:** A reagent of no less than 98 % (mass fraction) in purity.

f) **Boric acid:** A JIS Guaranteed Reagent specified in JIS K 8863 or a reagent of equivalent quality.

g) **Elute** (1) (2)**:** Weigh 0.083 g of phthalic acid, 0.552 g of *p*-hydroxybenzoic acid, 0.195 g of 1-sodium octane sulfonate, 0.376 g of 1-sodium hexane sulfonate, and 6.183 g of boric acid to a 1-L volumetric flask, add about 500 mL of water to dissolve and add water up to the marked line. Filter with membrane type filter (pore size: no more than 0.5 μm) made of hydrophilic PTFE

h) **Ammonium thiocyanate standard solution (1,000 mg/L)** (1)**:** Put 0.1 g of ammonium thiocyanate (3), specified in JIS K 9000 in weighing dish, and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.

i) **Ammonium thiocyanate standard solution (10 mg/L)** (1)**:** At the time of usage, put 10 mL of ammonium thiocyanate standard solution (1,000 mg/L) to a 100-mL volumetric flask and add water up to the marked line.

j) **Ammonium thiocyanate standard solution for the calibration curve preparation (0.3 mg/L - 3 mg/L):** At the time of usage, put 3 mL - 30 mL of sulfamic acid standard solutions (10 mg/L) to 100-mLvolumetric flasks step-by-step and add water up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(2) The concentrations of prepared solutions are phthalic acid 0.5 mmol/L, *p*-hydroxybenzoic acid 4.0 mmol/L, 1-sodium octane sulfonate 0.9 mmol/L, 1-sodium hexane sulfonate 2.0 mmol/L, and boric acid 100 mmol/L, respectively.

(3) It is recommended to store in a desiccator because of deliquescence.

(3) Apparatus and instruments: Apparatus and instruments are as shown below:

a) **Ion Chromatograph (IC) or High Performance Liquid Chromatograph (HPLC):** IC specified in JIS K 0127 or HPLC specified in JIS K 0124 that satisfies following requirements.

1) **Column:** A 4-mm inner diameter 100-mm long stainless steel column tube filled with hydrophilic methacrylate-gel, to which 5-μm particle diameter class 4 ammonium group chemically bonds (4).

2) **Column bath**: A column bath whose temperature can be adjusted to 55 ºC - 60 ºC.
3) **Detection unit**: An electric conductivity detector

b) **Membrane filters**: Pore size is no more than 0.5 µm, made of hydrophilic PTFE

Note (4) The column is commercially sold under the name of Shodex IC NI-424 etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as follows:

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, stopple the volumetric flask, and shake to dissolve, and then add water up to the marked line.
c) Transfer a predetermined amount of the solution, and dilute 12.5 times exactly with water.
d) Filter with a membrane filter (pore size no more than 0.5 µm) to make the sample solution.

(4.2) Measurement: Conduct measurement according to JIS K 0127 or JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the Ion Chromatograph (IC) or the High Performance Liquid Chromatograph (HPLC) method used in measurement.

a) Measurement conditions for the Ion Chromatograph (IC) or High Performance Liquid Chromatograph (HPLC): Set up the measurement conditions considering the following example of measurement conditions.

1) **Column:** A hydrophilic methacrylate-gel column (4-mm inner diameter, 100-mm long, 5-µm particle diameter) to which class 4 ammonium group chemically bonds.
2) **Column bath temperature:** 58 ºC
3) **Elute**: Prepared by the procedures in **(2) g)**
4) **Flow:** 1 mL/min
5) **Injection volume:** 20 µL
6) **Detection unit:** An electric conductivity detector

b) Calibration curve preparation

1) Inject 20 µL of respective standard solutions for the calibration curve preparation into an IC or HPLC, and record the chromatogram of electric conductivity to obtain peak area.
2) Prepare a curve for the relationship between the concentration and the peak area of electric conductivity of respective standard solutions for the calibration curve preparation.
   Prepare a calibration curve when the sample is measured.

Comment 1 In the measurement of sample solution, there is a possibility that the recovery rate becomes lower than actual due to the influence of matrix if the concentration is calculated with peak height. Therefore prepare a relationship line with peak area when calibration curve is prepared.

c) Sample measurement

1) Subject 20 µL of sample solution to the same procedure as in **b) 1)**
2) Obtain the ammonium thiocyanate content from calibration curve by peak area to calculate the concentration of ammonium thiocyanate in the analytical sample.

Comment 2 Calculate the concentration by the peak area to prevent the influence of matrix similarly as the calibration curve preparation,

Comment 3 Note that it takes time to stabilize the baseline due to the usage of the ion-pairing reagent in the elute. It is recommended to take about 120 minutes for stabilization time before starting measurement.

Comment 4 It is possible for the simultaneous measurement of ammonium thiocyanate and sulfamic acid in this testing method. In that case, mix a predetermined amount of sulfamic acid standard solution (1,000 mg/L) and ammonium thiocyanate standard solution (1,000 mg/L), dilute with water to prepare mixture standard solution (10 mg/L) [(1)] and use it instead of respective standard solutions (10 mg/L) in **(2) i)**. After that, conduct similarly as the procedure in **(4.2) b)**, and calculate the respective concentrations of materials subjected to measurement in the analytical sample.

Comment 5 A recovery testing of ammonium sulfate (3 brands) was conducted, as a result, the average recovery rate at additive level of 0.25 % (mass fraction) and 0.075 % (mass fraction) was 93.9 % - 103.7 %, and the repeatability was 0.6 % - 5.9 % relative standard deviation.
Additionally, the minimum limit of quantification of the test method is about 0.04 % (mass fraction).

Reference

1) Toshiaki HIROI and Yuji SHIRAI: Simultaneous Determination of Sulfamic Acid and Ammonium Thiocyanate in Ammonium Sulfate by Nonsuppressed Ion Chromatography, Research Report of Fertilizer,Vol.5, 2012, (1 - 23)

(5) Flow sheet for testing method: The flow sheet for ammonium thiocyanate in ammonium sulfate is shown below:

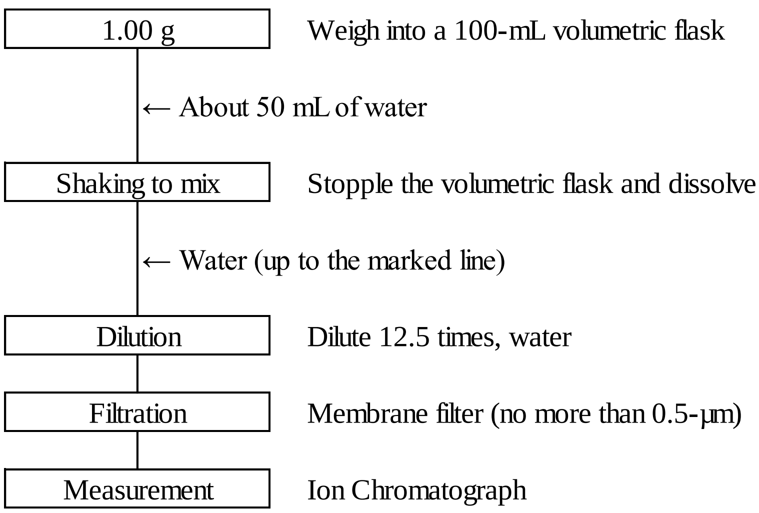
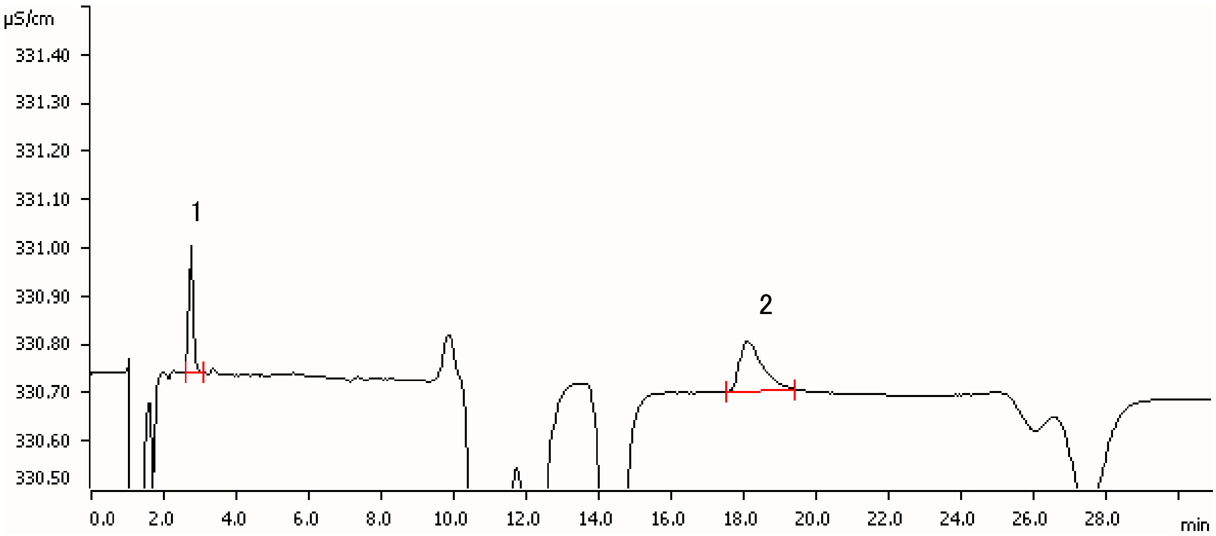
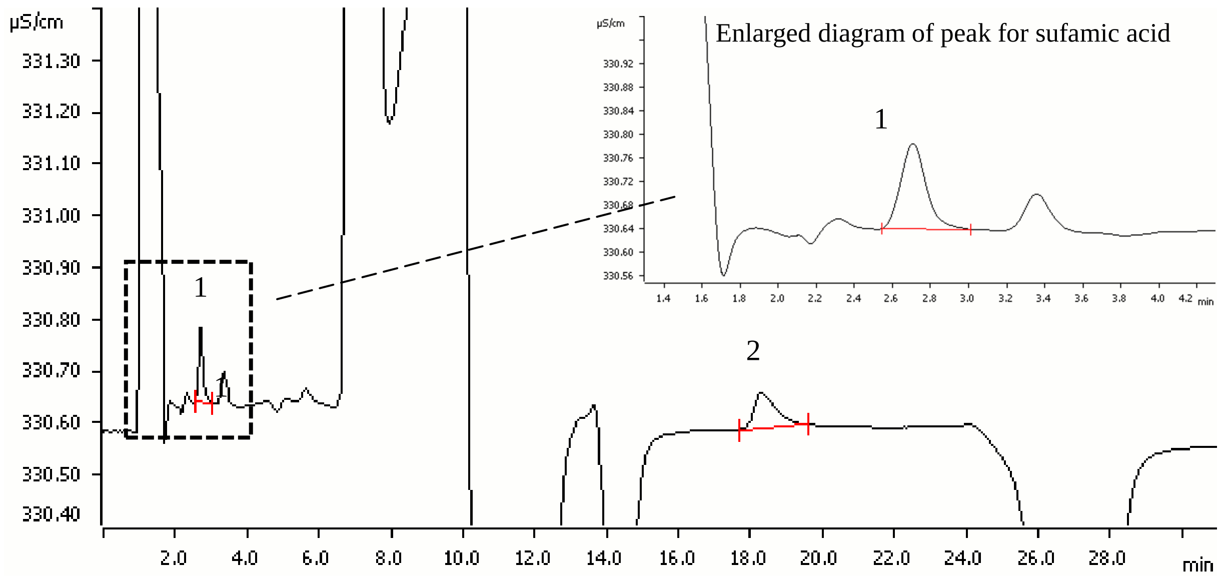


Figure flow sheet for ammonium thiocyanate in ammonium sulfate

Reference The IC chromatogram of sulfamic acid and thiocyanic acid of the standard solution for the calibration curve preparation and the sample solution (ammonium sulfate) are shown below.



(A) Mixture standard solution (the equivalents of 60 ng as sulfamic acid and ammonium thiocyanate (3 mg/L, 20 µL), respectively)



(B) Sample solution (the equivalents of 0.25% (mass fraction) as sulfamic acid and ammonium thiocyanate added in ammonium sulfate (2,500 µg/g), respectively)

Reference diagram: IC chromatogram of sulfamic acid and thiocyanic acid
(Peak: 1. Sulfamic acid, 2. Thiocyanic acid)

IC measurement conditions
Column: Shodex IC NI-424 (4.6-mm inner diameter, 100-mm long, 5-µm particle diameter)
Other conditions are according to the example of measurement conditions in **(4.2) a)**

5.8.b High Performance Liquid Chromatography <Reference method>

(1) Summary

This method is applicable to fertilizers.

Add water to an analytical sample, extract sulfurized cyanide (hereinafter referred to as "ammonium thiocyanate") and adjust pH as necessary. Introduce it into a High Performance Liquid Chromatograph (HPLC), isolate with a silica gel column to which amino group chemically bonds or a vinyl alcohol polymer column to which amino group chemically bonds, and measure at wavelength 210 nm to quantify ammonium thiocyanate.

With this method, nitrous acid and ammonium thiocyanate can be determined simultaneously. (Refer to Comment 4)

(2) Reagents: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557or equivalent quality.
- **b) Sodium hydroxide:** A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.
- **c) Disodium hydrogenphosphate dodecahydrate:** A JIS Guaranteed Reagent specified in JIS K 9019 or a reagent of equivalent quality.
- **d) Sodium dihydrogenphosphate dihydrate:** A JIS Guaranteed Reagent specified in JIS K 9009 or a reagent of equivalent quality.
- **e) Sodium perchlorate monohydrate:** A JIS Guaranteed Reagent specified in JIS K 8227 or a reagent of equivalent quality.
- **f) Ammonium thiocyanate standard solution (1 mg/mL)** (1)**:** Put 0.1 g of ammonium thiocyanate specified in JIS K 9000 in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mLvolumetric flask and add water up to the marked line.
- **g) Ammonium thiocyanate standard solution (100 µg/mL)** (1)**:** At the time of usage, put 10 mL of ammonium thiocyanate standard solution (1 mg/mL) to a 100-mL volumetric flask and add water up to the marked line.
- **h) Ammonium thiocyanate standard solution for the calibration curve preparation (1 µg/mL - 20 µg/mL):** At the time of usage, put 1 mL - 20 mL of ammonium thiocyanate standard solution (100 µg/mL) to 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are as shown below:

- **a) High Performance Liquid Chromatograph (HPLC):** HPLC specified in JIS K 0124 that satisfies following requirements.
  - **1) Column:** A 4-mm to 6-mm inner diameter 150-mm to 250-mm long stainless steel column tube filled with silica gel (2) or poly vinyl alcohol, to which 5-µm particle diameter amino group chemically bonds .
  - **2) Column bath:** A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
  - **3) Detector:** An absorptiometric detector that can measure at wavelength around 210 nm.
- **b) Magnetic stirrer**
- **c) Centrifugal separator:** A centrifugal separator that can centrifuge at 2,000 × *g*.
- **d) High speed centrifugal separator:** A centrifugal separator that can centrifuge at 8,000×*g*.
- **e) pH test paper:** A pH test paper infiltrated with indicator and dried, which can measure the value from pH 1 to pH 11 and a color change chart with the pH interval value 1 is attached.

Note (2) Remaining silanol group of silica gel affects the measurement of ion in some cases. Therefore use a column which does not affect the measurement of thiocyanic acid by treating the silanol group. As an example of the treatment, silica gel is to be entirely covered with the uniform membrane of silicone polymer.

Comment 1 A column is sold under the production names of CAPCELL PAK NH2 UG80 and Asahipak NH2P-50 4E from Shiseido and Showa Denko respectively.

Comment 2 pH test paper is sold under the production name of UNIV Shikenshi from ADVANTEC

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery analytical sample

a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and stir it with a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, transfer supernatant solution to a 50-mL ground-in stopper centrifugal precipitate tube.

d) Centrifuge at 2,000 × *g* centrifugal force for about five minutes (3) to make supernatant as the extract.

Note (3) 18.7-cm of rotor radius and 3,000 rpm of revolutions makes about 2,000 × g centrifugal force.

(4.1.2) Fluid analytical sample

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, stopple the volumetric flask and shake to mix and dissolve.

c) Add water up to the marked line to make the extract.

(4.2) pH adjustment: Conduct pH adjustment as shown below.

a) Transfer a small amount of the extract to confirm pH value using a pH-test paper.

b) If the pH value in **a)** is pH 5 or more, transfer the extract to a 1.5-mL ground-in stopper centrifugal precipitate tube(4) and conduct the procedure in **f)** to prepare sample solution.

c) If the pH value in **a)** is pH 4 or less, transfer 40 mL of the extract to a 100-mL beaker.

d) Add sodium hydroxide solution (5 mg/mL), adjust it to pH 5 to pH 7 with a pH meter and transfer to a 50-mL volumetric flask with water.

e) Add water to the marked line and transfer to a 1.5-mL ground-in stopper centrifugal precipitate tube (4).

f) Centrifuge at 8,000 × *g* centrifugal force for about five minutes (5) to make supernatant as the sample solution.

Note (4) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(5) 7-cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 3 Instead of procedures in **(4.2) b)** and **e) - f)**, it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(4.3) Measurement: Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of High Performance Liquid Chromatograph (HPLC) used in measurement.

a) Measurement conditions for High Performance Liquid Chromatograph (HPLC): Set up the measurement conditions for High Performance Liquid Chromatograph (HPLC) considering the following example:

1) Column: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which amino group chemically bonds or a vinyl alcohol polymer column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which amino group chemically bonds.

2) Temperature of Column bath: 30 ºC - 40 ºC

3) Eluent [(1)]: Dissolve 1.79 g of disodium hydrogenphosphate dodecahydrate, 0.78 g of sodium dihydrogenphosphate dihydrate and 14.04 g of sodium perchlorate monohydrate in water to make 1,000 mL. Filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE.

4) Flow rate: 0.9 mL/min

5) Injection rate: 10 µL

6) Detector: An absorptiometric detector, measurement wavelength: 210 nm

b) Calibration curve preparation

1) Inject 10 µL of respective standard solutions for the calibration curve preparation to HPLC, record chromatogram at wavelength 210 nm and obtain the peak area.

2) Prepare a curve for the relationship between the concentration and the peak area at wavelength 210 nm of the standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1)**.

2) Obtain the ammonium thiocyanate content from the peak area using the calibration curve to calculate the concentration of ammonium thiocyanate in the analytical sample.

Comment 4 This test method enables the simultaneous measurement of nitrous acid and ammonium thiocyanate. In this case, mix a predetermined amount of nitrous acid standard solution (1 mg/mL) and ammonium thiocyanate standard solution (1 mg/mL), dilute with water to prepare mixture standard solution (100 µg/mL) [(1)] and use it instead of **(2) h)** ammonium thiocyanate standard solution (100 µg/mL). After that, conduct the same procedure in **(4.3) b)** to calculate the respective concentrations of materials subjected to measurement in the analytical sample.

(5) Flow sheet for testing method: The flow sheet for ammonium thiocyanate in fertilizers is shown below:

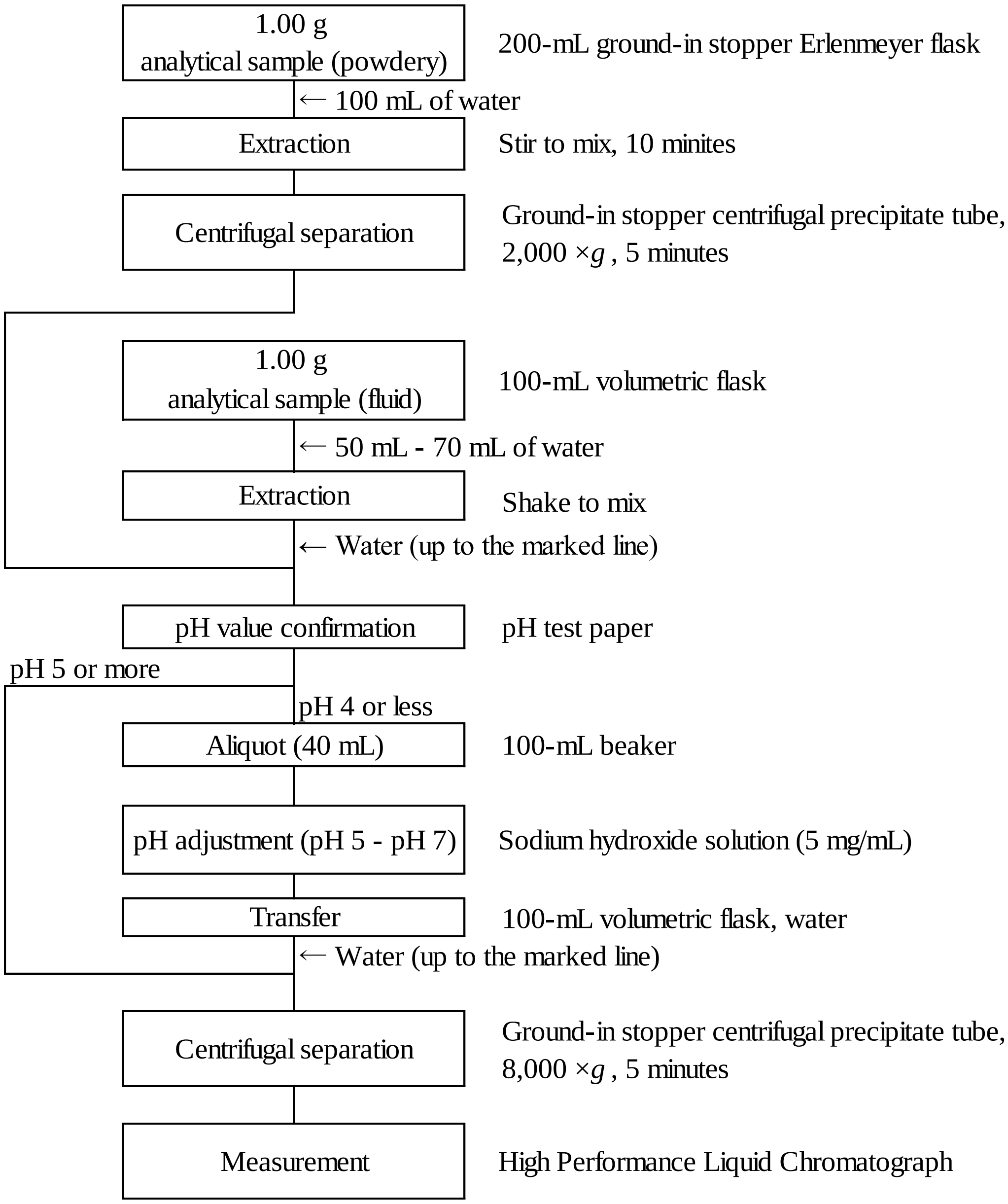


Figure Flow sheet for ammonium thiocyanate in fertilizers

5.9 Nitrous acid

5.9.a High Performance Liquid Chromatography <Reference method>

(1) Summary

This method is applicable to fertilizers.

Add water to an analytical sample, extract nitrous acid and adjust pH as necessary. Introduce it into a High Performance Liquid Chromatograph (HPLC), isolate with a silica gel column to which amino group chemically bonds or a vinyl alcohol polymer column to which amino group chemically bonds, and measure at wavelength 210 nm to quantify nitrous acid.

With this method, nitrous acid and ammonium thiocyanate can be measured simultaneously. (Refer to Comment **4**)

(2) Reagents: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557or equivalent quality.
- **b) Sodium hydroxide:** A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.
- **c) Disodium hydrogenphosphate dodecahydrate:** A JIS Guaranteed Reagent specified in JIS K 9019 or a reagent of equivalent quality.
- **d) Sodium dihydrogenphosphate dihydrate:** A JIS Guaranteed Reagent specified in JIS K 9009 or a reagent of equivalent quality.
- **e) Sodium perchlorate monohydrate:** A JIS Guaranteed Reagent specified in JIS K 8227 or a reagent of equivalent quality.
- **f) Nitrous Acid standard solution (1 mg/mL)** (1)**:** Put 0.147 g of sodium nitrite specified in JIS K 8019 in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.
- **g) Nitrous acid standard solution (100 µg/mL)** (1)**:** At the time of usage, put 10 mL of nitrous acid standard solution (1 mg/mL) to a 100-mL volumetric flask and add water up to the marked line.
- **h) Nitrous acid standard solution for the calibration curve preparation (1 µg/mL - 20 µg/mL):** At the time of usage, put 1 mL - 20 mL of nitrous acid standard solution (100 µg/mL) to 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are as shown below:

- **a) High Performance Liquid Chromatograph (HPLC):** HPLC specified in JIS K 0124 that satisfies following requirements.
  - **1) Column:** A 4-mm to 6-mm inner diameter 150-mm to 250-mm long stainless steel column tube filled with silica gel (2) or poly vinyl alcohol, to which 5-µm particle diameter amino group chemically bonds .
  - **2) Column bath:** A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
  - **3) Detector:** An absorptiometric detector that can measure at wavelength around 210 nm.
- **b) Magnetic stirrer**
- **c) Centrifugal separator:** A centrifugal separator that can centrifuge at 2,000 × *g*.
- **d) High speed centrifugal separator:** A centrifugal separator that can centrifuge at 8,000×*g*.
- **e) pH test paper:** A pH test paper infiltrated with indicator and dried, which can measure the value from pH 1 to pH 11 and a color change chart with the pH interval value 1 is attached.

Note (2) Remaining silanol group of silica gel affects the measurement of ion in some cases. Therefore use a column which does not affect the measurement of nitrous acid by treating the silanol group. As an example of the treatment, silica gel is entirely covered with the uniform membrane of silicone polymer.

Comment 1 A column is sold under the production names of CAPCELL PAK NH2 UG80 and Asahipak NH2P-50 4E from Shiseido and Showa Denko respectively.

Comment 2 pH test paper is sold under the production name of UNIV Shikenshi from ADVANTEC

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery analytical sample

a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and stir it with a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, transfer supernatant solution to a 50-mL ground-in stopper centrifugal precipitate tube.

d) Centrifuge at 2,000 × *g* centrifugal force for about five minutes (3) to make supernatant as the extract.

Note (3) 18.7-cm of rotor radius and 3,000 rpm of revolutions makes about 2,000 × g centrifugal force.

(4.1.2) Fluid analytical sample

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, stopple the volumetric flask and shake to mix and dissolve

c) Add water up to the marked line to make the extract.

(4.2) pH adjustment: Conduct pH adjustment as shown below.

a) Transfer a small amount of extract to confirm pH value using a pH-test paper.

b) If the pH value in **a)** is pH 5 or more, transfer the extract to a 1.5-mL ground-in stopper centrifugal precipitate tube (4) and conduct the procedure in **f)** to prepare sample solution.

c) If the pH value in **a)** is pH 4 or less, transfer 40 mL of the extract to a 100-mL beaker.

d) Add sodium hydroxide solution (5 mg/mL), adjust it to pH 5 to pH 7 with a pH meter and transfer to a 50-mL volumetric flask with water.

e) Add water to the marked line and transfer to a 1.5-mL ground-in stopper centrifugal precipitate tube (4).

f) Centrifuge at 8,000 × *g* centrifugal force for about five minutes (5) to make supernatant as the sample solution.

Note (4) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(5) 7-cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 3 Instead of procedures in **(4.2) b)** and **e) - f)**, it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(4.3) Measurement: Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of High Performance Liquid Chromatograph (HPLC) used in measurement.

a) Measurement conditions for High Performance Liquid Chromatograph (HPLC): Set up the measurement conditions for High Performance Liquid Chromatograph (HPLC) considering the following example:

1) **Column:** A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which amino group chemically bonds or a vinyl alcohol polymer column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which amino group chemically bonds.
2) **Temperature of Column bath:** 30 ºC - 40 ºC
3) **Eluent (1)**: Dissolve 1.79 g of disodium hydrogenphosphate dodecahydrate, 0.78 g of sodium dihydrogenphosphate dihydrate and 14.04 g of sodium perchlorate monohydrate in water to make 1,000 mL. Filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE.
4) **Flow rate:** 0.9 mL/min
5) **Injection rate:** 10 µL
6) **Detector:** An absorptiometric detector, measurement wavelength: 210 nm

b) Calibration curve preparation

1) Inject 10 µL of respective standard solutions for the calibration curve preparation to HPLC, record chromatogram at wavelength 210 nm and obtain the peak area.
2) Prepare a curve for the relationship between the concentration and the peak area at wavelength 210 nm of the standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1)**.
2) Obtain the nitrous acid content by the peak area using the calibration curve to calculate the concentration of nitrous acid in the analytical sample.

Comment 4 This test method enables the simultaneous measurement of nitrous acid and ammonium thiocyanate. In this case, mix a predetermined amount of nitrous acid standard solution (1 mg/mL) and ammonium thiocyanate standard solution (1 mg/mL), dilute with water to prepare mixture standard solution (100 µg/mL) (1) and use it instead of **(2) h)** nitrous acid standard solution (100 µg/mL). After that, conduct the same procedure in **(4.3) b)** to calculate the respective concentrations of materials subjected to measurement in the analytical sample.

(5) Flow sheet for testing method: The flow sheet for nitrous acid in fertilizers is shown below:

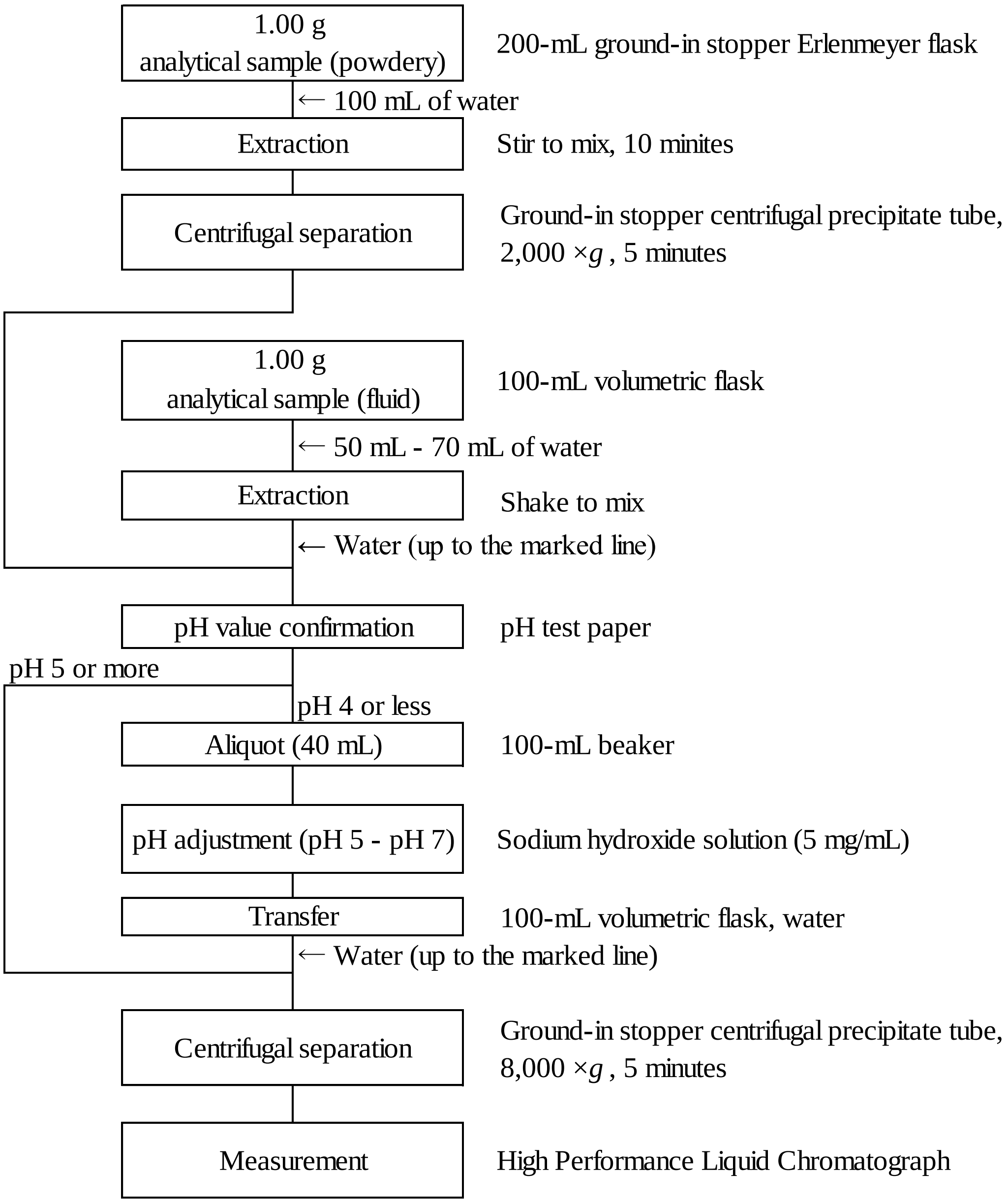


Figure Flow sheet for nitrous acid in fertilizers

6. Testing relating to the other limitations

6.1 Dicyandiamide nitrogen

6.1.a High Performance Liquid Chromatography

(1) Summary

The test method is applicable to nitrolime and fertilizers containing nitrolime.
Add methanol to an analytical sample to extract dicyandiamide (Dd), introduce it to a High Performance Liquid Chromatograph (HPLC), isolate with an amino propyl silica gel column and measure at wavelength 215 nm to calculate dicyandiamide nitrogen (Dd-N).

(2) Reagents: Reagents are as shown below:

a) Methanol: A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.

b) Methanol: Methanol used in eluent of HPLC is HPLC analysis grade or a reagent of equivalent quality.

c) Acetonitrile: A reagent of HPLC grade or equivalents.

d) Dicyandiamide standard solution (1 mg/mL) (1)**:** Put 0.1 g of dicyandiamide [$C_2H_4N_4$] (2) to a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of methanol to dissolve, transfer to a 100-mL volumetric flask and add the solvent up to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.

e) Dicyandiamide standard solution (100 µg/ mL): Put 10 mL of dicyandiamide standard solution (1 mg/ mL) to a 100-mL volumetric flask and add methanol up to the marked line.

f) Dicyandiamide standard solution (10 µg/ mL - 50 µg/ mL) for the calibration curve preparation: At the time of usage, put 5 mL - 25 mL of dicyandiamide standard solution (100 µg/ mL) to 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

g) Dicyandiamide standard solution (1 µg/mL - 10 µg/mL) for the calibration curve preparation: At the time of usage, put 2.5 mL - 25 mL of dicyandiamide standard solution (20 µg/mL) for the calibration curve preparation to 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 98 % (mass fraction) in purity as dicyanamide is commercially sold.

Comment 1 Dicyandiamide is commercially sold as dicyanodiamide by Wako Pure Chemical Industries, Ltd. and Kanto Chemical Co., Inc.

(3) Instruments: Instruments are as shown below:

a) High Performance Liquid Chromatograph: HPLC specified in JIS K 0124 that satisfies the following requirements.

1) Column: A column of 4 mm - 6 mm inner diameter and 150 mm - 250 mm long stainless steel column tube filled with silica gel, to which amino or amino propyl chemically bonds.

2) Column bath: A column bath whose temperature can be adjusted to at 30 ºC - 45 ºC.

3) Detector: An absorptiometric detector that can measure at wavelength around 215 nm.

b) Shaking apparatus

c) Centrifugal separator: A centrifugal separator that can centrifuge at 8,000×*g*.

Comment 2 A column is sold under production names such as Hibar LiChrosorb NH_2, Inertsil NH_2, Unison UK-Amino, Mightysil NH_2, Shim-pack CLC-NH_2, Shodex NH-5A, Unisil Q NH_2, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it in a 200-mL - 300-mL ground-in stopper Erlenmeyer flask.

b) Immediately add 100 mL of methanol (3) and shake to mix by using a shaking apparatus for about 10 minutes.

c) After allowing to stand still, transfer supernatant solution to a 1.5 mL of ground-in stopper centrifugal precipitate tube (4).

d) Centrifuge by 8,000 × *g* for about five minutes (5)

e) 1 mL of the supernatant is used as the sample solution.

Note (3) Add methanol immediately as the determined value becomes higher than usual if it is left in air.

(4) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(5) 7-cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 3 Instead of the procedures in **(4.1) c) - e)**, it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of PTFE and the filtrate can be the sample solution.

(4.2) Measurement: Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of High Performance Liquid Chromatograph (HPLC) used in measurement.

a) Measurement conditions for High Performance Liquid Chromatograph (HPLC): An example of measurement conditions for High Performance Liquid Chromatograph (HPLC) is shown below. Set up the measurement conditions considering it:

1) **Column:** A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which amino or amino propyl chemically bonds.
2) **Temperature of Column bath**: 30 ºC - 40 ºC
3) **Eluent**: Acetonitrile - methanol (6+1）
4) **Flow rate**: 1 mL/ min
5) **Detector**: An absorptiometric detector, measurement wavelength: 215 nm

b) Calibration curve preparation

1) Inject 10 µL of respective dicyandiamide standard solutions for the calibration curve preparation to HPLC, record chromatogram at wavelength 215 nm, and obtain the peak area or the height.
2) Prepare a curve for the relationship between the concentration and the peak area or the height at wavelength 215 nm of the dicyandiamide standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1)**.
2) Obtain dicyandiamide (Dd) content from the calibration curve to calculate the concentration of dicyandiamide (Dd) in the analytical sample.

3) Calculate the dicyandiamide nitrogen (Dd - N) by the following formula.

Dicyandiamide nitrogen (Dd - N) (% (mass fraction)) in an analytical sample
$= A \times (MW_1/MW_2)$
$= A \times 0.6664$

A: Dicyandiamide (Dd) (% (mass fraction)) in an analytical sample
MW_1: 4 atomic weight of nitrogen (56.027)
MW_2: Molecular weight of dicyandiamide (84.080)

Comment 4: Recovery testing was conducted using nitrolime (3 samples) and blended fertilizer containing nitrolime (2 samples), as a result, the recovery rate of dicyandiamide at concentration level of 6 and 0.6 % (mass fraction) was 94.9 % - 105.1 % and 95.6 % - 103.5 %, and the repeatability relative standard deviation were 0.7 % - 2.0 % and 0.4 % - 1.7 %, respectively.
In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 1
Additionally, the minimum limit of quantification of this test method is about 0.01 % (mass fraction).

Table 1 Results and analysis results from a collaborative study for the test method validation of dicyandiamide nitrogen

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Nitrolime 1	9	0.0321	0.0010	3.2	0.0012	3.8
Nitrolime 2	10	0.159	0.002	1.3	0.006	3.8
Nitrolime 3	11	0.245	0.002	0.7	0.008	3.3
Blended fertilizers 1	11	0.124	0.001	0.7	0.002	2.0
Blebded fertilizers 2	11	0.410	0.007	1.6	0.008	1.9

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories x number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masakazu SAIKI and Miyuki ASAO: Validation of High Performance Liquid Chromatography for Determination of Dicyandiamide in Nitrolime, Research Report of Fertilizers, Vol.2, 2009. (25 - 31)

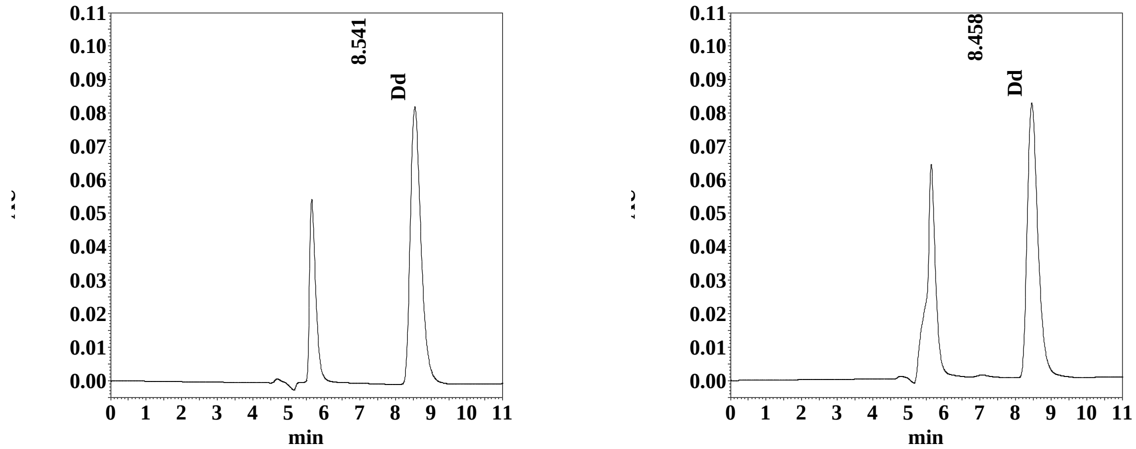
2) Masakazu SAIKI and Masayuki YOSHIMOTO: Determination of Dicyandiamide in Nitrolime by High Performance Liquid Chromatography: A Collaborative Study, Research Report of Fertilizers, Vol.2, 2009. (32 - 37)

(5) Flow sheet for dicyandiamide nitrogen: The flow sheet for dicyandiamide nitrogen in nitrolime and fertilizers containing nitrolime is shown below:

1.00 g analytical sample	200-mL - 300-mL ground-in stopper Erlenmeyer flask
← 100 mL of methanol	
Extraction	Shaking, 30 minutes
Centrifugal separation	Ground-in stopper centrifugal precipitate tube, 8,000 ×*g* , 5 minutes
Measurement	High Performance Liquid Chromatograph

Figure Flow sheet for dicyandiamide nitrogen in nitrolime and fertilizers containing nitrolime

Reference HPLC chromatographs of dicyandiamide standard solution and sample solution (nitrolime) for the calibration curve preparation are shown below.



1) Standard solution 2) Sample solution

Reference diagram: HPLC chromatogram of dicyandiamide

1) Dicyandiamide standard solution (the equivalents of 100 ng of dicyandiamide (10 µg/ mL, 10 µL))
2) Sample solution (nitrolime)

The measurement conditions of HPLC

Column: Hibar LiChrosorb NH_2 (4.6-mm inner diameter, 25-cm long, 5-µm particle diameter)

Other conditions are according to the example of the measurement condition of HPLC in **(4.2) a)**.

7. Nitrification inhibitor

7.1 2-amino-4-chloro-6 -methylpyrimidine (AM)

7.1.a High Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers containing 2-amino-4-chloro-6-methylpyrimidine (AM).

Add methanol - water (1+1) to an analytical sample to extract 2-amino-4-chloro-6-methylpyrimidine, introduce it into a High Performance Liquid Chromatograph (HPLC), isolate with an octadecyl silylation silica gel column, and measure at wavelength 295 nm to calculate 2-amino-4-chloro-6-methylpyrimidine.

(2) Reagents, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557 or equivalents.

b) Methanol: A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.

c) Methanol: Methanol used in eluent of HPLC is a regent of HPLC analysis grade or equivalents.

d) 2-amino-4-chloro-6-methylpyrimidine standard solution (1 mg/mL)(1): Put 0.1 g of 2-amino-4-chloro-6-methylpyrimidine [$C_5H_6ClN_3$](2) in a weighing dish and measure the mass to the order of 0.1 mg. Add methanol - water (1+1) to dissolve, transfer to a 100-mL volumetric flask and add the same solvent up to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.

e) 2-amino-4-chloro-6-methylpyrimidine standard solution (100 μg/mL): In the case of usage, transfer 10 mL of 2-amino-4-chloro-6-methylpyrimidine standard solution (1 mg/mL) to a 100-mL volumetric flask and add methanol - water (1+1) up to the marked line.

f) 2-amino-4-chloro-6-methylpyrimidine standard solution (10 μg/mL - 50 μg/mL) for the calibration curve preparation: In the case of usage, transfer 5 mL - 25 mL of 2-amino-4-chloro-6-methylpyrimidine standard solution (100 μg/mL) to 50-mL volumetric flasks step-by-step and add methanol - water (1+1) up to the marked line.

g) 2-amino-4-chloro-6-methylpyrimidine standard solution （1 μg/mL - 10 μg/mL）for the calibration curve preparation: In the case of usage, transfer 2.5 mL - 25 mL of 2-amino-4-chloro-6-methylpyrimidine standard solution (20 μg/mL) for the calibration curve preparation to 50-mL volumetric flasks step-by-step and add methanol - water (1+1) up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 98 % (mass fraction) in purity is commercially sold as 2-amino-4-chloro-6-methylpyrimidine.

Comment 1: 2-amino-4-chloro-6-methylpyrimidine is commercially sold by Wako Pure Chemical Industries, Ltd. and Kanto Chemical Co., Inc.

(3) Instruments: Instruments are as shown below:

a) High Performance Liquid Chromatograph: HPLC specified in JIS K 0124 that satisfies the following requirements.

1) Column: A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which octadecyl chemically bonds.

2) Column bath: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.

3) **Detector:** An absorptiometric detector that can measure at wavelength around 295 nm.

b) **Magnetic stirrer**

c) **Centrifugal separator:** A centrifugal separator that can centrifuge at 2,000 × *g*.

d) **High speed centrifugal separator:** A centrifugal separator that can centrifuge at 8,000×*g*.

e) **Acidic alumina cartridge column:** Link a 10-mL cylinder to a column [(3)] that is filled with 500 mg - 1 g of acidic alumina, put 3 mL of methanol and let it flow down.

Note (3) A cartridge with a 3-mL - 6-mL column filled with 500-mg - 1-g of silica gel can be used.

Comment 2 A column is sold under production names such as Inertsil ODS, Mightysil RP-18, L-column ODS, Shim-pack VP-ODS, Silica C18M 4D, Puresil C_{18}, COSMOSIL 5C18-MS-II, etc.

Comment 3 An acidic alumina cartridge is commercially sold under production names such as Bond Elut AL-A, Sep-Pak Alumina-A, Supelclean LC-Alumina-A.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of methanol - water (1+1) and stir it by using a magnetic stirrer for about 30 minutes.

c) After allowing to stand still, transfer supernatant solution to a 50-mL ground-in stopper centrifugal precipitate tube.

d) Centrifuge it at 2,000 × *g* centrifugal force for about five minutes [(4)] and use the supernatant as the extract [(5)].

Note (4) 18.7-cm of rotor radius and 3,000 rpm of revolutions makes about 2,000 × *g* centrifugal force.

(5) If there is a possibility that the 2-amino-4-chloro-6-methylpyrimidine in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of extract with methanol water (1+1).

(4.2) Cleanup: Conduct cleanup as shown below:

a) Transfer the extract to an acidic alumina cartridge column.

b) Dispose of about the first 3 mL of effluent and then transfer about the next 2 mL to a test tube.

c) Transfer the effluent to a 1.5-mL ground-in stopper centrifugal precipitate tube [(6)].

d) Centrifuge at 8,000×*g* centrifugal force for about five minutes [(7)] and use the supernatant as the sample solution.

Note (6) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(7) 7-cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 4 Instead of the procedures in **(4.2) c) - e)**, it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of PTFE and the filtrate can be the sample solution.

Comment 5 The test is possible by the following procedures in the case of fertilizers not containing organic matters.
The procedures in **(4.1) c) - d)** and **(4.2) a) - b)** are omitted and "Transfer effluent" in **(4.2) c)** is replaced with the "After allowing to stand still, transfer supernatant" to operate.

(4.3) Measurement: Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of High Performance Liquid Chromatograph (HPLC) used in measurement.

a) Measurement conditions for High Performance Liquid Chromatograph (HPLC): Set up the measurement conditions for High Performance Liquid Chromatograph (HPLC) considering the following example:

1) Column: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which octadecyl chemically bonds.

2) Temperature of column bath: 30 ºC - 40 ºC

3) Eluent: Methanol - water (4+6)

4) Flow rate: 1 mL/min

5) Detector: An absorptiometric detector, measurement wavelength: 295 nm

b) Calibration curve preparation

1) Inject 10 µL of respective 2-amino-4-chloro-6-methylpyrimidine standard solutions for the calibration curve preparation to HPLC, record chromatogram at wavelength 295 nm and obtain the peak area or height.

2) Prepare a curve for the relationship between the concentration and the peak area or height at wavelength 295 nm of the 2-amino-4-chloro-6-methylpyrimidine standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1)**.

2) Obtain 2-amino-4-chloro-6-methylpyrimidine content from the calibration curve to calculate the concentration of 2-amino-4-chloro-6-methylpyrimidine in the analytical sample.

Comment 6 Recovery testing was conducted using compound fertilizer (1 sample) and blended fertilizer (2 samples), as a result, the mean recovery rate of 2-amino-4-chloro-6-methylpyrimidine at concentration level of 1.0 %, 0.4 % and 0.1 % (mass fraction) was 99.1 % - 100.5 %, 99.3 % - 101.6 % and 100.2 % - 100.7 %, and repeatability relative standard deviation were 0.4 % - 1.8 %, 1.2 % - 2.5 % and 0.8 % - 3.0 %, respectively. Additionally, the minimum limit of quantification of this test method is about 0.005 % (mass fraction).

Reference

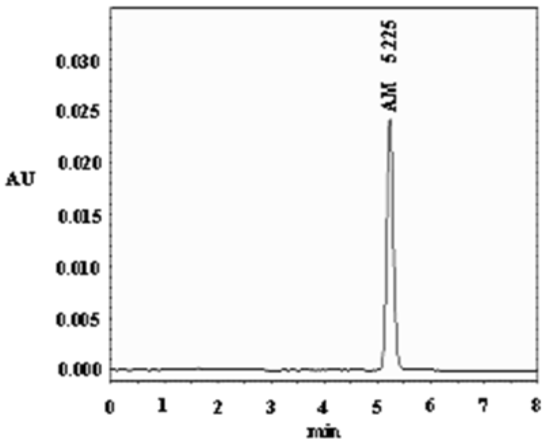
1) Yuji SHIRAI: The volumetric analysis of 2-amino-4-chloro-6-methylpyrimidine in fertilizers with High Performance Liquid Chromatograph, Validation Report of Fertilizers (in Japanese), 44 (3), 26 - 41(1991)

(5) Flow sheet for 2-amino-4-chloro-6-methylpyrimidine: The flow sheet for 2-amino-4-chloro-6-methylpyrimidine in fertilizers is shown below:

1.00 g analytical sample	200-mL of ground-in stopper Erlenmeyer flask
← 100 mL of methanol-water (1+1)	
Extraction	Shaking, 30 minutes
Centrifugal separation	Ground-in stopper centrifugal precipitate tube, 2,000 × *g* , 5 minutes
Cleanup	Acidic alumina cartridge column
Centrifugal separation	Ground-in stopper centrifugal precipitate tube, 8,000 × *g* , 5 minutes
Measurement	High Performance Liquid Chromatograph

Figure Flow sheet for 2-amino-4-chloro-6-methylprimidine in fertilizers.

Reference: HPLC chromatogram of 2-amino-4-chloro-6-methylpyrimidine standard solution for the calibration curve preparation is shown below.



Reference diagram: HPLC chromatogram of 2-amino-4-chloro-6-methylpyrimidine standard solution

Measurement conditions for HPLC
Column: Mightysil RP-18 GP
(4.6-mm inner diameter, 150-mm long, 5-µm particle diameter)
2-amino-4-chloro-6-methylpyrimidine standard solution (the equivalents of 100 ng)
Other conditions are according to the examples of the measurement conditions of **(4.3) a)** HPLC.

7.2 1-amidino-2-thiourea (ASU)

7.2.a High Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers containing 1-amidino-2-thiourea (ASU). Add water to an analytical sample to extract 1-amidino-2-thiourea, introduce it into a High Performance Liquid Chromatograph (HPLC), isolate with an octadecyl silylation silica gel column, and measure at wavelength 262 nm to calculate 1-amidino-2-thiourea.

(2) Reagents, etc.: Reagents and water are as shown below.

a) **Water:** Water of A3 specified in JIS K 0557 or equivalents.

b) **Methanol:** Methanol used in eluent of HPLC is a regent of HPLC analysis grade or equivalents.

c) **1-sodium hexasulfonate:** A reagent of ion pair chromatography analysis grade or equivalents.

d) **Acetic acid:** A reagent of HPLC analysis grade or equivalents.

e) **1-amidino-2-thiourea standard solution (1 mg/mL)(1):** Put 0.1 g of 1-amidino-2-thiourea [$C_2H_6N_4S$](2) in a weighing dish and measure the mass to the order of 0.1 mg. Add water to dissolve, transfer to a 100-mL volumetric flask and add water up to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.

f) **1-amidino-2-thiourea standard solution (100 µg/mL):** In the case of usage, transfer 10 mL of 1-amidino-2-thiourea standard solution (1 mg/mL) to a 100-mL volumetric flask and add water up to the marked line.

g) **1-amidino-2-thiourea standard solution (10 µg/mL- 50 µg/mL) for the calibration curve preparation:** In the case of usage, transfer 5 mL - 25 mL of 1-amidino-2-thiourea standard solution (100 µg/mL) to 50-mL volumetric flasks step-by-step and add water up to the marked line.

h) **1-amidino-2-thiourea standard solution (1 µg/mL- 10 µg/mL) for the calibration curve preparation:** In the case of usage, transfer 2.5 mL - 25 mL of 1-amidino-2-thiourea standard solution (20 µg/mL) for the calibration curve preparation to 50-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 98 % (mass fraction) in purity is commercially sold as 1-amidino-2-thiourea.

Comment 1 1-amidino-2-thiourea is sold under the name of guanylthiourea by Tokyo Chemical Industry Co., Ltd, and under the name of amidino thiourea by Kanto Chemical Co., Inc.

(3) Instruments: Instruments are as shown below.

a) **High Performance Liquid Chromatograph (HPLC):** HPLC specified in JIS K 0124 that satisfies the following requirements.

1) **Column:** A column of 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which octadecyl chemically bonds.

2) **Column bath:** A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.

3) **Detector:** An absorptiometric detector that can measure at wavelength around 262 nm.

b) **Magnetic stirrer**

c) High speed centrifugal separator: A centrifugal separator that can centrifuge at 8,000 × *g*.

Comment 2: A column is sold under the production names such as Inertsil ODS, Mightysil RP-18, L-column ODS, Shim-pack VP-ODS, Silica C18M 4D, Puresil C_{18}, COSMOSIL 5C18-MS-II.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and stir it with a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, transfer supernatant (3) solution to a 1.5-mL ground-in stopper centrifugal precipitate tube (4).

d) Centrifuge at 8,000 × *g* centrifugal force for about five minutes (5) to make supernatant as the sample solution.

Note **(3)** If there is a possibility that the 1-amidino-2-thiourea concentration in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of supernatant with water.

(4) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(5) 7-cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 3 Instead of procedures in **(4.1) c) - d)**, it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(4.2) Measurement: Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of High Performance Liquid Chromatograph (HPLC) used in measurement.

a) Measurement conditions for High Performance Liquid Chromatograph (HPLC): Set up the measurement conditions for High Performance Liquid Chromatograph (HPLC) considering the following example:

1) **Column:** A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which octadecyl chemically bonds.
2) **Temperature of Column bath:** 30 ºC - 45 ºC
3) **Eluent**: Dissolve 0.94 g of sodium 1-hexasulfonic acid in 1,000 mL of methanol water (2+8), adjust to pH 3.15 with acetic acid and filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE (1).
4) **Flow rate:** 1 mL/min
5) **Detector:** An absorptiometric detector, measurement wavelength: 262 nm

b) Calibration curve preparation

1) Inject 10 µL of respective 1-amidino-2-thiourea standard solutions for the calibration curve preparation to HPLC, record chromatogram at wavelength 262 nm and obtain the peak area or height.
2) Prepare a curve for the relationship between the concentration and the peak area or height at wavelength 262 nm of the 1-amidino-2-thiourea standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1)**.
2) Obtain the 1-amidino-2-thiourea content from the calibration curve to calculate the concentration of 1-amidino-2-thiourea in the analytical sample.

Comment 4 Recovery testing with triplicates measurement was conducted using compound fertilizer (2 samples), as a result, the mean recovery rate of 1-amidino-2-thiourea at concentration level of 1.0 %, 0.5 % and 0.25 % (mass fraction) were 99.0 % - 104.3 %, 97.7 % - 100.7 % and 99.7 % - 101.3 %, and repeatability relative standard deviation were 1.0 % - 3.9 %, 1.5 % - 2.6 % and 8.2 % - 8.4 %, respectively.

In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 1.

Additionally, the minimum limit of quantification of this test method is about 0.005 % (mass fraction)

Table 1 Results and analysis results from a collaborative study for the test method validation of 1-amidino-2-thiourea (ASU)

Sample name	Number of laboratories[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	s_R[6] (%)[3]	RSD_R[7] (%)
Compound fertilizer 1	10	0.093	0.009	9.1	0.010	11.2
Compound fertilizer 2	10	0.246	0.021	8.6	0.021	8.6
Compound fertilizer 3	10	0.511	0.018	3.6	0.025	4.9
Compound fertilizer 4	10	0.759	0.039	5.1	0.040	5.3
Compound fertilizer 5	10	1.020	0.039	3.8	0.044	4.3

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Reference

1) Kazunori CHIBA: Analysis method of 1-amidino-2-thiourea (ASU) of Nitrification inhibitor in fertilizers with High Performance Liquid Chromatograph, Validation Report of Fertilizers (in Japanese), **43 (4)**, 15 - 22 (1990)
2) Shigehiro KAI and Erina WATABE: Determination of 1-Amidino-2-thiourea as a Nitrification Inhibitor in Compound Fertilizer by High Performance Liquid Chromatography: A Collaborative Study, Research Report of Fertilizers, Vol.6, 2013. (36 - 42)

(5) Flow sheet for 1-amidino-2-thiourea: The flow sheet for 1-amidino-2-thiourea in fertilizers is shown below:

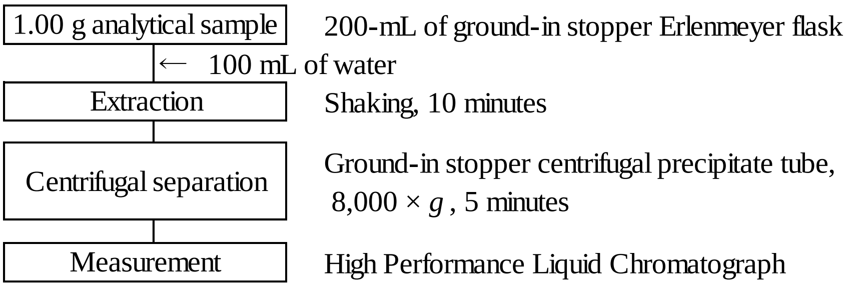
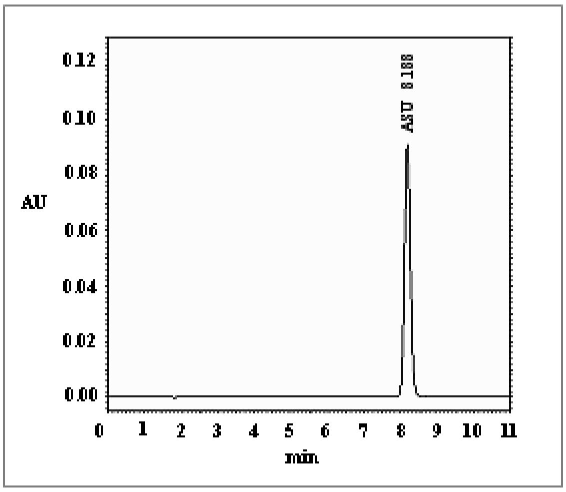


Figure Flow sheet for 1-amidino-2-thiourea in fertilizers.

Reference HPLC chromatogram of 1-amidino-2-thiourea standard solution for the calibration curve preparation is shown below.



Reference diagram: HPLC chromatogram of 1-amidino-2-thiourea standard solution

Measurement conditions for HPLC

Column: Mightysil RP-18 GP (4.6-mm inner diameter, 150-mm long, 5-µm particle diameter)

1-amidino-2-thiourea standard solution (the equivalents of 200 ng)

Other conditions are shown according to the examples of the measurement conditions of **(4.2) a)** HPLC.

7.3 4-amino-1,2,4-triazole hydrochloride (ATC)

7.3.a High Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers containing 4-amino-1,2,4-triazole hydrochloride (ATC) but not containing organic matters.

Add methanol to an analytical sample to extract 4-amino-1,2,4-triazole hydrochloride, introduce it into a High Performance Liquid Chromatograph (HPLC), isolate with an aminopropyl silica gel column, and measure at wavelength 220 nm to calculate 4-amino-1,2,4-triazole hydrochloride.

(2) Reagents: Reagents are as shown below.

a) **Methanol:** A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.

b) **Methanol:** Methanol used in eluent of HPLC is a regent of HPLC analysis grade or equivalents.

c) **Acetonitrile**: Acetonitrile used in eluent of HPLC is a regent of HPLC analysis grade or equivalents.

d) **4-amino-1,2,4-triazole standard solution (1 mg/mL)(1)(2):** Transfer 0.1 g of 4-amino-1,2,4-triazole [$C_2H_4N_4$](3) to a weighing dish and measure the mass to the order of 0.1 mg. Add methanol to dissolve, transfer to a 100-mL amber volumetric flask and add methanol to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.

e) **4-amino-1,2,4-triazole standard solution (100 µg/mL):**In the case of usage, transfer 10 mL of 4-amino-1,2,4-triazole standard solution (1 mg/mL) to a 100-mL volumetric flask and add methanol up to the marked line.

f) **4-amino-1,2,4-triazole standard solution (10 µg/mL - 50 µg/mL) for the calibration curve preparation:** In the case of usage, transfer 5 mL - 25 mL of 4-amino-1,2,4-triazole standard solution (100 µg/mL) to 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

g) **4-amino-1,2,4-triazole standard solution (1 µg/mL - 10 µg/mL) for the calibration curve preparation:** In the case of usage, transfer 2.5 mL - 25 mL of 4-amino-1,2,4-triazole standard solution for the calibration curve preparation (20 µg/mL) to 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

Note **(1)** The solution contains 1.434 mg/mL as 4-amino-1,2,4-triazole hydrochloride.

(2) This is an example of preparation; prepare an amount as appropriate.

(3) A reagent of no less than 98 % (mass fraction) in purity is commercially sold as 4-amino-1,2,4-triazole.

Comment 1 4-amino-1,2,4-triazole is sold under production names such as 4-amino-1,2,4-triazole by Wako Pure Chemical Industries., Ltd. and Tokyo Chemical Industry Co., Ltd, and 4-amino-*4H*-1,2,4-triazole by Kanto Chemical Co., Inc.

(3) Instruments: Instruments are as shown below:

a) **High Performance Liquid Chromatograph (HPLC)**: HPLC specified in JIS K 0124 that satisfies the next requirements.

1) **Column:** A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which amino or aminopropyl chemically bonds.

2) **Column bath:** A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
3) **Detector:** An absorptiometric detector that can measure at wavelength around 220 nm.

b) **Magnetic stirrer**

c) **High speed centrifugal separator**: A centrifugal separator that can centrifuge at 8,000 × *g*.

Comment 2 A column is commercially sold under the names such as Hibar LiChrosorb NH_2, Inertsil NH_2, Unison UK-Amino, Mightysil NH_2, Shim-pack CLC-NH_2, Shodex NH-5A, Unisil Q NH_2.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it to a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of methanol and stir it with using a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, transfer the supernatant solution to a 1.5-mL ground-in stopper centrifugal precipitate tube (4)

d) Centrifuge by 8,000 × *g* centrifugal force for about five minutes (5) to make the supernatant as the sample solution.

Notes (4) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(5) 7 cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 3 Instead of procedures in **(4.1) c) - d)**, it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of PTFE and the filtrate can be the sample solution.

(4.2) Measurement: Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of High Performance Liquid Chromatograph (HPLC) used in measurement.

a) **Measurement conditions for High Performance Liquid Chromatograph (HPLC):** Set up the measurement conditions for High Performance Liquid Chromatograph (HPLC) considering the following example:

1) **Column:** A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which amino or aminopropyl chemically bonds.
2) **Temperature of Column bath:** 30 ºC - 40 ºC
3) **Eluent:** Acetonitrile-methanol (9+1)
4) **Flow rate:** 1 mL/min
5) **Detector:** An absorptiometric detector, measurement wavelength: 220 nm

b) **Calibration curve preparation**

1) Inject 10 µL of respective 4-amino-1,2,4-triazole standard solutions for the calibration curve preparation to HPLC, record chromatogram at wavelength 220 nm and obtain the peak area or height.
2) Prepare a curve for the relationship between the concentration and the peak area or height at wavelength 220 nm of the 4-amino-1,2,4-triazole standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1)**.

2) Obtain the amount of 4-amino-1,2,4-triazole from the calibration curve to calculate the concentration of 4-amino-1,2,4-triazole in the analytical sample.

3) Calculate the 4-amino-1,2,4-triazole hydrochloride by the following formula.

4-amino-1,2,4-triazole hydrochloride in the analytical sample (% (mass fraction))
$= A \times 1.434$

A: 4-amino-1,2,4-triazole in the analytical sample (% (mass fraction))

Comment 4 Recovery testing was conducted using compound fertilizer (2 samples), as a result, the mean recovery rate of 4-amino-1,2,4-triazole hydrochloride at concentration level of 0.5 %, 0.3 % and 0.2 % (mass fraction) were 100.2 % - 104.9 %, 100.8 % - 103.0 % and 100.7 % - 104.2 %, and repeatability relative standard deviation were 0.8 % - 5.1 %, 3.3 % - 7.4 % and 0.9 % - 8.4 %, respectively. Additionally, the minimum limit of quantification of this test method is about 0.005 % (mass fraction)

Reference

1) Koichi SAKAGAMI: Analysis methods of 4-amino-1,2,4-triazole hydrochloride with High Performance Liquid Chromatography, Validation Report of Fertilizers (in Japanese), 40 (4), 9 - 16 (1987)

(5) Flow sheet for 4-amino-1,2,4-triazole hydrochloride: The flow sheet for 4-amino-1,2,4-triazole hydrochloride in fertilizers is shown below:

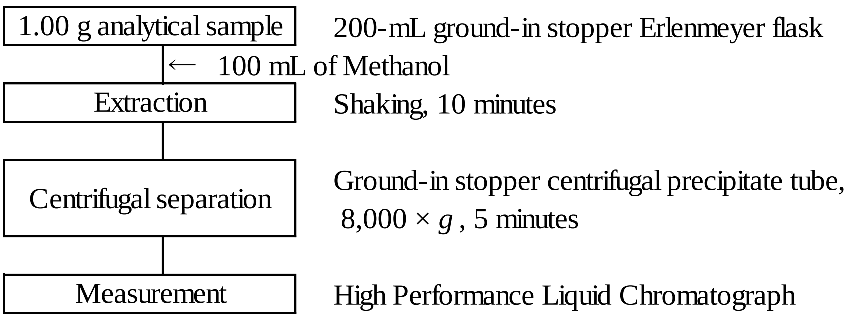


Figure Flow sheet for 4-amino-1,2,4-triazole hydrochloride in fertilizers.

7.4 N-2,5-dichlorophenyl succinamic acid (DCS)

7.4.a High Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers containing N-2,5-dichlorophenyl succinamic acid (DCS) but not containing organic matters.
Add methanol - phosphate (996+4) and water to an analytical sample to extract N-2,5-dichlorophenyl succinamic acid (DCS), introduce it into a High Performance Liquid Chromatograph (HPLC), isolate with octadecyl silylation silica gel column, and measure at wavelength 246 nm to calculate N-2,5-dichlorophenyl succinamic acid (DCS).

(2) Reagents, etc.: Reagents and water are as shown below.

- **a) Water:** Water of A3 specified in JIS K 0557 or water with equivalent quality.
- **b) Methanol:** A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.
- **c) Methanol:** Methanol used in eluent of HPLC is a regent of HPLC analysis grade or equivalents.
- **d) N-2,5-dichlorophenyl succinamic acid standard solution(1 mg/mL)** (1) **:** Transfer 0.1 g of N-2,5-dichlorophenyl succinamic acid [$C_{10}H_9Cl_2NO_3$] to a weighing dish and measure the mass to the order of 0.1 mg. Add methanol to dissolve, transfer to a 100-mL volumetric flask and add methanol to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.
- **e) N-2,5-dichlorophenyl succinamic acid standard solution(100 µg/mL):** In the case of usage, transfer 10 mL of N-2,5-dichlorophenyl succinamic acid standard solution (1 mg/mL) to a 100-mL volumetric flask and add methanol up to the marked line.
- **f) N-2,5-dichlorophenyl succinamic acid standard solution (10 µg/mL - 50 µg/mL) for the calibration curve preparation:** In the case of usage, transfer 5 mL - 25 mL of N-2,5-dichlorophenyl succinamic acid standard solution (100 µg/mL) to 50-mL volumetric flasks step-by-step and add methanol up to the marked line.
- **g) N-2,5-dichlorophenyl succinamic acid standard solution (1 µg/mL - 10 µg/mL) for the calibration curve preparation:** In the case of usage, transfer 2.5 mL - 25 mL of N-2,5-dichlorophenyl succinamic acid standard solution for the calibration curve preparation (20 µg/mL) to 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as shown below:

- **a) High Performance Liquid Chromatograph (HPLC):** HPLC specified in JIS K 0124 that satisfies the following requirements.
  - **1) Column:** A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which octadecyl chemically bonds.
  - **2) Column bath:** A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
  - **3) Detector:** An absorptiometric detector that can measure at wavelength around 246 nm.
- **b) Magnetic stirrer**
- **c) High speed centrifugal separator:** A centrifugal separator that can centrifuge at 8,000 × *g*.

Comment 1 A column is sold under the production names such as Inertsil ODS, Mightysil RP-18, L-column ODS, Shim-pack VP-ODS, Silica C18M 4D, Puresil C_{18}, COSMOSIL 5C18-MS-II.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of methanol - phosphate (996+4) and stir it with a magnetic stirrer for about 30 minutes.

c) After allowing to stand still, transfer supernatant solution [(2)] to 1.5-mL ground-in stopper centrifugal precipitate tube [(3)].

d) Centrifuge by 8,000 × *g* centrifugal force for about five minutes [(4)] to make supernatant as the sample solution.

Note **(2)** If there is a possibility that the N-2,5-dichlorophenyl succinamic acid concentration in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of the outflow solution with methanol.

(3) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(4) 7-cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 2 Instead of procedures in **(4.1) c) - d)**, it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(4.2) Measurement: Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of High Performance Liquid Chromatograph (HPLC) used in measurement.

a) Measurement conditions for High Performance Liquid Chromatograph (HPLC): Set up the measurement conditions for High Performance Liquid Chromatograph (HPLC) considering the following example:

1) **Column:** A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which octadecyl chemically bonds.
2) **Temperature of Column bath:** 30 ºC - 40 ºC
3) **Eluent:** Methanol - water [(5)] (55 + 45)
4) **Flow rate:** 0.8 mL/min
5) **Detector:** Absorptiometric detector, measurement wavelength: 246 nm

Note **(5)** Adjust the water used to pH 3 with phosphate in advance.

b) Calibration curve preparation

1) Inject 10 µL of respective N-2,5-dichlorophenyl succinamic acid standard solutions for the calibration curve preparation to HPLC, record chromatogram at wavelength 246 nm and obtain the peak area or height.
2) Prepare a curve for the relationship between the concentration and the peak area or height at wavelength 246 nm of the N-2,5-dichlorophenyl succinamic acid standard solution for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1)**.
2) Obtain the N-2,5-dichlorophenyl succinamic acid content from the calibration curve to calculate the concentration of N-2,5-dichlorophenyl succinamic acid in the analytical sample.

Comment 3 Recovery testing was conducted using compound fertilizer (2 samples) and blended fertilizer (1 sample), as a result, the mean recovery rate of N-2,5-dichlorophenyl succinamic acid at concentration level of 0.4 %, 0.2 % and 0.1 % (mass fraction) were 100.9 % - 101.4 %, 100.8 % - 101.4 % and 101.2 % - 103.4 %, and repeatability relative standard deviation were 0.5 % - 1.0 %, 1.1 % - 2.0 % and 0.8 % - 1.1 %, respectively. Additionally, the minimum limit of quantification of this test method is about 0.005 % (mass fraction)

Reference

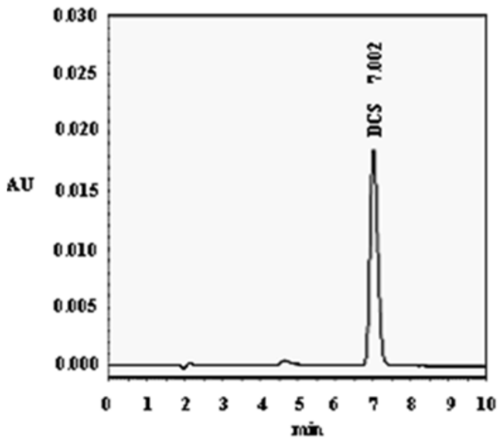
1) Akira KUBO: Analysis methods of N-2,5-dichlorophenyl succinamic acid (DCS) of Nitrification suppression materials in fertilizers with High Performance Liquid Chromatograph, Validation Report of Fertilizers (in Japanese), **44 (4)**, 25 - 36 (1991).

(5) Flow sheet for N-2,5-dichlorophenyl succinamic acid: The flow sheet for N-2,5-dichlorophenyl succinamic acid in fertilizers is shown below:

1.00 g analytical sample	200-mL ground-in stopper Erlenmeyer flask
← 100 mL of methanol phosphate (996+4)	
Extraction	Shaking, 30 minutes
Centrifugal separation	Ground-in stopper centrifugal precipitate tube, 8,000 × *g* , 5 minutes
Measurement	High Performance Liquid Chromatograph

Figure Flow sheet for N-2,5-dichlorophenyl succinamic acid in fertilizers.

Reference: HPLC chromatogram of N-2,5-dichlorophenyl succinamic acid standard solution for the calibration curve preparation is shown below.



Reference diagram: HPLC chromatogram of N-2,5-dichlorophenyl succinamic acid

Measurement conditions for HPLC

Column: Mightysil RP-18 GP (4.6-mm inner diameter, 150-mm long, 5-μm particle diameter)

N-2,5-dichlorophenyl succinamic acid standard solution (the equivalents of 100 ng)

Other conditions are according to the examples of the measurement conditions of **(4.2) a)** HPLC.

7.5 Dicyandiamide (Dd)

7.5.a High Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers containing dicyandiamide (Dd).

Add water to an analytical sample, leave at rest for a little while and add methanol to extract dicyandiamide. After removing interfering substances with a silica gel cartridge column, introduce it into a High Performance Liquid Chromatograph (HPLC), isolate with an aminopropyl silica gel column, and measure at wavelength 215 nm to calculate dicyandiamide.

(2) Reagents, etc.: Reagents and water are as shown below.

- **a) Water:** Water of A3 specified in JIS K 0557 or water with equivalent quality.
- **b) Methanol:** A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.
- **c) Methanol:** Methanol used in eluent of HPLC is a regent of HPLC analysis grade or equivalents.
- **d) Acetonitrile:** A regent of HPLC analysis grade or equivalents.
- **e) Dicyandiamide standard solution (1 mg/mL)** (1)**:** Transfer 0.1 g of dicyandiamide [$C_2H_4N_4$](2) to a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount methanol to dissolve, transfer to a 100-mL volumetric flask and add the same solvent up to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.
- **f) Dicyandiamide standard solution (100 µg/mL):** In the case of usage, transfer 10 mL of dicyandiamide standard solution (1 mg/mL) to a 100-mL volumetric flask and add methanol up to the marked line.
- **g) Dicyandiamide standard solution (10 µg/mL - 50 µg/mL) for the calibration curve preparation:** In the case of usage, transfer 5 mL - 25 mL of dicyandiamide standard solution (100 µg/mL) to 50-mL volumetric flasks step-by-step and add methanol up to the marked line.
- **h) Dicyandiamide standard solution (1 µg/mL - 10 µg/mL) for the calibration curve preparation:** In the case of usage, transfer 2.5 mL - 25 mL of dicyandiamide standard solution for the calibration curve preparation (20 µg/mL) to 50-mL volumetric flasks step-by-step and add methanol up to the marked line.
- **i) Sodium sulfate:** A JIS Guaranteed Reagent specified in JIS K 8987 or a reagent of equivalent quality.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 98 % in purity is commercially sold as dicyandiamide.

Comment 1 Dicyandiamide is commercially sold as Dicyanodiamide by Wako Pure Chemical Industries., Ltd and Kanto Chemical Co., Inc.

(3) Instruments: Instruments are as shown below.

- **a) High Performance Liquid Chromatograph (HPLC)**: HPLC specified in JIS K 0124 that satisfies the following requirements.
  - **1) Column**: A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which octadecyl chemically bonds.
  - **2) Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
  - **3) Detector:** An absorptiometric detector that can measure at wavelength around 215 nm.

b) Shaking apparatus
c) Centrifugal separator: A centrifugal separator that can centrifuge at 2,000 × *g*.
d) High speed centrifugal separator: A centrifugal separator that can centrifuge at 8,000 × *g*.
e) Silica gel cartridge column: Link a 10-mL cylinder to the column (3) filled with 500 mg - 1 g of silica gel, add 3 mL of methanol to let it flow down.

Note (3) A cartridge with a 3-mL - 6-mL column that is filled with 500 mg - 1 g of silica gel can be used.

Comment 2 A column is commercially sold under production names such as Hibar LiChrosorb NH_2 、Inertsil NH_2, Unison UK-Amino, Mightysil NH_2, Shim-pack CLC-NH_2, Shodex NH-5A, Unisil Q NH_2.
Comment 3 A silica gel cartridge column is commercially sold under production names such as Sep-Pak Plus Silica, InertSep Si.

(4) Test procedures
(4.1) Extraction: Conduct extraction as shown below.
a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.
b) Add 1 mL of water (4) and leave at rest for 5 minutes.
c) Add 100 mL of methanol and shake to mix with a shaking apparatus for about 10 minutes.
d) Add an appropriate amount of sodium sulfate (5).
e) After allowing to stand still, transfer supernatant solution to a 50-mL ground-in stopper centrifugal precipitate tube.
f) Centrifuge at 2,000 × *g* centrifugal force for about five minutes (6) to make the supernatant as the extract (7).

Note (4) Mix well until the whole sample comes in contact with water.
(5) About 5 g - 10 g.
(6) 18.7-cm of rotor radius and 3,000 rpm of revolutions makes about 2,000 × *g* centrifugal force.
(7) If there is a possibility that the concentration of dicyandiamide in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of extract with methanol.

(4.2) Cleanup: Conduct cleanup as shown below
a) Transfer extract to a silica gel cartridge column.
b) Dispose of about the first 3 mL of effluent and then transfer about the next 2 mL to a test tube.
c) Transfer effluent to a 1.5- mL of ground-in stopper centrifugal precipitate tube (8).
d) Centrifuge at 8,000 × *g* centrifugal force for about five minutes (9) to make supernatant as the sample solution.

Note (8) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.
(9) 7-cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 4 Instead of the procedures in **(4.2) c) - d)**, it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of PTFE and the filtrate can be the sample solution.

(**4.3**) **Measurement:** Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of High Performance Liquid Chromatograph (HPLC) used in measurement.

a) Measurement conditions for High Performance Liquid Chromatograph (HPLC): Set up the measurement conditions for High Performance Liquid Chromatograph (HPLC) considering the following example:

1) **Column:** A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which amino or aminopropyl chemically bonds.
2) **Temperature of column bath**: 30 ℃ - 40 ºC
3) **Eluent:** Acetonitrile – methanol (6+1)
4) **Flow rate**: 0.5 mL/min - 1 mL/min
5) **Detector**: An absorptiometric detector, measurement wavelength: 215 nm

b) Calibration curve preparation

1) Inject 10 µL of respective dicyandiamide standard solutions for the calibration curve preparation to HPLC, record chromatogram at wavelength 215 nm and obtain the peak area or height.
2) Prepare a curve for the relationship between the concentration and the peak area or height at wavelength 215 nm of the dicyandiamide standard solution for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1)**.
2) Obtain the dicyandiamide content from the calibration curve to calculate the concentration of dicyandiamide in the analytical sample.

Comment 5: Recovery testing was conducted using inorganic compound fertilizer (2 samples) and organic compound fertilizer (3 samples), as a result, the mean recovery at the concentration level of 2 % and 0.2% (mass fraction) were 101.2 % - 102.6 % and 98.4 % - 100.6 %, and repeatability relative standard deviation were 0.5 % - 1.8 % and 0.2 % - 1.3 %, respectively.
In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 1.
Additionally, the minimum limit of quantification of this test method is about 0.01 % (mass fraction).

Table 1 Results and analysis results from a collaborative study
for the test method validation of dicyandiamide

Sample name	Number of laboratories[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	s_R[6] (%)[3]	RSD_R[7] (%)
Compound fertilizer 1	11	0.263	0.009	3.2	0.019	7.4
Compound fertilizer 2	11	2.04	0.04	1.7	0.07	3.2
Compound fertilizer 3	13	0.548	0.011	2.0	0.033	6.0
Compound fertilizer 4	12	0.423	0.013	3.2	0.022	5.2
Compound fertilizer 5	12	1.02	0.01	1.4	0.04	4.3

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masakazu SAIKI: Development of High Performance Liquid Chromatography for Determination of Dicyandiamide as a Nitrification Inhibitor in Fertilizer. Research Report of Fertilizer, Vol.3, 2010 (43 - 50).
2) Masakazu SAIKI: Determination of Dicyandiamide as a Nitrification Inhibitor in Fertilizer by High Performance Liquid Chromatography: Collaborative Study, Research Report of Fertilizer, Vol.4, 2011 (16 - 22).

(5) **Flow sheet for dicyandiamide:** The flow sheet for dicyandiamide in fertilizers is shown

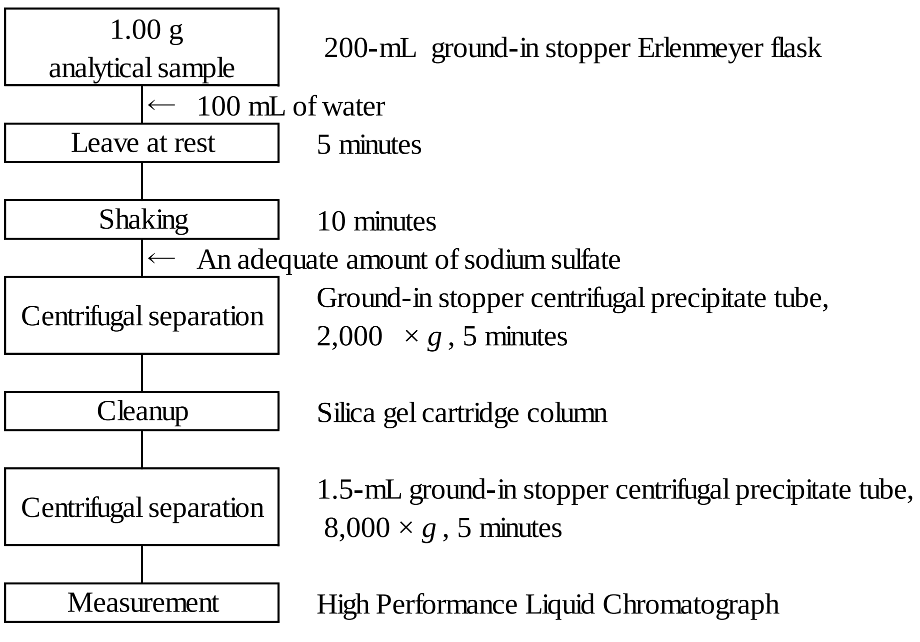
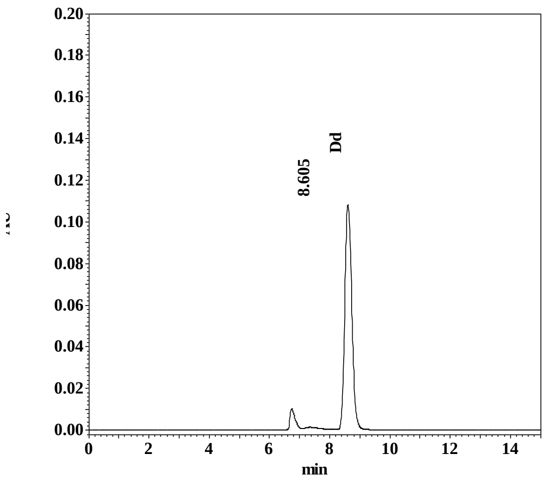
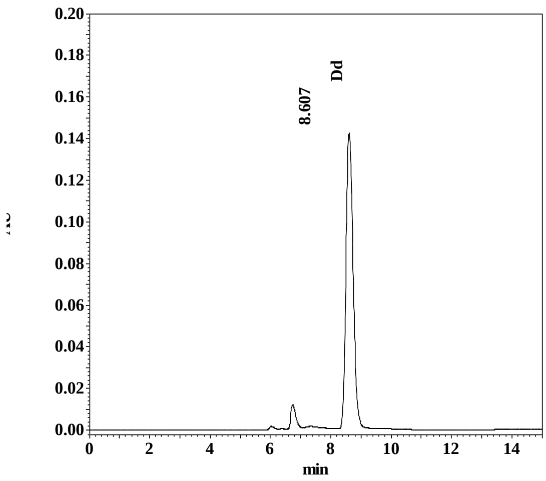


Figure Flow sheet for dicyandiamide in fertilizers

Reference: HPLC chromatogram of dicyandiamide standard solution for the calibration curve preparation and sample solution (compound fertilizer) are shown below.



1) Standard solution



2) Sample solution

Reference diagram　HPLC chromatogram of dicyandiamide

1) Dicyandiamide standard solution (the equivalents of 100 ng of dicyandiamide (10 µg/mL, 10 µL))
2) Sample solution (compound fertilizer)

Measurement conditions for HPLC

Column: Inertsil NH_2 (4.6-mm inner diameter, 250-mm long, 5-µm particle diameter)

Temperature of Column bath: 30 ºC

Flow rate: 0.5 mL/min

Other conditions are according to the examples of the measurement conditions of **(4.3) a)** HPLC.

7.6 2-sulfanilamide thiazole (ST)

7.6.a High Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers containing 2-sulfanilamide thiazole (ST).

Add methanol - water (1+1) to an analytical sample to extract 2-sulfanilamide thiazole, introduce it into a High Performance Liquid Chromatograph (HPLC), isolate with an octadecyl silylation silica gel column, and measure at wavelength 285 nm to calculate 2-sulfanilamide thiazole.

(2) Reagents, etc.: Reagents and water are as shown below.

- **a) Water:** Water of A3 specified in JIS K 0557 or water with equivalent quality..
- **b) Methanol:** A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.
- **c) Methanol:** Methanol used in eluent of HPLC is a reagent of HPLC analysis grade or equivalents.
- **d) 2-sulfanilamide thiazole standard solution (1 mg/mL)** (1) **:**Transfer 0.1 g of 2-sulfanilamide thiazole [$C_9H_9N_3O_2S_2$](2) to a weighing dish and measure the mass to the order of 0.1 mg. Add water to dissolve, transfer to a 1,000-mL volumetric flask and add methanol - water (1+1) to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.
- **e) 2-sulfanilamide thiazole standard solution (100 µg/mL)**: In the case of usage, transfer 10 mL of 2-sulfanilamide thiazole standard solution (100 µg/mL) to a 100-mL volumetric flask and add methanol - water (1+1) up to the marked line.
- **f) 2-sulfanilamide thiazole standard solution (10 µg/mL - 50 µg/mL) for the calibration curve preparation:** In the case of usage, transfer 5 mL - 25 mL of 2-sulfanilamide thiazole standard solution (100 µg/mL) to 50-mL volumetric flasks step-by-step and add methanol - water (1+1) up to the marked line.
- **g) 2-Sulfanilamide thiazole standard solution（1 µg/mL - 10 µg/mL）for the calibration curve preparation**: In the case of usage, transfer 2.5 mL - 25 mL of 2-sulfanilamide thiazole standard solution for the calibration curve preparation (20 µg/mL) to 50-mL volumetric flasks step-by-step and add methanol - water (1+1) up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 98 % (mass fraction) in purity is commercially sold as 2-sulfanilamide thiazole.

Comment 1: 2-sulfanilamide thiazole is sold under the production name of sulfathiazole by Tokyo Chemical Industry Co., Ltd, Wako Pure Chemical Industries., Ltd and Kanto Chemical Co., Inc.

(3) Instruments: Instruments are as shown below:

- **a) High Performance Liquid Chromatograph:** HPLC specified in JIS K 0124 that satisfies the following requirements.
  - **1) Column**: A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which octadecyl chemically bonds.
  - **2) Column bath:** A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
  - **3) Detector:** An absorptiometric detector that can measure at wavelength around 285 nm.
- **b) Magnetic stirrer**
- **c) Centrifugal separator**: A centrifugal separator that can centrifuge at 2,000 × *g*.

d) High speed centrifugal separator: A centrifugal separator that can centrifuge at 8,000 × *g*.

e) Acidic alumina cartridge column: Link a 10-mL cylinder to column (3) that is filled with 500 mg - 1 g of acidic alumina and add 3 mL of methanol to let it flow down.

Note (3) A cartridge with a 3-mL - 6-mL column filled with 500 mg - 1 g of silica gel can be used.

Comment 2 A column is sold under production names such as Inertsil ODS, Mightysil RP-18, L-column ODS, Shim-pack VP-ODS, Silica C18M 4D, Puresil C_{18}, COSMOSIL 5C18-MS-II, etc.

Comment 3 An acidic alumina cartridge is commercially sold under production names such as Bond Elut AL-A, Sep-Pak Alumina-A, Supelclean LC-Alumina-A.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of methanol - water (1+1) and stir it with a magnetic stirrer for about 15 minutes.

c) After allowing to stand still, transfer supernatant solution to a 50-mL ground-in stopper centrifugal precipitate tube.

d) Centrifuge at 2,000 × *g* centrifugal force for about five minutes (4) to make the supernatant as the extract (5).

Note (4) 18.7-cm of rotor radius and 3,000 rpm of revolutions makes about 2,000 × *g* centrifugal force.

(5) If there is a possibility that the 2-2-sulfanilamide thiazole sulfanilamide thiazole concentration in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of the extract with methanol.

(4.2) Cleanup: Conduct cleanup as shown below:

a) Transfer extract to an acidic alumina cartridge column.

b) Dispose of about first 3 mL of effluent and then transfer about the next 2 mL to a test tube.

c) Transfer effluent to a 1.5-mL ground-in stopper centrifugal precipitate tube (6).

d) Centrifuge at 8,000 × *g* centrifugal force for about five minutes (7) to make the supernatant as the sample solution.

Note (6) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(7) 7-cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 4 Instead of the procedures in **(4.2) c) - d)**, it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-μm) made of PTFE and the filtrate can be the sample solution.

Comment 5 The test is possible by the following procedures in the case of fertilizers not containing organic matters.

The procedures in **(4.1) c) - d)** and **(4.2) a) - b)** are omitted and “Transfer effluent” in **(4.2) c)** is replaced with the “After allowing to stand still, transfer supernatant” to operate.

(4.3) Measurement: Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of High Performance Liquid Chromatograph (HPLC) used in measurement.

a) Measurement conditions for High Performance Liquid Chromatograph (HPLC): Set up the measurement conditions for High Performance Liquid Chromatograph (HPLC) considering the following example:

1) Column: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which octadecyl chemically bonds.

2) Temperature of Column bath: 30 ºC - 40 ºC

3) Eluent: Methanol - water (2+8)

4) Flow rate: 1 mL/min

5) Detector: An absorptiometric detector, measurement wavelength: 285 nm

b) Calibration curve preparation

1) Inject 10 µL of respective 2-sulfanilamide thiazole standard solutions for the calibration curve preparation to HPLC, record chromatogram at wavelength 285 nm and obtain the peak area or height.

2) Prepare a curve for the relationship between the concentration and the peak area or height at wavelength 285 nm of the 2-sulfanilamide thiazole standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1)**.

2) Obtain the 2-sulfanilamide thiazole content from the calibration curve to calculate the concentration of 2-sulfanilamide thiazole in the analytical sample.

Comment 6 Recovery testing was conducted using compound fertilizer (1 sample) and blended fertilizer (2 sample), as a result, the mean recovery rate of 2-sulfanilamide thiazole at the concentration level of 1.0 %, 0.4 % and 0.1 % (mass fraction) were 101.2 % - 102.1 %, 99.6 % - 101.7 % and 99.4 % - 101.0 %, and repeatability relative standard deviation were 0.5 % - 1.2 %, 2.1 % - 3.2 % and 1.0 % - 3.4 %, respectively.

Additionally, the minimum limit of quantification of this test method is about 0.005 % (mass fraction).

Reference

1) Yuji SHIRAI: The volumetric analysis of 2-sulfanilamide thiazole in fertilizers with High Performance Liquid Chromatograph, Validation Report of Fertilizers (in Japanese), 44 (1), 10 - 20 (1991)

(5) Flow sheet for 2-sulfanilamide thiazole: The flow sheet for 2-sulfanilamide thiazole in fertilizers is shown below:

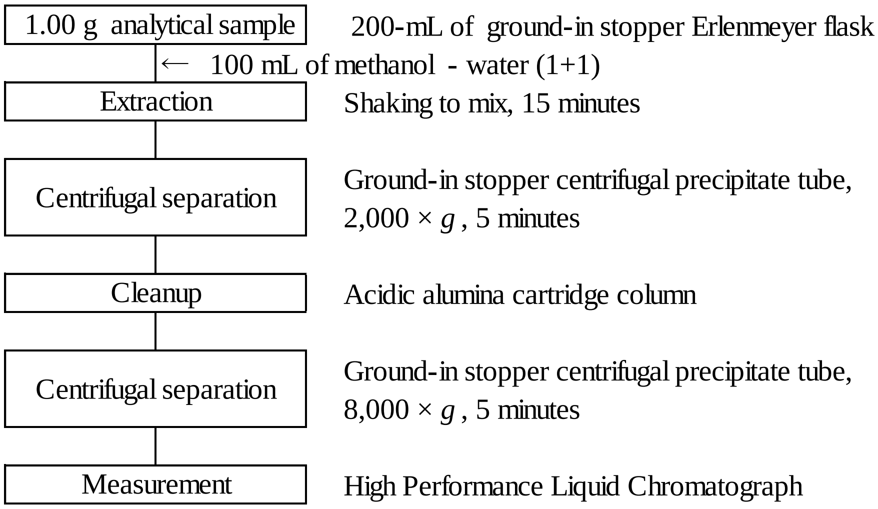
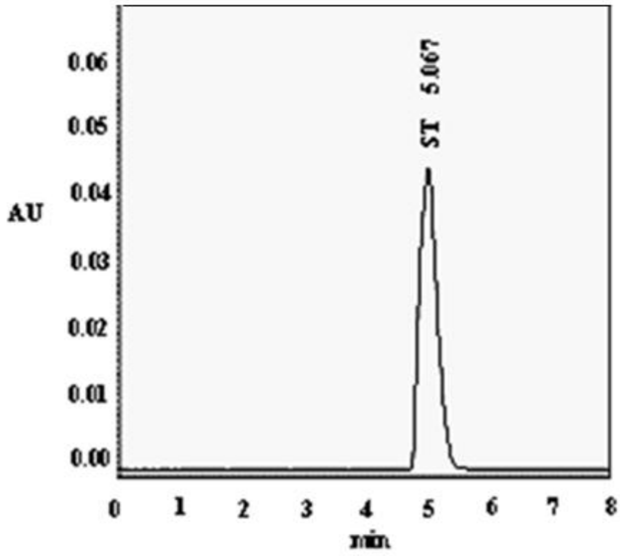


Figure Flow sheet for 2-sulfanilamide thiazole in fertilizers.

Reference: HPLC chromatogram of 2-sulfanilamide thiazole standard solution for the calibration curve preparation is shown below.



Reference diagram: HPLC chromatogram of 2-sulfanilamide thiazole

Measurement conditions for HPLC

Column: Mightysil RP-18 GP (4.6-mm inner diameter, 150-mm long, 5-µm particle diameter)

2-sulfanilamide thiazole standard solution (the equivalents of 200 ng)

Other conditions are according to the examples of the measurement conditions of **(4.3) a)** HPLC.

8. Others

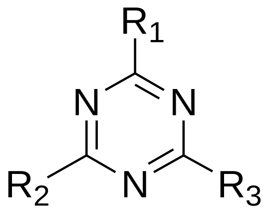
8.1 Melamine and its degradation products

8.1.a Gas Chromatography/Mass Spectrometry <Reference method>

(1) Summary

Extract melamine and its derivative substances (hereinafter referred to as “melamine derivations”) in organic matters and fertilizers containing organic matters with diethylamine-water-acetonitrile (1+4+5) and derivatize with BSTFA-TMCS (99+1) and then measure with a gas chromatography/mass spectrometer.

Comment 1 The structural formula of melamine and its degradation products is shown in the figure 1. During the production process of melamine, a by-product that replaces “-NH_2” of R_1-R_3 with “–OH” is formed in some cases.



	R_1	R_2	R_3	MW
Melamine	NH_2	NH_2	NH_2	126.12
Ammeline	OH	NH_2	NH_2	127.10
Ammelide	OH	OH	NH_2	128.09
Cyanuric acid	OH	OH	OH	129.07

Figure 1 Structural formula of melamine and its degradation products

(2) Reagents, etc.: Reagents and water are as shown below:

a) Water: Water of A3 specified in JIS K 0557or equivalent quality.

b) Acetonitrile: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8039 or a reagent of equivalent quality.

c) Diethylamine: A guaranteed reagent or a reagent of equivalent quality.

d) Pyridine (dehydration) (1)**:** A reagent of organic synthesis grade of no less than 99.5 % (mass fraction) in purity and no more than 0.05 mg/mL in moisture or a reagent of equivalent quality.

e) Derivatization reagent (2)**:** Bis (trimethylsilyl) trifluoroacetamide - trimethylchlorosilane (99+1).

f) Melamine derivations standard solution (0.5 mg/mL): Put about 0.05 g of melamine [$C_3H_6N_6$] (3), ammeline [$C_3H_5N_5O$] (3), ammelide [$C_3H_4N_4O_2$] (3) and cyanuric acid [$C_3H_3N_3O_3$] (3) into a weighing dish and measure the mass to the order of 0.1 mg. Dissolve in a small amount of diethylamine - water (1+4), transfer to a 100 mL volumetric flask respectively, and add the solvent up to the marked line.

g) Mixture standard solution (50 µg/mL) (3)**:** Put 5 mL of respective melamine derivations standard solutions (0.5 mg/mL) to 50-mL volumetric flasks and add diethylamine - water - acetonitrile (1+4+5) up to the marked line.

Note (1) After it is opened once, add a proper amount of sodium sulfate (anhydrous) and seal tightly to store.

(2) A mixed derivatization reagent is commercially sold under the name of BSTFA-TMCS (99+1).

(3) The respective standard reagents of melamine, ammeline, ammelide and cyanuric acid are commercially sold.

Comment 2 BSTFA-TMCS (99+1) is sold as 1-mL ampoule by SUPELCO. After it is opened once, use it on the same day.

Comment 3 The standard reagent of melamine, ammeline, ammelide and cyanuric acid are sold by Wako Pure Chemical Industries., Ltd and Kanto Chemical Co., Inc. and Hayashi Pure Chemical Industries., Ltd.

(**3**) **Instruments:** Instruments are as shown below.

a) **Gas Chromatograph/Mass Spectrometer (GC/MS):** GC/MS specified in JIS K 0123 that satisfies the following requirements.

1) Gas Chromatograph

(i) Sample injector: An injector that enables split less system.

(ii) Capillary column: A capillary column (0.25-mm - 0.32-mm inner diameter and 30-m long) made of fused silica. 5 % phenyl 95 % methyl polysiloxane chemically bonds to the inner surface of a capillary column with 0.25 μm thickness. The column is according to the specification of mass spectrometer.

(iii) Carrier gas: High purity helium of no less than 99.999 % (volume fraction) in purity.

2) Mass Spectrometer

(i) Ionization method: Electron-Impact ionization (EI) method

(ii) Ion detection method: Selected Ion Monitoring (SIM) method

b) **Ultrasonic generator:** An ultrasonic washer can be used.

c) **High speed centrifugal separator:** A centrifugal separator that can centrifuge at 8,000 × *g*.

d) **Concentrator:** A Centrifugal evaporator that can be adjusted to 70 ºC ± 2 ºC.

e) **Water bath:** A water bath that can be adjusted to 70 ºC ± 2 ºC.

Comment 4 A capillary column is commercially sold under the names such as DB-5ms, Rtx-5ms, HP-5ms, SLB-5ms, BPX-5, CP-Sil 8CB low Bleed/MS and TC-5HT for GC/MS.

(**4**) **Test procedures**

(**4.1**) **Extraction:** Conduct extraction as shown below.

a) Weigh 0.50 g of an analytical sample, and put it into a 200-mL - 300-mL ground-in stopper Erlenmeyer flask.

b) Add 160 mL - 200 mL of diethylamine - water - acetonitrile (1+4+5), and subject to ultra-sonication for about 30 minutes using an ultrasonic generator.

c) Transfer about 1.5 mL to a 1.5-mL ground-in stopper centrifugal precipitate tube (4), and centrifuge at 8,000 × *g* for about 5 minutes(5).

d) Transfer 1 mL of the supernatant solution to 5-mL - 50-mL volumetric flasks, add diethylamine - water - acetonitrile (1+4+5) up to the marked line to make the extract.

Note **(4)** Confirm that the tube is made of polypropylene, etc. to not affect testing results.

(5) 7-cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 5 Grind until it completely passes through a sieve of 500 μm aperture to prepare the test sample.

Comment 6 Weigh 0.5 g of an analytical sample, extract with 200 mL of diethylamine - water - acetonitrile (1+4+5). If it is diluted 50 times in the procedure in **d)**, the quantitative range of melamine derivations in the analytical sample is 0.2 % - 10 % (mass fraction). In the case of measuring melamine derivations which do not reach the quantitative range, make the dilution factor in the procedure in **d)** decrease. In addition, if the contents of melamine derivations exceeds 10 % (mass fraction) respectively, the sampling volume of an analytical sample should be reduced.

(**4.2**) **Derivatization:** Conduct derivatization as shown below.

a) Transfer 0.2 mL of the extract to a 5-mL - 10-mL test tube with a screw stopper.

b) Place a test tube in a concentrator, concentrate under reduced pressure at 70 ºC ± 2 ºC and vaporize the solvent completely (6).

c) Add 0.3 mL of pyridine (dehydration) (1) and 0.2 mL of derivatization reagent (2) to the residue to mix, and then stopple to seal tightly.

d) After heating in a water bath at 70 ºC ± 2 ºC for about 45 minutes (7), let it stand to cool to make the sample solution (8).

Note **(6)** A spraying type concentrator can be used.

(7) If moisture remains after the procedure in **b)** or the reagent used in the procedure in **c)** contains moisture, the reaction of the derivatization in **d)** does not advance enough in some cases.

(8) If necessary, transfer the sample solution into a 1.5-mL ground-in stopper centrifugal precipitate tube (4) to centrifuge at 8,000 × *g* for about 5 minutes (5).

(**4.3**) **Measurement:** Conduct measurement according to JIS K 0123 and as shown below. Specific measurement procedures are according to the operation method of the gas chromatograph/ mass spectrometer used in measurement.

a) **Measurement conditions for the gas chromatograph/ mass spectrometer**: Set up the measurement conditions considering the following example of measurement conditions for gas chromatograph/mass spectrometer.

1) **Gas chromatograph**:

(i) Sample injection method: split less injection method (1min)

(ii) Temperature of sample injector: 280 ºC

(iii) Capillary column: A capillary column (0.25-mm - 0.32-mm inner diameter, 30-m long, 0.25 µm layer thickness) made of fused silica. 5 % phenyl 95 % methyl polysiloxane chemically bonds to the inner surface of the capillary column.

(iv) Temperature of column bath: 100 ºC (1 min) → (15 ºC /min) → 320 ºC (3 min)

(v) Temperature of GC/MS: 250 ºC

(vi) Carrier gas: helium, flow rate: 1.5 mL/min

2) **Mass spectrometer**:

(i) Ionization method: Electron-Impact ionization (EI) method

(ii) Ionization voltage: 70 V

(iii) Temperature of ion source: 230 ºC

(iv) Ion detection method: Selected Ion Monitoring (SIM) method

(v) Measurement of ion: As shown in table 1

b) **Calibration curve preparation**

1) Transfer 5 mL of mixture standard solution (50 µg/mL) to a 50-mL volumetric flask and add diethylamine - water - acetonitrile (1+4+5) up to the marked line to make the mixture standard solution (5 µg/mL).

2) Transfer 1 mL - 20 mL of the mixture standard solution (5 µg/ mL) to 50 mL volumetric flasks step-by-step and add diethylamine - water - acetonitrile (1+4+5) up to the marked line to make the mixture standard solution (0.1 µg/mL - 2 µg/mL).

3) Conduct the procedures of **(4.2) b) - d)** for the mixture standard solution **(**0.1 µg/mL - 2 µg/mL) to make the mixture standard solution, the equivalents of 0.04 µg/mL - 0.8 µg/mL, for calibration curve.

4) Inject 1 µL of respective mixture standard solutions for calibration curve to GC/MS and record the chromatogram of ion (*m*/*z*) for determination and ion (*m*/*z*) for validation of materials subjected to measurement to obtain the peak area and height.

5) Calculate the peak area ratio or height ratio of ion (*m*/*z*) for determination and ion (*m*/*z*) for validation of respective materials subjected to measurement. .

6) Prepare a curve for the relationship between the concentration of material subjected to measurement and the peak area or height of ion (*m*/*z*) for determination of respective mixture solutions for the calibration curve preparation.

c) **Sample measurement**

1) Subject 1 µL of the sample solution to the same procedure as in **b) 4) - 5)** (9).

2) Obtain the content of respective materials subjected to measurement from the calibration curve to calculate the concentration of respective materials subjected to measurement in the analytical sample.

Note (9) Confirm that the ratio against the peak area ratio or height ratio of the standard solution is within the range of about ± 30 %. In addition, the peak area ratio or height ratio depends on the concentration.

Comment 7 If the variation of sensitivity of melamine derivations is observed, conduct measurement by the following method of **a**) or **b**).

a) In the procedure in **(4.3) c) 1**, inject the sample solution into GC/MS predetermined times, and then correct the calibration curve according to **(4.3) b) 4) - 6)**.

b) Add 2,6-diamino-4-chloropyrimidine (the equivalents of 0.5 µg) as an internal reference material to the standard solution and the sample solution, conduct the same procedures as **(4.2) c) - d)**, **(4.3) b) 4) - 6)** and **c) 1)**. However, prepare the calibration curve from the peak area ratio or height ratio of ion (*m*/*z*) for determination of respective materials subjected to measurement and internal reference material, and calculate the concentration of respective materials subjected to measurement in the analytical sample.

Table 1 Fragment ion of materials to be measured

Materials to be measured	Measured fragment ion (*m* /*z)*				
	Determination	Validation	Validation	Validation	Validation
Melamine	342	344	327	285	213
Ammeline	328	345	343	285	214
Ammelide	344	346	329	214	198
Cyanuric acid	345	347	330	215	188
DACP(I.S.)	288	289	290	273	275

Comment 8 Recovery testing of melamine derivations was conducted using soybean meal, fish meal, fish waste processed fertilizer, mixed organic fertilizer, blended fertilizer and compound fertilizer, as a result, the mean recovery rate at additive level of 10 and 0.2 % (mass fraction) were 92.1 % - 102.9 % and 90.3 % - 102.2 %, and the repeatability were 0.9 % - 10.5 % and 0.6 % - 9.1 % relative standard deviation.

Additionally, the minimum limit of quantification of melamine derivations of the test method is about 0.01 % (mass fraction).

Reference

1) Yuji SHIRAI, Jun OKI: Validation of Gas Chromatography/Mass Spectrometry for Determination of Melamine and Its Degradation Products in Fertilizers, Research Report of Fertilizer, Vol.1, 2008, (114 - 121)

(5) Flow sheet for melamine derivations: The flow sheet for melamine derivations in fertilizers is shown below.

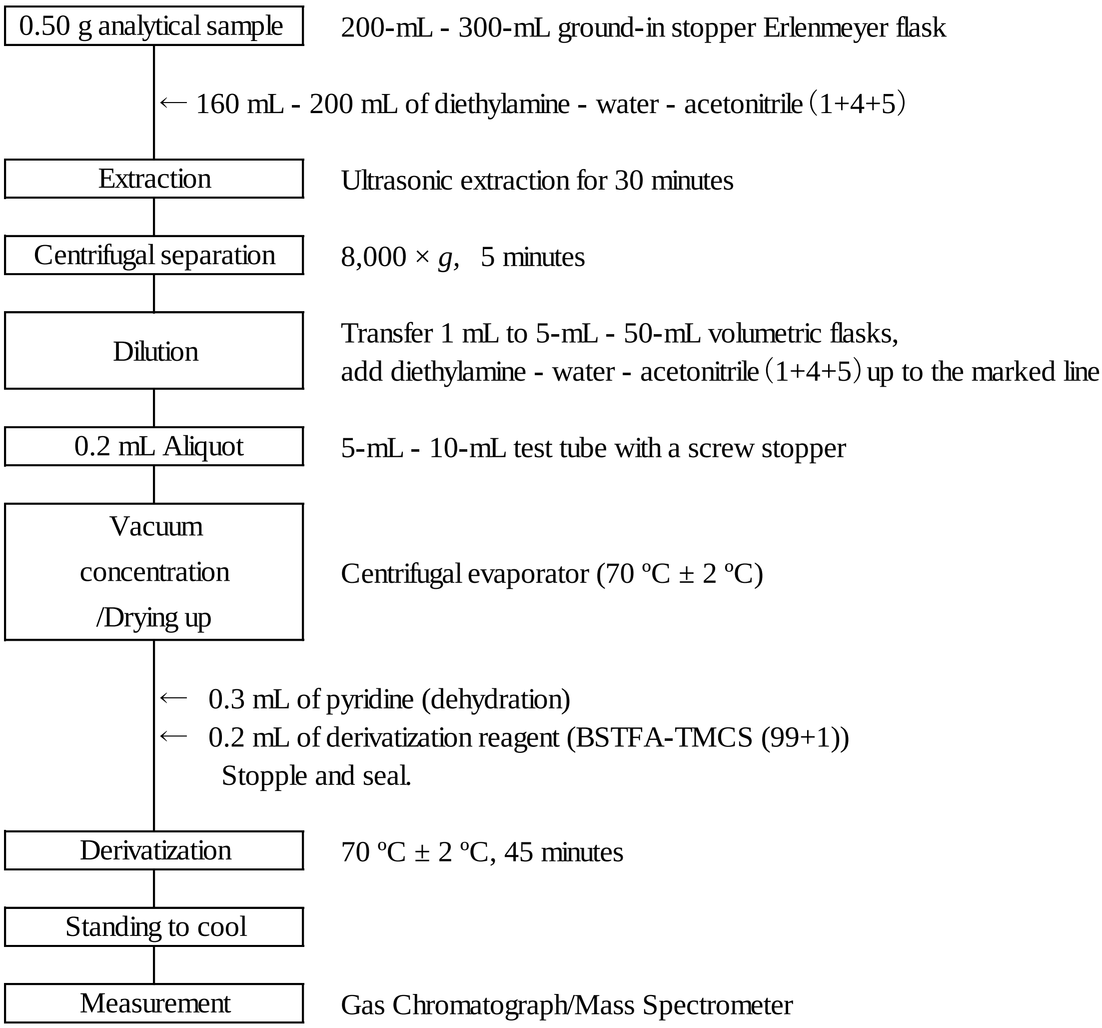


Figure 2 Flow sheet for melamine derivations in fertilizers

Reference Total Ion Chromatogram (TIC) of GC/MS of mixture standard solution for calibration curve preparation of melamine derivations is shown below.

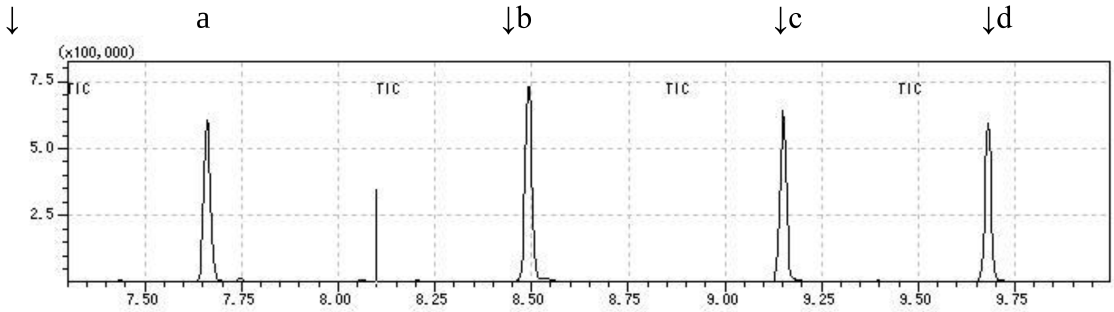


Figure 3 Total Ion Chromatogram (TIC) of GC/MS for melamine derivations

Measurement conditions of GC/MS

Capillary column: Rtx-5ms (0.25-mm inner diameter, 30-m long, 0.25 µm layer thickness)

Other conditions are according to the example of measurement conditions for gas chromatography/mass spectrometer in (**4.3) a**.

Peak name of respective total ion chromatograms

a) Cyanuric acid b) Ammelide

c) Ammeline d) Melamine

Sample and amount injected into GC/MS

Injected sample: Mixture standard solutions (the equivalents of 2 µg/mL) for the calibration curve preparation of respective melamine derivations.

Injected amount: 1 µL (the equivalents of 2 ng of respective melamine derivations)

8.1.b High Performance Liquid Chromatography (Nitrolime) <Reference method>

(1) Summary

This method is applicable to nitrolime.

Add acetonitrile - water - diethylamine (5+4+1) to an analytic sample, extract melamine, introduce it into a High Performance Liquid Chromatograph (HPLC), isolate with a silica gel column to which carbamoyl chemically bonds, and measure at wavelength 214 nm to calculate melamine. However it is difficult to extract cyanuric acid that is melamine derivations from nitrolime by this method and the recovery testing for ammeline and ammelide does not achieve good results, so that they are excluded from components subjected to measurement.

(**2) Reagents, etc.**: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557 or equivalent quality.

b) **Acetonitrile:** A JIS Guaranteed Reagent specified in JIS K 8032 or a reagent of equivalent quality.

c) Acetonitrile: Acetonitrile used in eluent of HPLC is a regent of HPLC analysis grade or equivalents.

d) **Diethylamine:** A guaranteed reagent or a reagent of equivalent quality.

e) **Phosphate buffer solution** (1)**:** Dissolve 0.237 g of disodium hydrogen-phosphate specified in JIS K 9020 and 0.520 g of sodium dihydrogenphosphate dihydrate specified in JIS K 9009 in water to make 1,000 mL (2). If it is used for eluent of HPLC, filter with a membrane filter (pore size: no more than 0.5-µm) made of hydrophilic PTFE.

f) Melamine derivations standard solution (0.5 mg/mL): Put about 0.05g of melamine $[C_3H_6N_6]$(3), ammeline $[C_3H_5N_5O]$(3)and ammelide $[C_3H_4N_4O_2]$(3) to weighing dishes respectively and measure the mass to the order of 0.1 mg. Dissolve in a small amount of diethylamine - water (1+4), transfer to respective 100 mL volumetric flasks, and add the solvent up to the marked line.

g) Mixture standard solution (50 µg/mL) (1)**:** Transfer 5 mL of respective melamine derivations standard solutions (0.5 mg/mL) to a 50-mL volumetric flask and add acetonitrile - phosphate buffer solution (4+1) up to the marked line.

h) Mixture standard solution for calibration curve preparation (**2 µg/mL - 20 µg/mL):** In the case of usage, transfer 2 mL - 20 mL of mixture standard solution (50 µg /mL) to 50-mL volumetric flasks step-by-step and add acetonitrile - phosphate buffer solution (4+1) up to the marked line.

i) Mixture standard solution for calibration curve preparation (0.1 µg/mL - 2 µg/mL): In the case of usage, transfer 1 mL - 20 mL of mixture standard solution (50 µg/ mL) to 50-mL volumetric flasks step-by-step, add acetonitrile - phosphate buffer solution (4+1) up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) pH of phosphate buffer solution becomes 6.7 ± 0.2.

(3) Standard reagents as melamine, ammeline and ammelide are commercially sold respectively.

Comment 1 The standard reagents of melamine, ammeline and ammelide are sold by Wako Pure Chemical Industries., Ltd, Kanto Chemical Co., Inc., Hayashi Pure Chemical Industries, Ltd and Tokyo Chemical Industry Co., Ltd.

(3) Instruments: Instruments are as shown below.

a) High Performance Liquid Chromatograph (HPLC): HPLC specified in JIS K 0124 that satisfies the following requirements.

1) **Column:** A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which carbamoyl chemically bonds.
2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
3) **Detector:** An absorptiometric detector that can measure at wavelength around 214 nm.

b) **Ultrasonic generator:** An ultrasonic washer can be used.
c) **Centrifugal separator**: A centrifugal separator that can centrifuge at 2,000 × *g*.
d) **High speed centrifugal separator**: A centrifugal separator that can centrifuge at 8,000 × *g*.

Comment 2 A column is sold under production name of TSKgel Amide-80, etc.

(4) Test procedures

(**4.1) Extraction:** Conduct extraction as shown below.

a) Weigh 0.50 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.
b) Add 100 mL of acetonitrile - water - diethylamine (5+4+1) and conduct ultra-sonication for about 30 minutes using an ultrasonic generator.
c) After allowing to stand still, transfer 50 mL of the supernatant solution to a ground-in stopper centrifugal precipitate tube.
d) Centrifuge at 2,000 × *g* for about 5minutes (4) to make supernatant as the extract.
e) Transfer 5 mL of the extract (5) into a 50-mL volumetric flask, and add acetonitrile - phosphate buffer solution (4+1) up to the marked line to dilute.
f) Transfer 1.5 mL of dilution liquid to a 1.5-mL ground-in stopper centrifugal precipitate tube (6).
g) Centrifuge at 8,000 × *g* for about 5 minutes (7) to make supernatant as the sample solution

Note **(4)** 18.7-cm of rotor radius and 3,000 rpm of revolutions makes about 2,000 × *g* centrifugal force.
(5) If there is a possibility that the concentration of melamine derivations in the sample solution exceeds the maximum limit of the calibration curb, the amount of supernatant solution to be transferred should be1 mL - 2.5 mL.
(6) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.
(7) 7cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 3 Instead of the procedures in **(4.2) f) - g)**, it is allowed to filter with a membrane filter (pore size: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(4.2) Measurement: Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High Performance Liquid Chromatograph (HPLC) used in measurement.

a) **Measurement conditions of High Performance Liquid Chromatograph (HPLC)**: Set up the measurement conditions considering the following example of measurement conditions for High Performance Liquid Chromatograph (HPLC).

1) **Column:** A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter column) to which carbamoyl chemically bonds.
2) **Temperature of column bath:** 30 ºC - 40 ºC

3) **Eluent:** Acetonitrile - phosphate buffer solution (4+1)
4) **Flow rate:** 1 mL/min
5) **Detector:** An absorptiometric detector, measurement wavelength: 214 nm

b) Calibration curve preparation

1) Inject 10 µL of respective mixture standard solutions for calibration curve preparation into HPLC, record chromatogram at wavelength 214 nm and obtain the peak area and height.
2) Prepare a curve for the relationship between the concentration and the peak area or height at wavelength 214 nm of respective mixture solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1).**
2) Obtain the melamine derivations content from the calibration curve to calculate the concentration of melamine derivations in the analytical sample.

Comment 4 Recovery testing was conducted using 3 brands of nitrolime, as a result, the recovery rate of melamine at concentration level of 4 % and 0.4 % were 95.7 % - 103.2 % and 93.6 % - 102.5 %, and the repeatability were 0.8 % - 2.3 % and 0.5 % - 1.0 % relative standard deviation.
Additionally, the minimum limit of quantification of the test method is about 0.01 % (mass fraction).

Reference

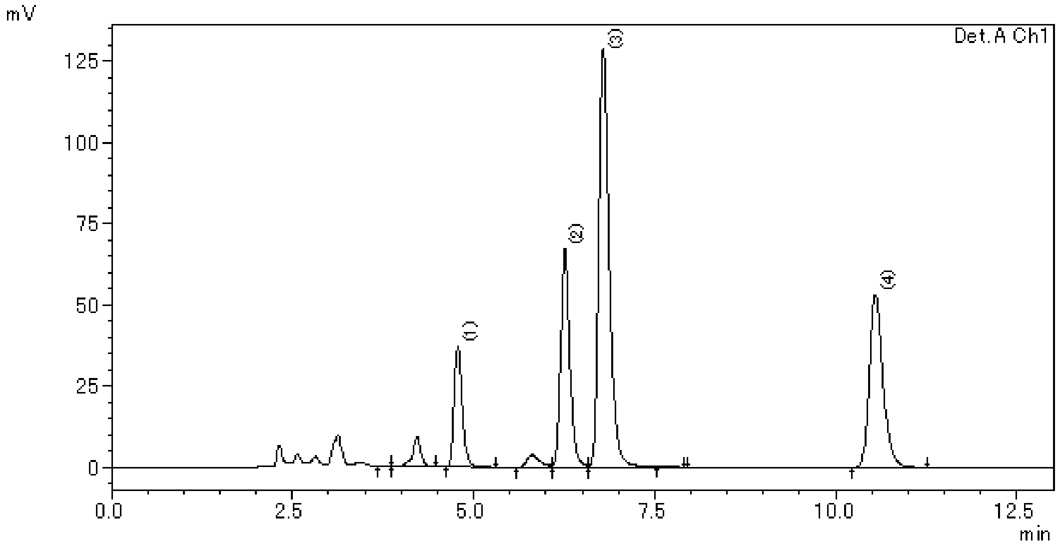
1) Etsuko BANDO, Toshiaki HIROI, Masahiro ECHI and Yuji SHIRAI: Validation of High Performance Liquid Chromatography (HPLC) for Determination of Melamine and Its Related Substances in Calcium Cyanamid, Research Report of Fertilizer,Vol.5, 2012, (24 - 30)

(5) Flow sheet for melamine derivations: The flow sheet for melamine derivations in nitrolime is shown below.

0.50 g analytical sample	200-mL ground-in stopper Erlenmeyer flask
←100 mL of acetonitrile - water - diethylamine (5+4+1)	
Extraction	Ultrasonic extraction for 30 minutes
Centrifugal separation	2,000 × *g* for 5 minutes
Dilution	Transfer 5 mL to a 50-mL volumetric flask, add acetonitrile - phosphate buffer solution (4+1) up to the marked line
Centrifugal separation	8,000 × *g* for 5 minutes
Measurement	High Performance Liquid Chromatograph

Figure Flow sheet for melamine derivations in fertilizers

Reference HPLC chromatogram of the mixture standard solution for calibration curve preparation of melamine derivations is shown below.



Reference diagram HPLC chromatogram of melamine derivations

The names of materials for respective peaks

(1) cyanuric acid (reference) (2) ammelide (3) melamine (4) ammeline

Measurement conditions of HPLC

Column: TSKgel Amide-80 (4.6-mm inner diameter, 250-mm long, 5-µm particle diameter)

Mixture standard solutions (the equivalents of 100 ng (10 µg/mL, 10 µL)) for calibration curve preparation of respective melamine derivations

Other conditions are according to the examples of the measurement conditions in (**4.2) a**) HPLC.

8.1.c High Performance Liquid Chromatography (Fertilizers not containing organic matters)

(1) Summary

This method is applicable to fertilizers not containing organic matters.
Add hydrochloric acid (1+15) to an analytic sample, extract melamine and its degradation products (hereinafter referred to as “melamine derivations”), introduce it into a High Performance Liquid Chromatograph (HPLC), isolate with a silica gel column to which carbamoyl chemically bonds, and measure at wavelength 214 nm to calculate melamine derivations.

(2) Reagents, etc.: Reagents and water are as shown below.

- **a) Water:** Water of A3 specified in JIS K 0557.
- **b) Acetonitrile:** A JIS Guaranteed Reagent specified in JIS K 8032 or a reagent of equivalent quality. In addition, acetonitrile used in eluent of HPLC is a regent of HPLC analysis grade.
- **c) Hydrochloric acid:** A JIS Guaranteed Reagent or a reagent of equivalent quality.
- **d) Phosphate buffer solution** (1)**:** Dissolve 0.237 g of disodium hydrogen-phosphate specified in JIS K 9020 and 0.520 g of sodium dihydrogenphosphate dihydrate specified in JIS K 9009 in water to make 1,000 mL (2). If it is used for eluent of HPLC, filter with a membrane filter (pore size: no more than 0.5-µm) made of hydrophilic PTFE.
- **e) Melamine derivations standard solution (0.5 mg/mL):** Put about 0.05g of melamine [$C_3H_6N_6$](3), ammeline [$C_3H_5N_5O$](3), ammelide [$C_3H_4N_4O_2$](3) and cyanuric acid [$C_3H_3N_3O_3$] (3) to weighing dishes respectively and measure the mass to the order of 0.1 mg. Dissolve them with a small amount of hydrochloric acid (1+15), transfer to 100 mL volumetric flasks respectively, and add the solutions up to the marked line.
- **f) Mixture standard solution (50 µg/mL)** (1)**:** Transfer 5 mL of respective melamine derivations standard solutions (0.5 mg/mL) to 50-mL volumetric flasks and add acetonitrile - phosphate buffer solutions (4+1) up to the marked line.
- **g) Mixture standard solution for calibration curve preparation (1 µg/mL - 5 µg/mL):** In the case of usage, transfer 1 mL - 5 mL of mixture standard solution (50 µg /mL) to 50-mL volumetric flasks step-by-step and add acetonitrile - phosphate buffer solutions (4+1) up to the marked line.
- **h) Mixture standard solution for calibration curve preparation (0.05 µg/mL – 0.5 µg/mL):** In the case of usage, transfer 2.5 mL - 25 mL of mixture standard solution (1 µg/ mL) to 50-mL volumetric flasks step-by-step, add acetonitrile - phosphate buffer solutions (4+1) up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) pH of phosphate buffer solution becomes 6.7 ± 0.2.
(3) Standard reagents as melamine, ammeline, ammelide and cyanuric acid are commercially sold respectively.

Comment 1 The standard reagents of melamine, ammeline, ammelide and cyanuric acid are sold by Wako Pure Chemical Industries., Ltd, Kanto Chemical Co., Inc., Hayashi Pure Chemical Industries, Ltd and Tokyo Chemical Industry Co., Ltd.

(3) Instruments: Instruments are as shown below.

- **a) High Performance Liquid Chromatograph (HPLC):** HPLC specified in JIS K 0124 that satisfies the following requirements.

1) **Column:** A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which carbamoyl chemically bonds.
2) **Column bath**: A column bath whose temperature can be adjusted to 40 ºC ± 1 ºC.
3) **Detector:** An absorptiometric detector that can measure at wavelength around 214 nm.

b) **Ultrasonic generator:** An ultrasonic washer can be used.
c) **Centrifugal separator**: A centrifugal separator that can centrifuge at 2,000 × *g*.
d) **High speed centrifugal separator**: A centrifugal separator that can centrifuge at 8,000 × *g*.

Comment 2 Column is sold under production name of TSKgel Amide-80, etc. A column which has actually isolated melamine, ammeline, ammelide and cyanuric acid should be used.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 0.50 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.
b) Add 100 mL of hydrochloric acid (1+15) and conduct ultra-sonication for about 30 minutes using an ultrasonic generator.
c) After allowing to stand still, transfer supernatant solution to a 50-mL ground-in stopper centrifugal precipitate tube.
d) Centrifuge at 2,000 × *g* for about 5minutes (4) to make supernatant as the extract.
e) Transfer 5 mL of the extract (5) into a 50-mL volumetric flask, and add acetonitrile - phosphate buffer solution (4+1) up to the marked line to dilute.
f) Transfer dilution liquid to a 1.5-mL ground-in stopper centrifugal precipitate tube (6).
g) Centrifuge at 8,000 × *g* for about 5 minutes (7) to make supernatant as the sample solution

Note **(4)** 18.7cm of rotor radius and 3,000 rpm of revolutions makes about 2,000 × *g* centrifugal force.
(5) If there is a possibility that the concentration of melamine derivations in the sample solution exceeds the maximum limit of the calibration curb, the amount of supernatant solution to be transferred should be1 mL - 2.5 mL.
(6) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.
(7) 7cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 3 Instead of the procedures in **(4.1) f) - g)**, it is allowed to filter with a membrane filter (pore size: no more than 0.5-μm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(4.2) Measurement: Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High Performance Liquid Chromatograph (HPLC) used in measurement.

a) **Measurement conditions of High Performance Liquid Chromatograph (HPLC)**: Set up the measurement conditions considering the following example of measurement conditions for High Performance Liquid Chromatograph (HPLC).

1) **Column:** A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-μm particle diameter column) to which carbamoyl chemically bonds.

2) **Temperature of column bath:** 40 ºC ± 1 ºC
3) **Eluent:** Acetonitrile - phosphate buffer solution (4+1)
4) **Flow rate:** 1 mL/min
5) **Detector:** An absorptiometric detector, measurement wavelength: 214 nm

b) Calibration curve preparation

1) Inject 10 µL of respective mixture standard solutions for calibration curve preparation into HPLC, record chromatogram at wavelength 214 nm and obtain the peak area or height.
2) Prepare a curve for the relationship between the concentration and the peak area or height at wavelength 214 nm of respective mixture solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1).**
2) Obtain the melamine derivations content from the calibration curve to calculate the concentration of respective melamine derivations in the analytical sample.

Comment 4 Recovery testing was conducted using 3 brands of nitrolime, 1 brand of compound fertilizer containing nitrolime, 2 brands of compound fertilizers not containing nitrolime, 1 brand of ammonium sulfate and 1 brand of urea, as a result, the recovery rate of melamine derivations at concentration level of 4 % (mass fraction) and 0.1 % (mass fraction) were 90.5 % - 106.3 % and 92.2 % - 107.0 %, and the repeatability were 0.5 % - 4.7 % and 0.3 % - 4.2 % as relative standard deviation.

In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 1.

Additionally, the minimum limit of quantification of the test method is about 0.02 % (mass fraction) for melamine and cyanuric acid and about 0.01 % (mass fraction) for ammeline and ammelide. In the case of ammelide and cyanuric acid, the sufficient reproducibility was observed in the range of 0.188 % - 1.10 % and 0.105 % - 1.15 % respectively.

Table 1 Results and analysis results from a collaborative study for the test method validation of melamine deivations

Agrichemical name	Sample name	Number of laboratories[1)]	Mean[2)] (%)[7)]	s_r[3)] (%)[7)]	RSD_r[4)] (%)	s_R[5)] (%)[7)]	RSD_R[6)] (%)
Melamine	Nitrolime 1	9	2.83	0.04	1.4	0.12	4.3
	Nitrolime 2	10	0.391	0.003	0.8	0.023	5.8
	Compound fertilizer containing nitrolime	9	0.845	0.019	2.2	0.036	4.2
	Compounf ferttilizer	11	0.198	0.005	2.6	0.012	6.2
	Ammonium sulfate	10	0.0343	0.0015	4.5	0.0040	11.6
Ammeline	Nitrolime 1	9	1.60	0.02	1.3	0.06	3.8
	Nitrolime 2	10	0.105	0.001	1.3	0.002	2.3
	Compound fertilizer containing nitrolime	9	0.629	0.027	4.3	0.023	3.7
	Compounf ferttilizer	11	0.195	0.004	2.1	0.009	4.5
	Ammonium sulfate	10	0.0346	0.0013	3.7	0.0024	6.9
Ammelide	Nitrolime 1	9	1.10	0.02	2.1	0.08	7.6
	Nitrolime 2	11	0.361	0.008	2.2	0.023	6.5
	Compound fertilizer containing nitrolime	9	0.188	0.004	2.2	0.014	7.5
	Compounf ferttilizer	11	0.718	0.028	3.9	0.052	7.2
	Ammonium sulfate	11	0.0345	0.0031	8.9	0.0056	16.1
Cyanuric acid	Nitrolime 1	9	1.15	0.06	4.8	0.09	7.7
	Nitrolime 2	10	0.390	0.018	4.5	0.029	7.4
	Compound fertilizer containing nitrolime	9	0.105	0.003	2.9	0.014	13.2
	Compounf ferttilizer	9	0.788	0.026	3.2	0.054	6.8
	Ammonium sulfate	10	0.0365	0.0015	4.2	0.0067	18.3

1) Number of laboratories used in analysis
2) Total mean (number of laboratories × 2 samples replicate analysis)
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Reproducibility standard deviation
6) Reproducibility relative standard deviation
7) Mass fraction

Reference

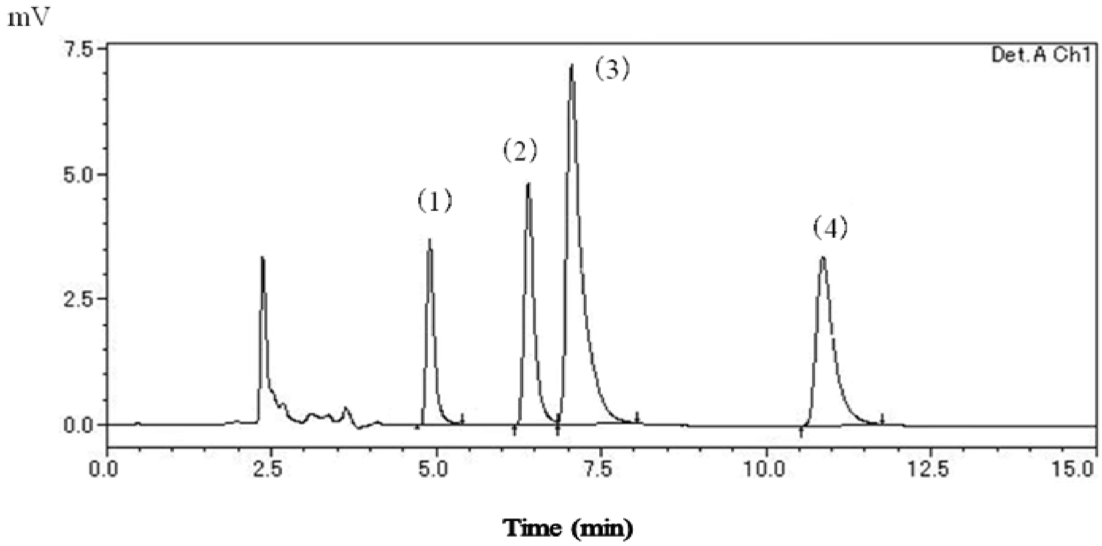
1) Etsuko BANDO and Yuji SHIRAI: Validation of High Performance Liquid Chromatography (HPLC) for Determination of Melamine and Its Related Substances in Fertilizer, Research Report of Fertilizer,Vol.6, 2013, (27 - 35)

(5) Flow sheet for melamine derivations: The flow sheet for melamine derivations in fertilizers is shown below.

0.50 g analytical sample	200-mL ground-in stopper Erlenmeyer flask
←100 mL of hydrochloric acid (1+15)	
Extraction	Ultrasonic extraction for 30 minutes
Centrifugal separation	2,000 × *g* for 5 minutes
Dilution	Transfer 5 mL to a 50-mL volumetric flask, add acetonitrile - phosphate buffer solution (4+1) up to the marked line
Centrifugal separation	8,000 × *g* for 5 minutes
Measurement	High Performance Liquid Chromatograph

Figure 2 Flow sheet for melamine derivations in fertilizers

Reference HPLC chromatogram of the mixture standard solution for calibration curve preparation of melamine derivations is shown below.



Reference diagram HPLC chromatogram of melamine derivations

The names of materials for respective peaks

(1) cyanuric acid (2) ammelide (3) melamine (4) ammeline

Measurement conditions of HPLC

Column: TSKgel Amide-80 (4.6-mm inner diameter, 250-mm long, 5-µm particle diameter)

Mixture standard solutions (respective equivalents of 10 ng (1 µg/mL, 10 µL)) for calibration curve preparation of respective melamine derivations

Other conditions are according to the examples of the measurement conditions in (**4.2) a**) HPLC.

8.2 Clopyralid and its degradation products

8.2.a High Performance Liquid Chromatography/Tandem Mass Spectrometry <Reference method>

(1) Summary

This method is applicable to compost and composted sludge fertilizers.
Extract clopyralid, aminopyralid and picloram with methanol under alkaline condition, refine with a cleanup cartridge by taking advantage of characteristics that the behavior of elution varies between acidity and alkaline, and then measure with a High Performance Liquid Chromatograph Tandem Mass Spectrometer.

Comment 1 Structural formulas of clopyralid, aminopyralid and picloram are as shown in Figure 1.

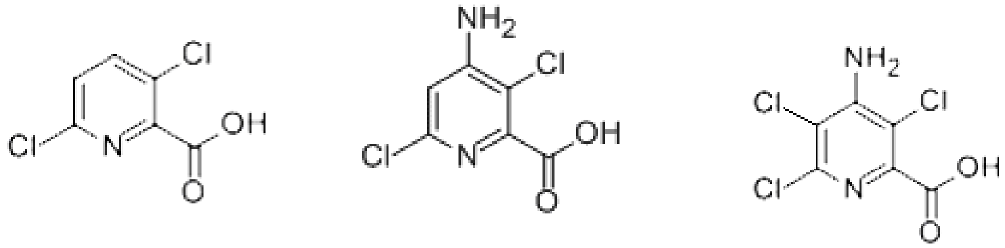


Figure 1 Structural formula of clopyralid, aminopyralid and picloram

(2) Reagents, etc.: Reagents and water are as shown below:

- **a) Water:** Water of A3 specified in JIS K 0557or equivalent quality. Note that water of A4 or equivalent quality should be used as the eluent which is introduced to LC-MS/MS.
- **b) Acetonitrile**: A regent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8039 or a reagent of equivalent quality.
- **c) Methanol:** A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.
- **d) Methanol:** Methanol used in eluent of LC/MS/MS is a regent of LC-MS analysis grade or equivalents.
- **e) Sodium hydroxide:** A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.
- **f) Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **g) Ammonia solution:** A JIS Guaranteed Reagent of 25 % (mass fraction) specified in JIS K 8085 or a reagent of equivalent quality.
- **h) Formic acid:** A JIS Guaranteed Reagent specified in JIS K 8264 or a reagent of equivalent quality.
- **i) Ammonia solution (0.0025 % (mass fraction))** (1)**:** Add 0.1 mL of ammonia solution to 1,000 mL of water.
- **j) Respective agrichemical standard solutions (0.1 mg/mL)**(1)**:** Put about 0.01 g of clopyralid [$C_6H_3C_{12}NO_2$](2), aminopyralid [$C_6H_4C_{12}N_2O_2$](2), picloram [$C_6H_3C_{13}N_2O_2$] (2) to weighing dishes and measure the mass to the order of 0.1 mg. Dissolve with a small amount of acetonitrile, transfer to 100-mL volumetric flasks and add the solvent up to the marked line.

- **k) Mixture standard solution (250 ng/mL):** Dilute a predetermined amount of respective agrichemical standard solutions (0.1 mg/mL) with formic acid (1+1,000) to prepare mixture standard solution (250 ng/mL).
- **l) Mixture standard solution for calibration curve preparation (5 ng/mL - 50 ng/mL):** In the case of usage, transfer 2.5 mL - 25 mL of mixture standard solution (100 ng/mL) to 50 mL volumetric flasks step-by-step, and add formic acid (1+1,000) up to the marked line.
- **m) Mixture standard solution for calibration curve preparation (0.5 ng/mL - 5 ng/mL):** In the case of usage, transfer 2.5 mL - 25 mL of mixture standard solution (10 ng/mL) to 50-mL volumetric flasks step-by-step, and add formic acid (1+1,000) up to the marked line.

Notes **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) A standard reagent is commercially sold.

Comment 2 Standard reagents of clopyralid, aminopyralid and picloram are sold by Wako Pure Chemical Industries., Ltd, Kanto Chemical Co., Inc. and Hayashi Pure Chemical Ind., Ltd.

(**3**) **Apparatus and instruments:** Apparatus and instruments are as shown below:

- **a) High Performance Liquid Chromatograph/Mass Spectrometer (LC-MS/MS)**: LC/MS/MS specified in JIS K 0136 that satisfies the following requirements.
  - **1) High Performance Liquid Chromatograph:**
    - (i) Column bath: A column bath whose temperature can be adjusted at 30 ºC - 45 ºC.
    - (ii) Column: A 2-mm - 3-mm inner diameter 50-mm - 150-mm long 1.6-µm - 2.2-µm particle diameter stainless steel column tube filled with silica gel, to which octadecyl chemically bonds. The specification is according to the mass spectrometer specification.
  - **2) Mass Spectrometer**:
    - (i) Ionization method: Electro Spray Ionization (ESI)
    - (ii) Ion detection method: Selected Reaction Monitoring
- **b) Shaking apparatus**
- **c) Manifold**
- **d) Centrifugal separator:** A centrifugal separator that can centrifuge at 1,300 × *g*.
- **e) High speed centrifugal separator:** A centrifugal separator that can centrifuge at 8,000 × *g*.
- **f) Concentrator:** An evaporator that can adjust to 40 ºC ± 2 ºC.
- **g) Copolymer cartridge column:** A divinylbenzene-N-vinylpyrrolidone copolymer mini column (200 mg)

Comment 3 A column is sold under the production names such as ACQUITY UPLC HSS C18.
Comment 4 A copolymer cartridge is sold under the production names such as Oasis HLB 6cc (200 mg).

(**4**) **Test procedures**

(**4.1**) **Extraction:** Conduct extraction as shown below.

- **a**) Weigh 5.00 g of an analytical sample and put it into a 200-mL - 300-mL ground-in stopper Erlenmeyer flask.

b) Add 1 mL of sodium hydroxide solution (40 g/L) and 99 mL of methanol, shake to mix with a shaking apparatus for about 30 minutes.
c) After allowing to stand still, transfer the supernatant solution to a 50-mL ground-in stopper centrifugal precipitate tube.
d) Centrifuge at 1,300 × *g* for about 5 minutes (3) to make supernatant as the sample solution.

Note **(3)** 13-cm of rotor radius and 3,000 rpm of revolutions makes about 1,300 × *g* centrifugal force.

Comment 5 Grind until it completely passes through a sieve of 500 µm aperture to prepare the test sample.

(4.2) **Cleanup (1):** Conduct cleanup (1) as shown below.
a) Wash a cartridge column quickly with about 5 mL of methanol and 5 mL of water in advance.
b) Place a 50-mL round-bottom flask under the cartridge column, transfer 5 mL of the extract to the cartridge column and allow the extract to overflow quickly until the surface of the liquid reaches the top of the packing materials.
c) Add about 5 mL of sodium hydroxide solution (0.4 g/L) - methanol (1+1) to the cartridge column 2 times and allow the liquid to overflow in the same manner in **b)**.

Note **(4)** If there is a possibility to foam by the concentration procedure in **(4.3) b)**, a 100-mL round-bottom flask can be used.

(**4.3**) **Cleanup (2):** Conduct cleanup (2) as shown below.
a) Wash a new cartridge column quickly with about 5 mL of acetonitrile and 5 mL of hydrochloric acid (1+120) in advance.
b) After conducting vacuum concentration of the effluent in (**4.2**) **c**) until no more than 5 mL on a water bath at no more than 40 °C, add 3 mL of hydrochloric acid (1 +11).
c) Put the concentrated effluent into the cartridge column and allow the effluent quickly to overflow until the surface of liquid reaches the top of packing materials.
d) Wash a round-bottom flask with about 5 mL of hydrochloric acid (1+120) 2 times and add washing into the cartridge successively.
e) Then, add about 5 mL of hydrochloric acid (1+120) - acetonitrile (9+1) and about 5 mL of water into the cartridge successively and allow the liquid to overflow quickly.
f) Place a 5-mL volumetric flask under the cartridge column, add 4 mL of ammonia solution (0.0025 % (mass fraction)) - acetonitrile (9+1) to the cartridge column and allow clopyralid, aminopyralid and picloram to elute quickly.
g) Add formic acid (1+1,000) up to the marked line (5) and transfer it to 1.5-mL ground-in stopper centrifugal precipitate tube.
h) Centrifuge at 8,000 × *g* centrifugal force for about 5 minutes (7) to make supernatant as the sample solution.

Note **(5)** If there is a possibility that the clopyralid, aminopyralid and picloram concentration in the sample solution exceed the maximum limit of the calibration curve, dilute a predetermined amount of effluent with formic acid (1 +1,000).
(6) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(7) 7-cm of rotor radius and 10,000 rpm of revolutions makes about 8,000 × *g* centrifugal force.

Comment 6 The procedures in **(4.2)** and **(4.3)** should be quickly carried out by using a suction apparatus.

(4.4) Measurement: Conduct measurement according to JIS K 0136 and as shown below. Specific measurement procedures are according to the operation method of the High Performance Liquid Chromatograph/Mass Spectrometer used in measurement.

a) Measurement conditions of High Performance Liquid Chromatograph/Mass Spectrometer: Set up the measurement conditions considering the following example of measurement conditions for High Performance Liquid Chromatograph/Mass Spectrometer.

1) High Performance Liquid Chromatograph:

(i) Column: A silica gel column (2-mm - 3-mm inner diameter, 50-mm - 150-mm long, 1.6-µm - 2.2-µm particle diameter column) to which octadecyl chemically bonds.

(ii) Flow rate: 0.2 mL/min - 0.5 mL/min

(iii) Eluent: A: Formic acid (1+1,000) B: methanol

(iv) Gradient: 0 min (5 % B) → 5 min (60 % B) → 6 min (95 % B) → 7 min (5 % B)

(v) Temperature of column bath: 40 ºC

(vi) Injection volume: 5 µL

2) Mass Spectrometer:

(i) Ionization method: Electro Spray Ionization (ESI) method

(ii) Mode: Positive

(iii) Capillary voltage: 1.0 kV

(iv) Ion source temperature: 120 ºC

(v) Desolvation temperature: 400 ºC

(vi) Cone voltage: Shown in Table 1

(vii) Collision energy: Shown in Table 1

(viii) Monitor ion: Shown in Table 1

Table 1 Monitor ion, etc. of respective agrichemicals

Substance name	Precussor ion (m/z)	Product ion (Determination) (m/z)	Product ion (Validation) (m/z)	Cone voltage (v)	Collision energy (Determination) (eV)	Collision energy (Validation) (eV)
Clopyralid	192	146	110	20	20	30
Aminipyralid	207	161	189	22	22	16
Oicrloram	241	195	223	28	22	16

b) Calibration curve preparation

1) Inject 5 µL of respective mixture standard solutions for calibration curve into the LC-MS/MS, record the chromatogram of ion (*m*/*z*) for determination and ion (m/z) for validation of clopyralid, aminopyralid and picloram and obtain respective peak areas or heights.

2) Calculate the peak area ratio or height ratio of ion (*m*/*z*) for determination and ion (*m*/*z*) for validation of clopyralid, aminopyralid and picloram.

3) Prepare a curve for the relationship between the concentration of respective agrichemicals and the peak area or height of ion (*m*/*z*) for determination of respective mixture standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 5 µL of the sample solution to the same procedure as in **b) 2) - 3)** (8).

2) Obtain the content of material subjected to measurement from the calibration curve to calculate the concentration of material subjected to measurement in the analytical sample.

Note (8) Confirm that the ratio against the peak area ratio or height ratio of the standard solution is within the range of about ± 30 %. In addition, the peak area ratio or height ratio depends on the concentration.

Comment 7 Additive recovery testing of clopyralid, aminopyralid and picloram was conducted using cow dung compost (2 kinds), composted sludge fertilizers containing cow manure (2 kinds) and composted sludge fertilizers containing pig manure (1 kind), as a result, the mean recovery rates at additive level of 1000 µg/kg, 400 µg/kg and 40 µg/kg were 78.1 % - 90.0 %, 81.0 % - 117.6 % and 71.2 % - 101.3 % and the repeatability were 1.5 % - 8.7 %, 1.1 % - 5.1 % and 2.4 % - 10.7 % relative standard deviation.

Additionally, the minimum limits of quantification of clopyralid, aminopyralid and picloram of the test method are about 10 µg/ kg respectively.

Reference

1) Toshiharu YAGI, Yuko SEKINE, Yuji SHIRAI: Determination of Clopyralid in Fertilizer by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), Research Report of Fertilizer Vol. 3, 2010, (51 - 59)

(5) Flow sheet for clopyralid and its derivative substances: The flow sheet for clopyralid and its derivative substances in fertilizer is shown below

5.00 g analytical sample — 200-mL - 300-mL ground-in stopper Erlenmeyer flask

← 1 mL of sodium hydroxide solution (4 g/L)

← 99 mL of methanol

shaking — 30 minutes

Centrifugal separation — 50 mL centrifugal precipitate tube, 1,300 × *g*, 5 minutes

← 5 mL of extract

Cleanup (1) — copolymer cartridge column (1)
(Wash with about 5 mL of methanol, then 5 mL of water in advance)
Cleanup procedures should be quickly carried out
by using a suction apparatus.

[Collection, 50-mL eggplant-shaped flask]

← About 5 mL of sodium hydroxide solution (0.04 w/v %) - methanol [1+1] × 2 times

[Collection, same as above vessel]

Vacuum concentration — 40 °C

← 3 mL of hydrochloric acid (1+11)

Cleanup (2) — copolymer cartridge column (1)
(Wash with about 5 mL of acetonitrile,
then 5 mL of hydrochloric acid in advance)
Clean up procedures should be quickly carried out
by using a suction apparatus.

← Wash the vessel 2 times with about 5mL of hydrochloric acid (1+120)

← Wash with about 5mL of hydrochloric acid (1+120) -acetonitrile [9+1]

← Wash with about 5mL of water

← 4 mL of ammonia solution (0.0025 w/w %)- acetonitrile [9+1]

[Elute, 5-mL volumetric flask]

Filling up

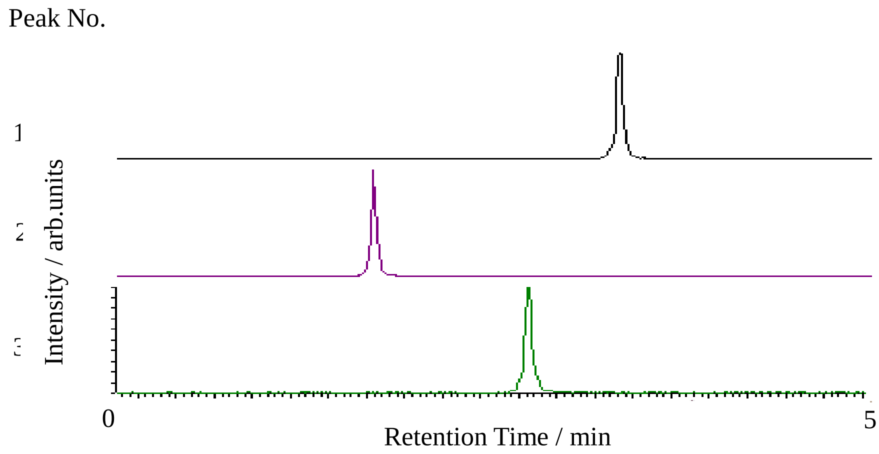
← Formic acid (1+1,000) (up to the marked line)

Centrifugal separation — 1.5-mL ground-in stoppered centrifugal precipitate tube,
8,000 × *g* , 5 minutes

Measurement — LC-MS/MS

Figure Flow sheet for clopyralid and its derivative substances in fertilizers

References Selected Reaction Monitoring chromatograms of clopyralid standard solution for calibration curve preparation and sample solution (cow dung compost) are shown below



1) Standard solution 2) Sample solution

Peak No1: Picloram
No2: Aminopyralid
No3: Clopyralid

Reference diagram: SRM chromatograms of respective agrichemicals

Mixture standard solution (the equivalents of 200 pg as respective agrichemicals)

Measurement conditions of LC-MS/MS

Column: ACQUITY UPLC HSS C18 (2.1-mm inner diameter, 100-mm long, 1.8-µm particle diameter)

Other conditions are according to the examples of the measurement conditions in (**4.4**) **a**) LC-MS/MS.

8.3 Residue agrichemicals multi component analysis

8.3.a High Performance Liquid Chromatography/Tandem Mass Spectrometry

(1) Compounds subjected to analysis: Abamectin: Abamectin B1a, ivermectin: 22, 23-dihydro avermectin B1a (Another name: ivermectin B1a), eprinomectin: eprinomectin B1a, rotenone: rotenone, piperonylbutoxide: piperonylbutoxide, pyrethrin: pyrethrin I and pyrethrin II

(2) Summary

This method is applicable to fluid home garden-use mixed fertilizer and fluid mixed fertilizer. Dissolve respective agricultural chemicals in fertilizers with acetonitrile and water, and extract. Refine by using 2 kinds of cleanup cartridge, and then measure with a High Performance Liquid Chromatograph/Mass Spectrometer.

(3) Reagents, etc.: Reagents and water are as shown below:

a) **Water:** Water of A3 specified in JIS K 0557or equivalent quality.

b) **Acetonitrile:** A regent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8039 or a reagent of equivalent quality.

c) **Methanol:** A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.

d) **Methanol:** Methanol used in eluent of HPLC is a regent of LC/MS analysis grade or equivalents.

e) **Ethyl acetate:** A JIS Guaranteed Reagent specified in JIS K 8361 or a reagent of equivalent quality.

f) **Toluene:** A JIS Guaranteed Reagent specified in JIS K 8680 or a reagent of equivalent quality.

g) **Ammonium formate:** A JIS Guaranteed Reagent (no less than 95 % (mass fraction) in purity) or a reagent of equivalent quality.

h) **Ammonium formate solution (0.1 mol/L)** [(1)]**:** Add 6.306 g of ammonium formate into 1,000 mL of water

i) **Ammonium formate solution (0.1 mmol/L)** [(1)]: Add 1mL formic acid ammonium solution (0.1 mol/L) into 1,000 mL water

j) **Formic acid**: A JIS Guaranteed Reagent specified in JIS K 8264 or a reagent of equivalent quality.

k) **Formic acid solution (0.1 v/ v %)** [(1)]**:** Add 1 mL of formic acid to 1,000 mL of water

l) **Acetonitrile formate solution (0.1 v/v %)** [(1)]**:** Add 1 mL of formic acid to 1,000 mL of acetonitrile

m) **Respective agricultural chemicals standard solutions (0.1 mg/mL)** [(1)]**:** Put about 0.01 g of abamectin [$C_{48}H_{72}O_{14}$] [(2)], ivermectin [$C_{48}H_{74}O_{14}$] [(2)], eprinomectin [$C_{50}H_{75}NO_{14}$] [(2)], rotenone [$C_{23}H_{22}O_6$] [(2)], piperonylbutoxide [$C_{19}H_{30}O_5$] [(2)] and pyrethrin [pyrethrin I: $C_{21}H_{28}O_3$ and pyrethrin II: $C_{22}H_{28}O_5$] [(2)] to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve with a small amount of methanol, transfer to a 100-mL volumetric flask and add the solvent up to the marked line. (However 0.1 mg/mL of pyrethrin contains total of pyrethrin I/II)

n) **Mixture standard solution (10 µg/mL):** Transfer 10 mL of respective agricultural chemicals standard solutions to a 100-mL volumetric flask and add methanol up to the marked line.

o) **Mixture standard solution (1,000 ng/mL):** Transfer 10 mL of mixture standard solution (10 µg/mL) to a 100-mL volumetric flask and add methanol up to the marked line.

p) Mixture standard solution for calibration curve preparation (50 ng/mL - 500 ng/mL): In the case of usage, transfer 2.5 mL - 25 mL of mixture standard solution (1,000 ng/ mL) to 50-mL volumetric flasks step-by-step, and add methanol up to the marked line.

q) Mixture standard solution for calibration curve preparation (5 ng/mL - 50 ng/mL): In the case of usage, transfer 2.5 mL - 25 mL of mixture standard solution (100 ng/mL) to 50-mL volumetric flasks step-by-step, and add methanol up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) A standard reagent is commercially sold.

Comment 1 A standard reagent of respective agricultural chemicals is sold by Wako Pure Chemical Industries., Ltd, Kanto Chemical Co., Inc. and Hayashi Pure Chemical Industries., Ltd.

(4) Apparatus and instruments: Apparatus and instruments are as shown below.

a) High Performance Liquid Chromatograph/Mass Spectrometer (LC/MS/MS): LC/MS/MS specified in JIS K 0136 that satisfies the following requirements.

1) High Performance Liquid Chromatograph:
- (i) Column bath: A column bath whose temperature can be adjusted at 30 ºC - 45 ºC.
- (ii) Column: A 2-mm - 3-mm inner diameter 50-mm - 150-mm long 1.6-µm - 3.0-µm particle diameter stainless steel column tube filled with silica gel, to which octadecyl chemically bonds. The specification is according to the mass spectrometer specification (3).

2) Mass Spectrometer:
- (i) Ionization method: Electro Spray Ionization (ESI)
- (ii) Ion detection method: Selected Reaction Monitoring

b) Ultrasonic generator: An ultrasonic washer can be used.

c) Concentrator: An evaporator that can keep test temperature ± 2 ºC

d) Porous diatomaceous earth cartridge column: A column that is filled with the porous diatomaceous earth (capacity: 5 mL) (4)

e) Graphite carbon-NH_2 laminate cartridge column: A 6-mL cylinder on which 500 mg of graphite carbon and 500 mg of aminopropyl silylation silica gel is laminated (5).

Note **(3)** The column is sold under the names of ACQUITY UPLC HSS C18, etc.
(4) The column is sold under the names of Chem Elut (5 mL), etc.
(5) The column is sold under the names of Envi-carb/LC-NH_2 (500 mg/500 mg, 6 mL), etc.

(5) Test procedures

(5.1) Extraction: Conduct extraction as shown below.

a) Put about 5.00 mL (6) of an analytical sample into a 10-mL volumetric flask.

b) Add 3 mL of acetonitrile to the same volumetric flask, and add water up to the marked line to shake to mix well.

c) Conduct ultra-sonication for about 30 minutes using an ultrasonic generator (7) to make the extract.

Note **(6)** After measuring the specific gravity of sample, calculate the concentration of materials subjected to measurement in the analytical sample.

(7) Note that the volume of solution may expand as a result of ultra-sonication. It is recommended to leave it at room temperature for a while when it expands.

Comment 2 The specific gravity (density) can be calculated by placing a 10-mL volumetric flask on an electric balance, aligning the scale to zero, transferring 5.00 mL of the analytical sample to the volumetric flask and reading the weighing value.

(5.2) Cleanup (1): Conduct cleanup (1) as shown below.

a) Put 5 mL of extract into a porous diatomaceous earth cartridge column and keep it in the column for about 5 minutes.

b) Place a 100-mL round-bottom flask under the same cartridge column, add about 5 mL of ethyl acetate into the same cartridge column 4 times successively and allow the solution to elute until the surface of solution reaches the top of packing materials (8).

c) After conducting vacuum concentration of elute in a water bath of no more than 40 ºC until most of the elute dries up, send a nitrogen gas to dry up the elute **(9)**, and add 2 mL of acetonitrile-toluene (3+1) to dissolve the residue.

Note **(8)** Confirm the solution to elute before conducting the testing.

(9) There is a possibility for agricultural chemicals to vaporize if it is dried up excessively.

(5.3) Cleanup (2): Conduct cleanup (2) as shown below.

a) Wash the graphite carbon-NH_2 laminate cartridge column with about 10 mL of acetonitrile - toluene (3+1) in advance

b) Place a 100-mL round-bottom flask under the same cartridge column, put the solution in (**5.2**) **c**) to the same cartridge column, and allow the solution to overflow until the surface of solution reaches the top of packing materials.

c) Wash the vessel with about 5 mL of acetonitrile-toluene (3+1) 5 times and add washing to the same cartridge successively to allow it to overflow.

d) After conducting vacuum concentration of elute in a water bath of no more than 40 ºC until most of the elute dries up, send a nitrogen gas to dry up the elute **(10)**, and add 2 mL of methanol to dissolve the residue. Transfer a predetermined amount of the solution precisely and dilute with methanol exactly 5 times to make the solution as the sample solution.

Note **(10)** There is a possibility for agricultural chemicals to vaporize if it is dried up excessively.

(11) If there is a possibility that the concentration of agricultural chemicals in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of the sample solution with methanol.

(5.4) Measurement: Conduct measurement according to JIS K 0136 and as shown below. Specific measurement procedures are according to the operation method of High Performance Liquid Chromatograph/Mass Spectrometer used in measurement.

a) **The measurement conditions of High Performance Liquid Chromatograph/Mass spectrometer:** Set up the measurement conditions for High Performance Liquid Chromatograph/Mass Spectrometer considering the following:

1) **High Performance Liquid Chromatograph**:

(i) **Column:** A silica gel column (2-mm - 3-mm inner diameter, 50-mm - 150-mm long, 1.6-µm - 3.0-µm particle diameter column) to which octadecyl chemically bonds.
(ii) Flow rate: 0.2 mL/min - 0.5 mL/min
(iii) Eluent: A: Ammonium formate solution (0.1 mmol/L) - formic acid solution (0.1 v/v %) [1+1]
B: Acetonitrile formate solution (0.1 v/v %)
(iv) Gradient: 0 min (50 % B) → 15 min (95 % B) → 20 min (98% B) → 30 min (50 % B)
(v) Temperature of column bath: 40 ºC
(vi) Injection volume: 5 µL

2) Mass Spectrometer:
(i) Ionization method: Electro Spray Ionization (ESI) method
(ii) Mode: Positive
(iii) Capillary voltage: 3.0 kV
(iv) Ion source temperature: 120 ºC
(v) Desolvation temperature: 400 ºC
(vi) Cone voltage: Shown in table 1
(vii) Collision energy: Shown in table 1
(viii) Monitor ion: Shown in table 1

Table 1 Monitoring ion conditions, etc. of respective agrichemicals

Agrichemicals	Precursor ion (*m/z*)	Product ion (determination) (*m/z*)	Product ion (validation) (*m/z*)	Cone voltage (V)	Collision energy (eV)
Abamectin B1a	891	305	567	20	25
Ivermectin B1a	893	307	551	25	25
Eprinomectin B1a	915	186	298	20	20
Rotenone	395	213	192	35	25
Piperonylbutoxide	356	177	147	20	15
Pyrethrin Ⅰ	329	161	133	20	10
Pyrethrin Ⅱ	373	161	133	20	10

b) Calibration curve preparation

1) Inject 5 µL of respective mixture standard solutions for calibration curve preparation into the LC/MS/MS, record the chromatogram of ion (*m*/*z*) for determination and ion (*m*/*z*) for validation of materials subjected to measurement and obtain respective peak areas or heights.

2) Calculate the peak area ratio or height ratio of ion (*m*/*z*) for determination and ion (*m*/*z*) for validation of materials subjected to measurement.

3) Prepare a curve for the relationship between the concentration of materials subjected to measurement and the peak area or height of ion (*m*/*z*) for determination of respective mixture standard solutions for the calibration curve preparation.
Prepare a calibration curve when the sample is measured.

c) Sample measurement

1) Subject 5 µL of the sample solution to the same procedure as in **b) 2) - 3)** [12]**.**

2) Obtain the content of materials subjected to measurement from the calibration curve of the peak area or height to calculate the concentration of materials subjected to measurement in the analytical sample.

Note (12) Confirm that the ratio against the peak area ratio or height ratio of the standard solution is within the range of about ± 30 %. In addition, the peak area ratio or height ratio depends on the concentration.

(5.5) Calculation

Calculate the respective concentration of agricultural chemicals in the analytical sample by the following formula.

Respective concentration of agricultural chemicals in the analytical sample (µg/kg)
= $(A \times B \times 10)/C$

A: Concentration (ng/mL) of respective materials subjected to measurement in the final sample solution obtained from the calibration curve

B: Dilution factor in the case that the final sample solution is further diluted because it exceeds the upper limit of the calibration curve.

C: Specific gravity of the analytical sample (density) (g/mL)

Comment 3 Recovery testing was conducted using fluid home garden-use mixed fertilizer (3 kinds) and fluid mixed fertilizer (2 kinds), as a result, the mean recovery rate at additive level of 4,000 µg/kg and 400 µg/kg (pyrethrin, however, is 4,000 µg/kg and 400 µg/kg as a total amount of pyrethrin I/II) were 77.0 % - 107.9 % respectively, and the repeatability was 0.1 % - 9.1 % relative standard deviation.

In addition, the results and analysis results of a collaborative study for test method validation are shown in Table 2.

Additionally, the minimum limit of quantification of the test method is about 10 µg/ kg.

Table 2　Analysis results of a collaborative study for the testing method validation of multicomponent analysis of pesticide residue

Agrichemicals	Sample name	Number of laboratories[1)]	Mean[2)] (μg/kg)	Additive amount (μg/kg)	Recovery (%)	RSD_r[3)] (%)	RSD_R[4)] (%)
Abamectin B1a	Home garden-use mixed fertilizer1	8	286.8	333.3	86.1	13.3	14.4
	Home garden-use mixed fertilizer2	8	358.9	416.7	86.1	13.4	14.8
	Home garden-use mixed fertilizer3	8	425.8	500.0	85.2	8.6	11.6
	Fluid mixed fertilizer1	8	288.6	333.3	86.6	7.1	8.5
	Fluid mixed fertilizer2	8	405.5	500.0	81.1	7.1	7.2
Ivermectin B1a	Home garden-use mixed fertilizer1	8	298.9	333.3	89.7	14.9	15.0
	Home garden-use mixed fertilizer2	8	382.5	416.7	91.8	14.1	19.3
	Home garden-use mixed fertilizer3	8	431.1	500.0	86.2	9.8	10.9
	Fluid mixed fertilizer1	8	298.8	333.3	89.6	10.1	12.8
	Fluid mixed fertilizer2	8	405.2	500.0	81.0	3.8	5.8
Eprinomectin B1a	Home garden-use mixed fertilizer1	8	293.5	333.3	88.1	7.0	10.4
	Home garden-use mixed fertilizer2	8	361.9	416.7	86.9	9.2	14.3
	Home garden-use mixed fertilizer3	8	425.3	500.0	85.1	7.0	10.0
	Fluid mixed fertilizer1	8	277.3	333.3	83.2	9.0	12.0
	Fluid mixed fertilizer2	8	398.2	500.0	79.6	7.5	11.6
Rotenone	Home garden-use mixed fertilizer1	8	276.8	333.3	83.1	5.7	7.8
	Home garden-use mixed fertilizer2	8	353.5	416.7	84.8	9.8	12.5
	Home garden-use mixed fertilizer3	8	426.6	500.0	85.3	6.6	8.5
	Fluid mixed fertilizer1	8	263.5	333.3	79.1	11.0	12.3
	Fluid mixed fertilizer2	8	385.2	500.0	77.0	5.7	12.1
Piperonylbutoxide	Home garden-use mixed fertilizer1	8	318.2	333.3	95.5	8.1	13.2
	Home garden-use mixed fertilizer2	8	395.6	416.7	94.9	8.4	13.6
	Home garden-use mixed fertilizer3	8	450.3	500.0	90.1	4.6	9.3
	Fluid mixed fertilizer1	8	299.7	333.3	89.9	7.4	11.0
	Fluid mixed fertilizer2	8	435.8	500.0	87.2	5.8	7.4
Pyrethrin I	Home garden-use mixed fertilizer1	8	160.7	186.0	86.4	9.3	11.9
	Home garden-use mixed fertilizer2	8	202.2	232.5	87.0	12.6	12.8
	Home garden-use mixed fertilizer3	8	228.6	279.0	81.9	5.4	8.8
	Fluid mixed fertilizer1	8	158.2	186.0	85.1	6.8	10.4
	Fluid mixed fertilizer2	8	223.1	279.0	80.0	8.5	9.1
Pyrethrin II	Home garden-use mixed fertilizer1	8	131.1	147.3	89.0	6.5	9.7
	Home garden-use mixed fertilizer2	8	163.2	184.2	88.6	10.8	13.6
	Home garden-use mixed fertilizer3	8	182.0	221.0	82.4	5.4	8.9
	Fluid mixed fertilizer1	8	126.2	147.3	85.7	7.8	11.4
	Fluid mixed fertilizer2	8	180.2	221.0	81.5	6.3	8.3

1) Number of laboratories used in analysis
2) Mean (n= number of laboratories x number of samples (2))
3) Repeatability (relative standard deviation)
4) Reproducibility (relative standard deviation)

Reference

1) Toshiharu YAGI, Masayuki YAMANISHI, Yuji SHIRAI: Simultaneous Determination of Agricultural chemicals in Fluid Fertilizer by Liquid Chromatography/Tandem Mass Spectrometry, Research Report of Fertilizer,Vol.4, 2011, (36 - 48)
2) Toshiharu YAGI, Masayuki YAMANISHI, Yuji SHIRAI and Masato SHIBATA: Simultaneous Determination of Six Kind of Agricultural Chemicals in Fluid Fertilizer by Liquid Chromatograph-Tandem Mass Spectrometer (LC-MS/MS): A Collaborative Study, Research Report of Fertilizer,Vol.5, 2012, (48 - 59)

(6) Flow sheet for simultaneous analysis of 6 kinds of agrichemicals: The flow sheet for simultaneous analysis of 6 kinds of agrichemicals in fertilizer is shown below.

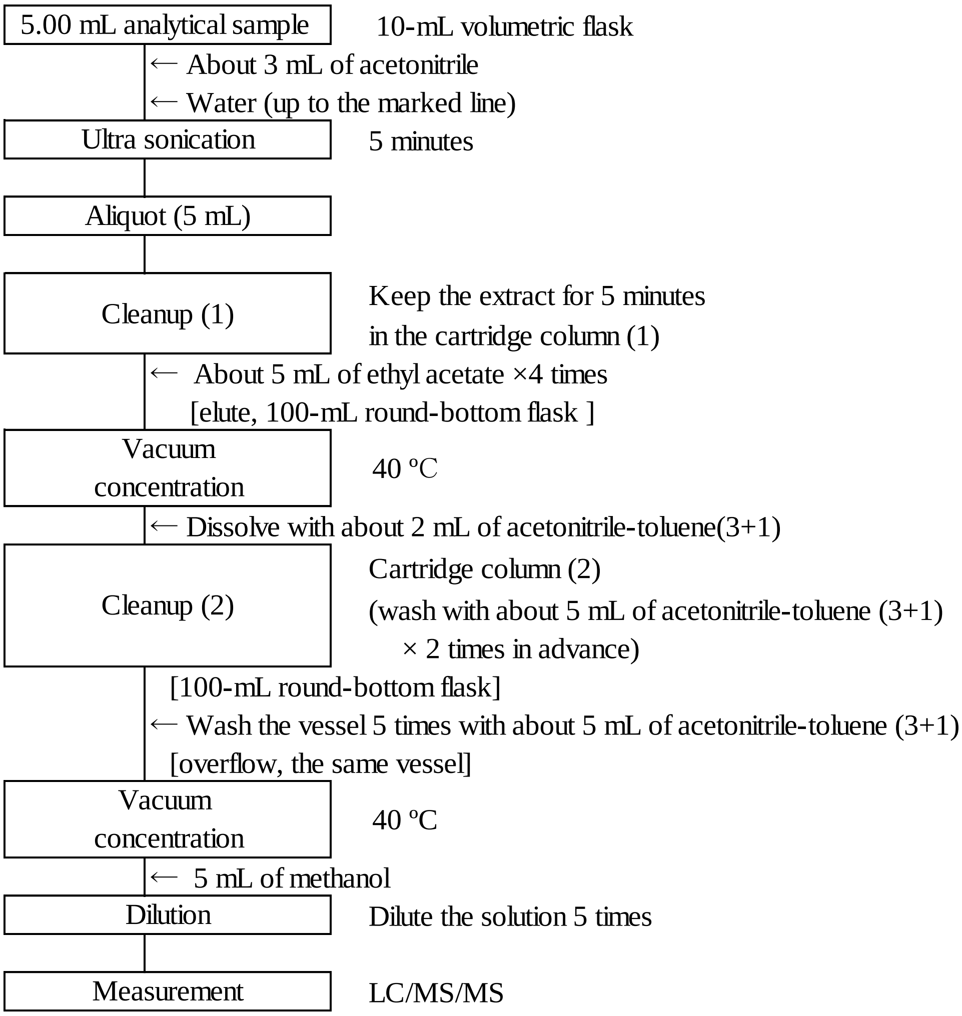
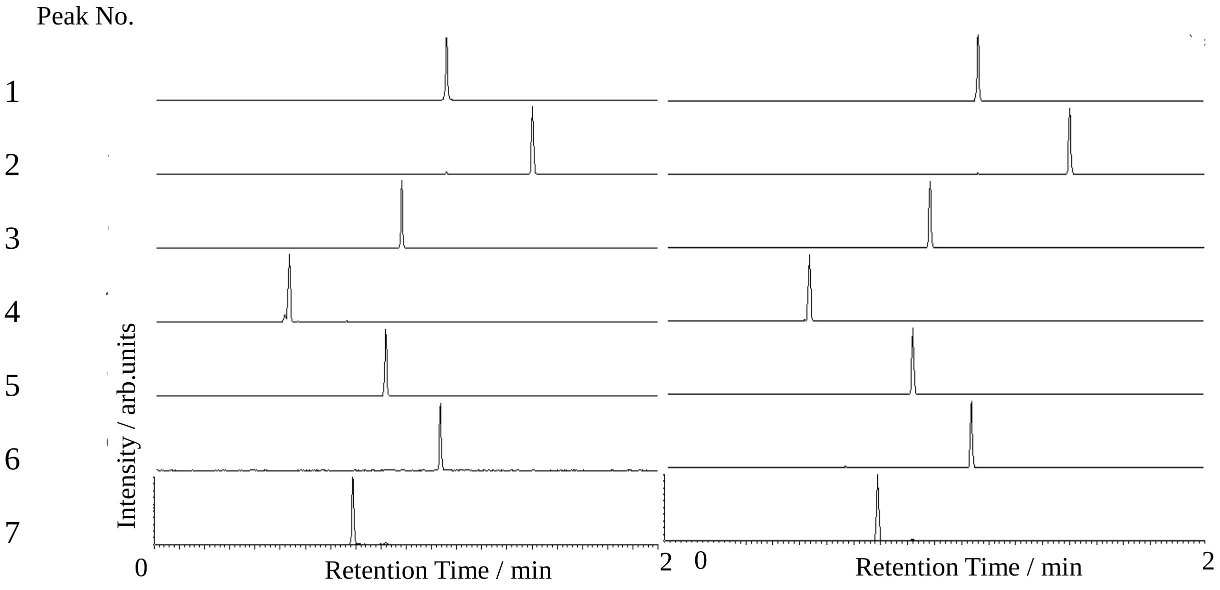


Figure Flow sheet for simultaneous analysis of 6 kinds of agrichemicals in fertilizers

Reference Selected Reaction Monitoring chromatograms of mixture standard solution for calibration curve preparation and sample solution (fluid home garden-use mixed fertilizer) are shown below.



Peak No.1: AbamectinB1a
No.2: IvermectinB1a
No.3: Eprinomectin B1a
No.4: Rotenone
No.5: Piperonylbutoxide
No.6: Pyrethrin I
No.7: Pyrethrin II

1) Mixture standard solution 2) Sample solution

Reference diagram Selected Reaction Monitoring chromatograms of respective agricultural chemicals

1) Mixture standard solution (the equivalents of 2,500 pg of respective agricultural chemicals)
(For pyrethrin, the equivalents of 2,500 pg of the total of pyrethrin I/II)
2) Sample solution (fluid home garden-use mixed fertilizer, additive of the equivalents of 400 µg/ kg in the sample)
(For pyrethrin, the equivalents of 400 µg/kg of the total of pyrethrin I/II)

Measurement conditions of LC/MS/MS
Column: ACQUITY UPLC HSS C18 (2.1-mm inner diameter, 100-mm long, 1.8-µm particle diameter)
Flow rate: 0.2 mL/min
Other conditions are according to the examples of the measurement conditions of (**5.4**) **a**) LC/MS/MS.

Appendix. The procedure to validate characteristics of testing methods

(1) Purposes

This article explains the procedure to validate characteristics of testing methods which will be listed in the Testing Methods of Fertilizers. In addition, when testing institutes conduct a test which is not included in the Testing Methods of Fertilizers, a procedure to evaluate the validity of the test method should conform to a method stipulated in this article.
Additionally, this article targets chemical testing methods. However, this article is not applicable to the extraction method of the content of effective figures (acid- , alkaline- , citrate- and water-soluble) in a powdery sample or a solidified fertilizer.

Comment 1 The contents of effective figures (acid- , alkaline- , citrate- and water-soluble) are stipulated in a notification of the Ministry of Agriculture, Forestry and Fisheries. In addition, the change of measurement conditions such as an extraction temperature may affect an observed value in some cases. Therefore, no changes will be implemented in the extraction method of the contents of effective figures in a powdery fertilizer and a solidified fertilizer for the present and the application of this article is limited to the change of a measurement method (including refining of extract, etc.).

(2) Definition of terminology

The definition of terminology in this article is as shown below.

- **a) Selectivity:** Capability to accurately measure components subjected to analysis under the existence of materials which seem to exist in a sample.
- **b) Trueness:** The degree of agreement between the mean obtained from multiple measurement results and the true value [(1)].
- **c) Precision:** The degree of agreement among the independent measurement results which are repeatedly measured under the determined conditions.
- **d) Repeatability:** The precision of the measurement results of analytical samples, which are regarded to be all identical, obtained under condition (repeatability conditions) that independent measurement results are measured in a short time, using the same method, in the same laboratory, by the same operator and with the same instrument.
- **e) Intermediate precision:** The precision of a measurement result of analytical samples, which are regarded to be all identical, obtained under condition (intermediate conditions) that independent measurement results are measured, using the same method, in the same laboratory and in different factors (such as different time and a different operator).
- **f) Reproducibility:** The precision of a measurement result of analytical samples, which are regarded to be all identical, obtained under condition (reproducibility conditions) that independent measurement results are measured, using the same method, in different laboratories, by different operators and with different instruments.
- **g) Minimum Limit of Quantification (LOQ):** The quantifiable lowest volume or minimum concentration of a component subjected to analysis which is contained in an analytical sample.
- **h) Minimum Limit of Detection (LOD):** The detectable lowest volume or minimum concentration of a component subjected to analysis which is contained in an analytical sample.
- **i) Reference material:** A material which is uniform and stable enough for one or more prescribed properties, and is made suitable for the purpose of use in a measurement process.

- **j)** **Certified reference material:** A reference material, whose values of one or more prescribed properties are characterized by a reasonable metrological procedure, having a certificate of attestation on which the characteristics of prescribed properties and their uncertainty and metrological traceability are stated.
- **k)** **Blank sample:** An analytical sample not containing components subjected to analysis [(2)].
- **i)** **Addition sample:** An analytical sample the content of whose components subjected to analysis is known, or an analytical sample to which reference materials are added [(3) (4)] or compounded [(3)].
- **m)** **Natural contamination sample:** An analytical sample prepared from fertilizers which naturally contain the components subjected to analysis such as harmful components.
- **n)** **Distribution sample:** An analytical sample prepared from fertilizers[(5)] which are manufactured in a fertilizer production factory, etc.
- **o)** **Surrogate:** A material which is added to an analytical sample in order to conduct a pre-process operation, correct yields in respective steps of analysis operation and confirm recovery, whose chemical structure is identical or similar to a target component.
- **p)** **SN ratio:** Intensity ratio of a signal (response value) S originated from the analysis target and a signal (usually noise) N based on the other factors.

Note **(1)** In reality, the certified value of a certified reference material, the chemical composition of a compound, the added content of a reference material, etc. and others.

(2) Regents, etc. containing a target matrix can be used in the case that there is no distribution fertilizer used as a blank sample for a recovery test and the confirmation of the minimum limit of quantification, etc.

(3) Mix a component subjected to analysis with a mortar, etc. to sufficient uniformity

(4) In the case of adding standard solution, vaporize the solvent sufficiently conducting measures such as letting it stand for one night.

(5) A fertilizer containing components subjected to analysis whose formation or form changed due to a chemical or physical process (a granulation process, etc.).

References

1) JIS K 0211: Technical terms for analytical chemistry (General part) (2013)

2) JIS K 0214: Technical terms for analytical chemistry (Chromatography part) (2013)

3) JIS Q 0035: Reference materials−General and statistical principles for certification (2008)

4) JIS Z 8101-2: Statistics−Vocabulary and symbols−Part 2: Statistical quality control terms (1999)

5) JIS Z 8402-1: Accuracy (trueness and precision) of measurement methods and results - Part 1: General principles and definitions (1999)

6) ALINORM 09/32/23 Joint FAO/WHO Food Standards Programme: Repot of the Thirtieth Session of the Codex Committee on Methods of Analysis and Sampling, Codex Almentarius Commission Thirty-second Session (2009)

7) ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2005)

(3) Validation method

Test necessary items of **(3.1)** to **(3.8)** in a planned manner and estimate performance parameters from the obtained results.
Confirm whether the estimated values of performance parameters are suitable to target values (performance norm) respectively, and evaluate that the test method is validated if they are all suitable.

(3.1) Scope of application

As a result of a validation test in a single laboratory and a collaborative study, if the result is suitable up to reproducibility, the test method is evaluated as a validated test method as far as the kind of a fertilizer used in the test and the range of concentration are concerned. Therefore a laboratory where the said test is conducted can use the performance (reproducibility, etc.) as a validated method through implementing internal quality control, etc.

As a result of a validation test in a single laboratory, if the result is suitable to trueness, repeatability and intermediate precision, etc., the test method is evaluated as a validated test method as far as the laboratory where the test was conducted and as far as the kind of a fertilizer used in the test and the range of concentration are concerned. Therefore another laboratory which wants to introduce the test method is required to carry out the validation anew in an individual laboratory with the above test method.

(3.2) Selectivity

(3.2.1) Case of Chromatography

Conduct a procedure for a blank sample and confirm that there is no peak (interference peak) which affects the measurement of components subjected to analysis [(6)]. In addition, in the case of the simultaneous measurement of multi components, confirm that adjacent peaks are sufficiently separated [(6)].

Note (6) Resolution (R) should be 1.0 or more at minimum though 1.5 or more is preferable.

Comment 2 Resolution (R) is used as a separation indicator of peaks. If Resolution (R) is 1.5 or more, the adjacent two peaks are sufficiently separated and they do not affect a measurement, whether a peak height or a peak area is used. If Resolution (R) is 1.0 or more, a measurement using a peak height is not a problem though the adjacent two peaks may overlap to some degree.
Resolution (R) can be obtained using a peak width by the formula (1a). In addition, if the peak is a normal distribution, it can be obtained using a peak width at half height by the formula (1b). With the data processing device of a chromatograph, the formula (1b) is often used to obtain Resolution (R).

$$\text{Resolution } (R) = \frac{t_2 - \mathrm{t}_1}{\frac{1}{2} \times (\mathrm{W}_1 + \mathrm{W}_2)} \qquad \cdots (1a)$$

$$\text{Resolution}(R) = \frac{1.18 \times (t_2 - t_1)}{\left(W_{\frac{1}{2},1} + W_{\frac{1}{2},2}\right)} \qquad \cdots (1b)$$

t_1: Retention time of Peak 1 t_2: Retention time of Peak 2
W_1: Peak width of Peak 1 W_2: Peak width of Peak 2

$W_{\frac{1}{2},1}$: Peak width at half height of Peak 1
$W_{\frac{1}{2},2}$: Peak width at half height of Peak 2

(3.2.2) Case of a method other than Chromatography [7]

Conduct a procedure for a blank sample and confirm that there is no response which originates from other components than a component subjected to analysis and can be a factor of positive error of a quantification value [8].

Note **(7)** A test method such as Molecular absorption spectrometry, Atomic absorption spectrometry or Titration analysis which does not isolate with a measurement instrument.
(8) Absorbance, titer, etc.

References

1) AOAC Official Methods of Analysis Appendix K: Guidelines for Dietary Supplements and Botanicals, AOAC INTERNATIONAL (2012)
2) JIS K 0114: General rules for gas chromatography (2012)
3) JIS K 0124: General rules for high performance liquid chromatography (2011)
4) The notification by the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, the Ministry of Health, Labour and Welfare: "Guideline on Bioanalytical Method Validation in Pharmaceutical Development", July 11, 2013, *Yaku-shoku-Sinsa-Hatsu*-0711 No.1 (2013)

(3.3) Calibration curve

Measure respective standard solutions for the calibration curve preparation of the concentration or the content [9] of level 6 to 8 a few times [10] to make a figure plotting the obtained signals [11] as a function of the concentration or the content of a component subjected to analysis and evaluate its linearity visually using the figure.
If linearity is recognized, calculate the inclination (b) and the intercept (a) of a calibration curve, its confidence interval and the coefficient of determination (r^2) using a statistical method such as the calculation of a regression equation by the least square method. Moreover make the plot of residuals [12] in respective levels.

Note **(9)** Blank test solution for the calibration curve preparation can be included.
(10) In order to avoid nonlinear confusion due to the variation of sensitivity, etc., conduct measurements randomly for each replicate determination.
(11) Absorbance, fluorescence intensity, peak height, peak area, etc.
(12) The difference between a signal obtained by measurement and a signal estimated using a regression equation.

Comment 3 It is recommended that the 95% confidence interval of an intercept (a) includes the origin (0).
Comment 4 Though it is usable if the coefficient of determination (r^2) is 0.99 or more, it is recommended that the coefficient of determination (r^2) is 0.999 or more for a precise analysis. If it is less than 0.99, use the equation of a higher order or study the conversion of a numerical value.
Comment 5 The mean of residuals is 0 and the residuals indicate a random pattern.

References

1) AOAC Official Methods of Analysis Appendix K: Guidelines for Dietary Supplements and Botanicals, AOAC INTERNATIONAL (2012)

2) Thompson, M., Ellison, S.L.R, Wood, R., Harmonized guidelines for single-laboratory validation of methods of analysis, Pure & Appl. Chem. 74 (5), 835–855 (2002)

3) CLSI EP9 A2 Ed. 2, Method Comparison and Bias Estimation Using Patient Samples, Clinical and Laboratory Standards Institute (2002)

(3.4) Trueness

As the estimation method of trueness, the methods are recommended in the following order. (1) Use of a certified reference material **(3.4.1)**, (2) Comparison with an observed value by a validated method **(3.4.2)** and (3) Recovery test **(3.4.3)**.
In addition, if a surrogate is used, it is recommended that a recovery is about 40% or more.

(3.4.1) Use of a certified reference material

With regard to a component which has matrixes similar to a fertilizer subjected to test and can use a certified reference material containing components subjected to measurement of the concentration in a measurement level, conduct repeatability testing using 3 or more analytical samples (n) according to the test method of the certified reference material. As a result, the mean of the observed values should be within the warning level to the certified value (characteristic value) or the absolute value of the difference between the mean of the observed values and the certified value (characteristic value) should not exceed 2 times of the standard uncertainty composed of respective uncertainties of the mean of the observed values and the certified value [13].

Comment 6 A warning limit is given using the formula (2) which is obtained from a collaborative study for the characterization of a certified reference material.

A warning limit for a certified value (μ)

$$= \mu \pm 2 \times \sqrt{(s_R{}^2 - s_r{}^2) + \frac{s_r{}^2}{n}} = \mu \pm 2 \times \sqrt{s_L{}^2 + \frac{s_r{}^2}{n}} \qquad \cdots (2)$$

μ: Certified value
s_R: Reproducibility standard deviation in a collaborative study
s_r: Repeatability standard deviation in a collaborative study [14]
n: The number of analytical samples to repeatability test
s_L: Pure between-laboratory standard deviation in a collaborative study

Note (13) The evaluation procedure of the difference between a measurement result and a certified value (characteristic value) is shown in Reference 1.
(14) It may be expressed as within-laboratory standard deviation (s_W) in some cases.

(3.4.2) Use of another validated test method

For a component for which a certified reference material is not usable but another validated test method (hereinafter referred to as “a standard test method”) is applicable, confirm that the condition **a)** or **b)** is satisfied.

a) In case 12 or more samples are available: Conduct respective tests of 12 or more test samples composed of addition samples, natural contamination samples or distribution samples according to a new test method and a standard test method, create the correlation

chart of observed values with two methods for each sample and calculate the inclination (*b*) and the intercept (*a*) of a regression line, and a correlation coefficient (*r*). Further confirm a prediction interval.
However, in case that the width between the minimum and the maximum observed value is small, conduct the paired samples *t*-test to confirm that a significant difference is not observed.

Comment 7 It is recommended that the 95% confidence interval of an inclination (*b*) includes 1, the 95% confidence interval of an intercept (*a*) includes the origin (0) and the correlation coefficient (*r*) is no less than 0.99.

b) In case fewer samples are available: With regard to 3 or more test samples of different concentration, conduct respective repeatability addition tests using 4 analytical samples according to the new test method and a standard test method, confirm the homoscedasticity of the results of 2 groups and conduct a *t*-test for each concentration to confirm that significant difference is not observed under the two-sided significant level of 5%.

(3.4.3) In case neither certified reference material nor other validated test methods are usable

For 3 or more test samples of different concentration, conduct respective repeatability tests using 3 analytical samples and evaluate by obtaining the recovery using the mean of the observed values. The criteria of the trueness are shown in a Separate sheet.

References

1) AOAC Official Methods of Analysis Appendix K: Guidelines for Dietary Supplements and Botanicals, AOAC INTERNATIONAL (2012)

2) Thompson, M., Ellison, S.L.R, Wood, R.,: Harmonized guidelines for single-laboratory validation of methods of analysis, Pure & Appl. Chem. 74 (5), 835–855 (2002)

3) Linsinger, T.,: Comparison of a measurement result with the certified value, European Reference Materials' application note 1, European Commission - Joint Research Centre Institute for Reference Materials and Measurements (IRMM) (2010)

4) Joint FAO/WHO Food Standards Programme: Procedural manual Twenty-second edition, Codex Almentarius Commission (2014)

5) ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2005)

(3.5) Precision

Evaluate reproducibility and repeatability by a collaborative study **(3.5.1)**. Or evaluate an intermediate precision and repeatability by a repeatability test **(3.5.2)**.

(3.5.1) Reproducibility and repeatability by a collaborative study

The number of laboratories to obtain effective data should be 8 or more [(15)]. Conduct undisclosed duplicate collaborative studies for 5 or more kinds of samples with different concentration. Obtain reproducibility and repeatability from the observed values [(16)] to evaluate.
Criteria to evaluate these precisions are shown in a Separate sheet.

Note (15) In case the number of laboratories which have required facility/instruments is limited, this should be 5 or more.

(16) The calculation method is shown in Reference 2.

(3.5.2) Intermediate precision and repeatability by a repeatability test in a single laboratory on different days

Conduct a duplicate test [17] per test day for 5 to 7 days using two analytical samples of different concentration which is included in a normal range [18]. Obtain intermediate precision and repeatability from the observed values [19] to evaluate.
Criteria to evaluate these precisions are shown in Separate sheet.

Note **(17)** The data of internal quality control can be used.
(18) It is not necessary for the same tester to conduct a test through 5 to 7 days.
(19) The calculation method is shown in Reference 2.

References

1) AOAC Official Methods of Analysis Appendix K: Guidelines for Dietary Supplements and Botanicals, AOAC INTERNATIONAL (2012)
2) Thompson, M., Ellison, S.L.R, Wood, R.,: Harmonized guidelines for single-laboratory validation of methods of analysis, Pure & Appl. Chem. 74 (5), 835-855 (2002)
3) AOAC Official Methods of Analysis Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis, AOAC INTERNATIONAL (2005)
4) Horwitz, W.: Protocol for the Design, Conduct and Interpretation of Method-Performance Studies, Pure & Appl. Chem., 67 (2), 331-343 (1995)

(3.6) Minimum Limit of Quantification (LOQ)

Estimate Minimum Limit of Quantification according to **(3.6.1)** to **(3.6.3)**. Prepare test samples which include the concentration estimated to be near the Minimum Limit of Quantification step by step as necessary. And conduct respective repeatability tests using 3 analytical samples and define the concentration of a prepared test sample as the Minimum Limit of Quantification, where the mean of the obtained values using the prepared test sample is suitable to the target value of trueness.

Comment 8 In case permissible content and equivalent level is 1.0 mg/kg or more, the Minimum Limit of Quantification (LOQ) of harmful components and restricted components, etc. should be no more than 1/5 of the permissible content and equivalent level. In case permissible content and equivalent level is no more than 1.0 mg/kg, the Minimum Limit of Quantification (LOQ) should be no more than 2/5 of the permissible content. Moreover, it is recommended that the Minimum Limit of Quantification of main components/major components and material components should be no more than 1/5 of minimum volume to be contained and the minimum content of a distribution fertilizer. In addition, in case the Minimum Limit of Quantification exceeds 1/5 of these minimum volumes, conduct the above mentioned repeatability test, confirm the Minimum Limit of Quantification and state clearly the fact in the applicable range of a test method.

Comment 9 There are some methods to estimate Minimum Limit of Quantification. The methods differ depending on whether they are based on an instrument analysis or not and depending on instruments used. A method different from the methods shown in **(3.6.1)** to **(3.6.3)** is allowed. However, the definition of a method and Minimum Limit of Quantification by the method should be clearly stated.

(3.6.1) Estimation method by a repeatability test
With regard to a test sample with concentration near Minimum Limit of Quantification, conduct a repeatability test using 7 to 10 analytical samples, obtain repeatability standard deviation and estimate Minimum Limit of Quantification in an analytical sample by the formula (3).

$$\text{Estimated value of Minimum Limit of Quantification (LOQ)} = 10 \times s_r \quad \cdots \ (3)$$

s_r: Repeatability standard deviation

(3.6.2) Estimation method using a calibration curve
In case a calibration curve is linear, estimate Minimum Limit of Quantification (LOQ) in an analytical sample by the formula (4) using the standard deviation of the residuals of a calibration curve or estimated signals in concentration 0 and the inclination of a calibration curve.

$$\text{Estimated value of Limit of Quantitation (LOQ)} = \frac{10 \times s}{b} \quad \cdots \ (4)$$

s: The standard deviation of residuals. Or the standard deviation of signasls in concentration 0, which are estimated from a regression line

b: The inclination of a calibration curve

(3.6.3) Estimation method using an SN ratio
In a test method such as Chromatography, etc. which has a baseline noise, calculate from a concentration in an analytical solution whose SN ratio is 10 to 1 at the peak and estimate Minimum Limit of Quantification (LOQ) in an analytical sample.

References

1) ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2005)
2) The notification by the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, the Ministry of Health, Labour and Welfare: The text (operation method) on Bioanalytical Method Validation", October 28, 1997, *Iyaku-Shin* No.338 (1997)

(3.7) Minimum Limit of Detection (LOD)
Estimate Minimum Limit of Detection according to **(3.7.1)** to **(3.7.3)**.

Comment 10 There are some methods to estimate Minimum Limit of Detection. The methods differ depending on whether they are based on an instrument analysis or not and depending on instruments used. A method different from the methods shown in **(3.7.1)** to **(3.7.3)** is allowed. However, the definition of the method and Minimum Limit of Detection by the method should be clearly stated.

(3.7.1) Estimation method by a repeatability test

With regard to a test sample or a blank sample with concentration near Minimum Limit of Quantification, conduct repeatability tests using 7 to 10 analytical samples, obtain repeatability standard deviation and estimate Minimum Limit of Detection in an analytical sample by the formula (5).

$$\text{Estimated value of Minimum Limit of of Detection (LOD)in an analytical sample} \\ = 2 \times t(n-1, 0.05) \times s_r \qquad \cdots (5)$$

s_r: Repeatability standard deviation

$t(n-1, 0.05)$: The Student value of Significance Level 5% (one side) [20]

n: The number of analytical samples in a repeatability test

Note (20) In case of a repeatability test using 7 analytical samples, the value is 1.94. In case of using 10 analytical samples, the value is 1.83.

(3.7.2) Estimation method using a calibration curve

In case a calibration curve is linear, estimate Minimum Limit of Detection (LOD) in an analytical sample by the formula (6) using the standard deviation of the residuals of a calibration curve or estimated signals in concentration 0 and the inclination of a calibration curve.

$$\text{Estimated value of Limit of of Detection (LOD)} \\ = \frac{2 \times t(\mathrm{n}-2, 0.05) \times s}{b} \qquad \cdots (6)$$

s: The standard deviation of residuals. Or the standard deviation of signasls in concentration 0, which are estimated from a regression line

b: The inclination of a calibration curve

$t(\mathrm{n}-2, 0.05)$: The Student value of Significance Level 5% (one side)

n: The number of a measurement point on a calibration curve

(3.7.3) Estimation method using an SN ratio

In a test method such as Chromatography, etc. which has a baseline noise, calculate from a concentration in an analytical solution whose SN ratio is 3 to 1 at the peak and estimate Minimum Limit of Detection (LOD) in an analytical sample.

References

1) ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2005)

2) The notification by the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, the Ministry of Health, Labour and Welfare: The text (operation method) on Bioanalytical Method Validation", October 28, 1997, *Iyaku-Shin* No.338 (1997)

(3.8) Robustness

Robustness should be studied when an analysis method is developed, and the estimation method depends on the type of analysis method to be developed. Robustness expresses the reliability of an analysis method when its analysis conditions are intentionally changed. If an observed value tends to be easily affected by the variation of an analysis condition, it is necessary to consider a method to control an analysis condition appropriately or to state the fact as a precaution in a testing method. The evaluation of robustness enables the establishment of a series of parameters such as Resolution related to system conformance. Similarly, the confirmation of these parameters ensures that the validation of an analysis method is maintained in a daily analysis.

Typical variation factors are as follows.

(3.8.1) Common variation factors: Typical variation factors common to various kinds of test methods are as follows.

- **a)** Extraction time, extraction temperature
- **b)** Stability of a test solution in respective steps
- **c)** Regent's grade

(3.8.2) Variation factors in Chromatography, etc.: Typical variation factors of measurements by Chromatography or refining by solid phase extraction are as follows.

- **a)** Change of a column or a cartridge (A different lot or a different brand)
- **b)** Influence by the variation of pH and composition of an eluent or a wash
- **c)** Temperature
- **d)** Flow rate
- **e)** Influence of a matrix and effect of dilution

References

1) ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2005)

2) The notification by the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, the Ministry of Health, Labour and Welfare: The text (operation method) on Bioanalytical Method Validation ", October 28, 1997, *Iyaku-Shin* No.338 (1997)

3) Thompson, M., Ellison, S.L.R, Wood, R.: Harmonized guidelines for single-laboratory validation of methods of analysis, Pure & Appl. Chem. 74 (5), 835–855 (2002)

Reference 1: A procedure to compare an observed value and a certified value

Obtain the total mean (m) of the replication test results and the certified value (μ), and the absolute value (Δ_m) of the difference of the two values by the formula (R1.1). Next, obtain the standard uncertainty (u_{CRM}) of the certified value of a certified reference material by the formula (R1.2), and obtain the standard uncertainty (u_m) of the total mean by the formula (R1.3). Calculate the combined standard uncertainty ($u_{C(\Delta_m)}$) of Δ_m by the formula (R1.4) using the obtained u_m and u_{CRM}. Further, calculate an expanded uncertainty (U_{Δ_m}) by the formula (R1.5) using the coverage factor (k = 2).
Compare Δ_m and U_{Δ_m} to confirm that the criterion (the formula (R1.6)) is satisfied, that is, Δ_m is no more than U_{Δ_m}.

The absolute value (Δ_m) of the difference of the total mean of repeatability test results and a certified value $= |m - \mu|$ ··· (R1.1)

The standard uncertainty (u_{CRM}) of the certified value $= \dfrac{U_{95\,\%}}{k_{CRM}}$ ··· (R1.2)

The standard uncertainty of the measurement of a total mean $= \dfrac{s_r}{\sqrt{n}}$ ··· (R1.3)

The combined standard uncertainty $\left(u_{C(\Delta_m)}\right)$ of $\Delta_m = \sqrt{{u_m}^2 + {u_{CRM}}^2}$ ··· (R1.4)

The expanded uncertainty $\left(U_{\Delta_m}\right)$ of $\Delta_m = k_{C(\Delta_m)} \times u_{C(\Delta_m)} = 2 \times u_{C(\Delta_m)}$ ··· (R1.5)

Criterion $\Delta_m \leqq U_{\Delta_m}$ ··· (R1.6)

m : The total mean of observed values
μ : A certified value
$U_{95\,\%}$: The expanded uncertainty of a certified value
k_{CRM} : The coverage factor of an expanded uncertainty of a standard reference material
s_r : Repeatability standard deviation
n : The number of repeatability test samples
$k_{C(\Delta_m)}$: The coverage factor of an expanded uncertainty of Δ_m ($k_{C(\Delta_m)} = 2$)

Reference 2: Calculation of a reproducibility or an intermediate precision and a repeatability

(1) Structure of an observed value

An observed value (x_{ij}) in Table 1, as is shown in the formula (R2.1), consists of a true value (μ), a variation (β) by a factor and a variation (e) by an accidental error under repeatability conditions (hereinafter referred to as "an accidental error"). When p laboratories conduct a collaborative study in which respective laboratories conduct repeatability tests using n samples, the formula (R2.2) is introduced on the assumption that the distribution of β is equivalent to N (0, ${\sigma_L}^2$) which depends on a pure between-laboratory variation and the distribution of e is equivalent to N (0, ${\sigma_r}^2$) which depends on an accidental error. In addition, when the same laboratory conducts replicate tests for p days using n samples on respective test days, the formula (R2.3) is introduced on the assumption that the distribution of β is equivalent to N (0, ${\sigma_{(T)}}^2$) which depends on test days variation (factor T) and the distribution of e is equivalent to N (0, ${\sigma_r}^2$) which depends on an accidental error.

$$\text{Ovserved value } (x_{ij}) = \mu + \beta_i + e_{ij} \qquad \cdots \text{(R2.1)}$$

$$\text{Observed value } (x_{ij}) = \mu + N(0, {\sigma_L}^2) + N(0, {\sigma_r}^2) \qquad \cdots \text{(R2.2)}$$

$$\text{Observed value } (x_{ij}) = \mu + N(0, {\sigma_{(T)}}^2) + N(0, {\sigma_r}^2) \qquad \cdots \text{(R2.3)}$$

μ: True value

β_i: Variation of a factor $\qquad e_{ij}$: Accidental error

$N(0, {\sigma_L}^2)$: Normal distribution of β_i with the mean 0 and standard deviation σ_{L_i}

$N(0, {\sigma_r}^2)$: Normal distrubution of e_{ij} with the mean 0 and standard deviation σ_r

${\sigma_L}^2$: Pure between − laboratory variance $\qquad {\sigma_r}^2$: Repeatability variance

$N(0, {\sigma_{(T)}}^2)$: Normal distribution of β_i with the mean 0 and standard deviation $\sigma_{(T)}$

${\sigma_{(T)}}^2$: Test days variance

Table 1 Test results of collaborative studies or repeatability tests on different days

Laboratory or test day (Factor)	Analytical sample number						
	1	2	3	⋯	j	⋯	n
1	x_{11}	x_{12}	x_{13}	⋯	x_{1j}	⋯	x_{1n}
2	x_{21}	X_{22}	x_{23}	⋯	x_{2j}	⋯	x_{2n}
3	x_{31}	X_{32}	x_{33}	⋯	x_{3j}	⋯	x_{3n}
⋯	⋯	⋯	⋯	⋯	⋯	⋯	⋯
i	x_{i1}	X_{i2}	x_{i3}	⋯	x_{ij}	⋯	x_{in}
⋯	⋯	⋯	⋯	⋯	⋯	⋯	⋯
p	x_{p1}	X_{p2}	x_{p3}	⋯	x_{pj}	⋯	x_{pn}

(2) Calculation procedure of reproducibility and repeatability of the results of a collaborative study

(2.1) Estimation of true value and variance

In an actual statistical analysis, a true value (μ), a true and pure between-laboratory variance (${\sigma_L}^2$) and a true repeatability variance (${\sigma_r}^2$) are unknown. Therefore they are replaced with estimated values obtained from the results of a collaborative study and are expressed as a mean (m), a pure between-laboratory variance (${s_L}^2$) and a repeatability variance (${s_r}^2$) respectively.

(2.2) One-way analysis of variance

Exclude ineffective observed values which have clearly objective reasons such as deviation from a protocol and malfunction of instruments from the report values by laboratories which participated in a collaborative study. Further exclude outliers by conducting Cochran's test and Grubb's test. And conduct one-way analysis of variance for the remaining results to obtain the unbiased variance (*V*) of respective variation factors in Table 2.

Table 2 Table of one-way analysis of variance

Variation factor	Sum of squares	Degree of freedom	Unbiased variance (V)	Expectation of variance $E(V)$
Between-laboratory (L)	SS_L	$p-1$	V_L	${\sigma_r}^2 + n \times {\sigma_L}^2$
Accidental error (e)	SS_r	$p \times (n-1)$	V_r	${\sigma_r}^2$

Comment 1 It is possible to conduct one-way analysis of variance easily using a statistical program or a tool of a spreadsheet program. In this case, it should be noted that different terminologies may be used (Between-laboratory (L) → Between-group, Accidental error (e) → Within-group, Unbiased variance → Mean square, etc.).

Comment 2 Unbiased variance (*V*) is calculated by (Sum of squares)/ (Degree of freedom).

(2.3) The calculation of reproducibility and repeatability

The relation of the expectation of variance E (*V*) of respective factors in Table 2 holds true. Therefore calculate repeatability variance (${s_r}^2$) and pure between-laboratory variance (${s_L}^2$) by the formula (R2.4) and (R2.5), and further calculate reproducibility variance (${s_R}^2$) by the formula (R2.6) [(1) (2)].

$$\text{Repeatability variance } ({s_r}^2) = V_r \qquad \cdots \text{(R2.4)}$$

$$\text{Pure between} - \text{laboratory variance } ({s_L}^2) = \frac{V_L - V_r}{n} \qquad \cdots \text{(R2.5)}$$

$$\text{Reproducibility variance } ({s_R}^2) = {s_L}^2 + {s_r}^2 \qquad \cdots \text{(R2.6)}$$

V_r: The unbiased variance of a variation factor (accidental error (e)) in the table of one − way analysis of variance (Table 2)
V_L: The unbiased variance of a variation factor (between − laboratories (L)) in the table of one − way analysis of variance (Table 2)

Calculate a repeatability standard deviation (s_r) and a reproducibility standard deviation (s_R) by the formula (R2.7) and (R2.8) using the obtained repeatability variance and reproducibility variance, and further calculate a repeatability relative standard deviation (RSD_r) and a reproducibility relative standard deviation (RSD_R) by the formula (R2.9) and (R2.10) [(2) (3)].

$$\text{Repeatability standard deviation } (s_r) = \sqrt{{s_r}^2} \qquad \cdots \text{(R2.7)}$$

$$\text{Reproducibility standard deviation } (s_R) = \sqrt{{s_R}^2} \qquad \cdots \text{(R2.8)}$$

$$\text{Repeatability relative standard deviation}(RSD_r, \%) = \frac{s_r}{m} \times 100 \qquad \cdots \text{(R2.9)}$$

$$\text{Reproducibility relative standard deviation}(RSD_R, \%) = \frac{s_R}{m} \times 100 \qquad \cdots \text{(R2.10)}$$

m: the total mean $(m)$of the effective data of collaborative study results

Note **(1)** In case $V_L < V_r$, assume $V_L = V_r$ (that is, the pure between-laboratory variance ($s_L{}^2$) = 0 in the formula (R2.5)) and let the formula (R2.6) form $s_R{}^2 = s_r{}^2$.

(2) The rounding of a numerical value is not executed in the middle of the calculation.

(3) The mean and the standard deviation are expressed rounding to the digit of the observed value. The relative standard deviation is expressed rounding to the first decimal place.

(3) Calculation procedure of intermediate precision and repeatability by the replicate test results on different days.

(3.1) Estimation of a true value and a variance

In an actual statistical analysis, a true value (μ), a true test days variance ($\sigma_{(T)}{}^2$) and a true repeatability variance ($\sigma_r{}^2$) are unknown. Therefore they are replaced with estimated values obtained from the replicate test results on different days and are expressed as a mean (m), test days variance ($s_{(T)}{}^2$) and repeatability variance ($s_r{}^2$) respectively.

(3.2) One-way analysis of variance

Conduct one-way analysis of variance for the replicate test results on different days to obtain the unbiased variance (V) of respective variation factors in Table 3.

Table 3 Table of one-way analysis of variance

Variation factor	Sum of squares	Degree of freedom	Unbiased variance (V)	Expectation of variance $E(V)$
Test days (T)	SS_T	$p-1$	V_T	$\sigma_r{}^2 + n \times \sigma_{(T)}{}^2$
Accidental error (e)	SS_r	$p \times (n-1)$	V_r	$\sigma_r{}^2$

Comment 3 It is possible to conduct one-way analysis of variance easily using a statistical program or a tool of a spreadsheet program. In this case, it should be noted that different terminologies may be used (Test days (T) → between-group, Accidental error (e) → within-group, Unbiased variance → Mean square, etc.).

Comment 4 Unbiased variance (V) is calculated by (Sum of squares)/ (Degree of freedom).

(3.3) The calculation of intermediate precision and repeatability

The relation of the expectation of variance E (V) of respective factors in Table 3 holds true. Therefore calculate repeatability variance ($s_r{}^2$) and test days variance ($s_{(T)}{}^2$) by the formula (R2.11) and (R2.12), and further calculate intermediate variance ($s_{I(T)}{}^2$) by the formula (R2.13) [(2) (4)].

$$\text{Repeatability variance } (s_r{}^2) = V_r \quad \cdots \text{(R2.11)}$$

$$\text{Test days variance } \left(s_{(T)}{}^2\right) = \frac{V_T - V_r}{n} \quad \cdots \text{(R2.12)}$$

$$\text{Intermediate Variance } \left(s_{I(T)}{}^2\right) = s_{(T)}{}^2 + s_r{}^2 \quad \cdots \text{(R2.13)}$$

V_r: The unbiased variance of a variation factor (accidental error (e)) in the table of one − way analysis of variance (Table 3)

V_T: The unbiased variance of a variation factor (test days (T))

$$\text{in the table of one} - \text{way analysis of variance (Table 3)}$$

Calculate a repeatability standard deviation (s_r) and an intermediate standard deviation ($s_{I(T)}$) by the formula (R2.14) and (R2.15) using the obtained estimated values of repeatability variance and intermediate variance, and further calculate a repeatability relative standard deviation (RSD_r) and an intermediate relative standard deviation ($RSD_{I(T)}$) by the formula (R2.16) and (R2.17) [(2) (3)].

$$\text{Repeatability standard deviation } (s_r) = \sqrt{s_r{}^2} \qquad \cdots \text{(R2.14)}$$

$$\text{Internediate standard deviation } \left(s_{I(T)}\right) = \sqrt{s_{I(T)}{}^2} \qquad \cdots \text{(R2.15)}$$

$$\text{Repeatability relative standard deviation}(RSD_r, \%) = \frac{s_r}{m} \times 100 \qquad \cdots \text{(R2.16)}$$

$$\text{Internediate relative standard deviation } \left(RSD_{I(T)}, \%\right) = \frac{s_I}{m} \times 100 \qquad \cdots \text{(R2.17)}$$

m: Total mean of the replicate test results on different days

Note (4) In case $V_T < V_r$, assume $V_L = V_r$ (that is, the test days variance ($s_{(T)}{}^2$) in the formula (R2.12) = 0) and let the formula (R2.13) form $s_{I(T)}{}^2 = s_r{}^2$.

(4) Examples of the calculation of intermediate precision and repeatability by the replicate test results on different days.

An example of repeatability test results on different days of citric acid-soluble phosphoric acid using sample 1 and sample 2 containing phosphite is shown in Table 4. Conduct one-way analysis of variance for the test results of respective samples to obtain the unbiased variance (V) of respective variation factors (Table 5).

Examples of the calculation of intermediate precision and repeatability for the sample 1 and sample 2 using the formula (R2.11) to the formula (R2.17) are shown in Table 6-1 and 6-2. In addition, the results of respective standard deviation are expressed rounding to the digit of the observed value and the results of the respective relative standard deviations are expressed rounding to the first decimal place.

Table 4 Example of repeatability test results on different days (mass fraction (%))

Sample No	Test day (factor)							Total mean (m)[1)]
	1	2	3	4	5	6	7	
Sample 1	51.20 51.45	52.15 51.85	51.00 51.09	51.35 51.28	51.35 51.10	51.38 51.38	51.28 51.43	51.38
Sample 2	5.18 5.00	4.90 5.12	5.01 5.06	5.15 5.14	5.14 5.07	5.13 5.11	5.21 5.18	5.10

1) The mean is expressed rounding to the digit of the observed value.

Table 5 Table of one-way analysis of variance

Sample No	Variation factor	Sum of squares	Degree of freedom	Unbiased varianc (V)	Expectation of variance $E(V)$
Sample 1	Test days (T)	1.0570	6	0.17616	$\sigma_r{}^2 + 2 \times \sigma_{(T)}{}^2$
	Accidental error (e)	0.1253	7	0.01789	$\sigma_r{}^2$
Sample 2	Test days (T)	0.0478	6	0.00797	$\sigma_r{}^2 + 2 \times \sigma_{(T)}{}^2$
	Accidental error (e)	0.0448	7	0.00640	$\sigma_r{}^2$

Table 6-1 Calculation of intermediate precision and repeatability using the sample 1 replicate test results on different days[1)]

Variation factor	Unit	Formula	Calculation	Result
Repeatability variance (s_r^2)		$= V_r$	$= 0.01789$	0.01789
Repeatability standard deviation(s_r)[2)]	Mass fraction (%)	$=\sqrt{s_r^2}$	$= \sqrt{0.01789}$	0.13
Repeatability relative standard deviation (RSD_r)[3)]	%	$= \frac{s_r}{m} \times 100$	$= \frac{0.1338}{51.38} \times 100$	0.3
Test days variance $(s_{(T)}^2)$		$= \frac{V_T - V_r}{n}$	$= \frac{0.17616 - 0.01789}{2}$	0.07914
Intermediate variance $(s_{I(T)}^2)$		$= s_{(T)}^2 + s_r^2$	$= 0.07914 + 0.01789$	0.09703
Intermediate standard deviation $(s_{I(T)})$[2)]	Mass fraction (%)	$= \sqrt{s_{I(T)}^2}$	$= \sqrt{0.09703}$	0.31
Intermediate relative standard deviation $(RSD_{I(T)})$[3)]	%	$= \frac{s_{I(T)}}{m} \times 100$	$= \frac{0.3115}{51.38} \times 100$	0.6

1) The rounding of a numerical value is not executed in the middle of the calculation.
2) The standard deviation is expressed rounding to the digit of the observed value.
3) The relative standard deviation is expressed rounding to the first decimal place.

Table 6-2 Calculation of intermediate precision and repeatability using the sample 2 replicate test results on different days[1)]

Variation factor	Unit	Formula	Calculation	Result
Repeatability variance (s_r^2)		$= V_r$	$= 0.00640$	0.00640
Repeatability standard deviation(s_r)[2)]	Mass fraction (%)	$=\sqrt{s_r^2}$	$= \sqrt{0.00640}$	0.08
Repeatability relative standard deviation (RSD_r)[3)]	%	$= \frac{s_r}{m} \times 100$	$= \frac{0.0800}{5.10} \times 100$	1.6
Test days variance $(s_{(T)}^2)$		$= \frac{V_T - V_r}{n}$	$= \frac{0.00797 - 0.00640}{2}$	0.00078
Intermediate variance $(s_{I(T)}^2)$		$= s_{(T)}^2 + s_r^2$	$= 0.00078 + 0.00640$	0.00718
Intermediate standard deviation $(s_{I(T)})$[2)]	Mass fraction (%)	$= \sqrt{s_{I(T)}^2}$	$= \sqrt{0.00718}$	0.08
Intermediate relative standard deviation $(RSD_{I(T)})$[3)]	%	$= \frac{s_{I(T)}}{m} \times 100$	$= \frac{0.0848}{5.10} \times 100$	1.7

Footnote: Refer to Table 6-1

Separate sheet: Criteria of trueness and precision in respective concentration levels

The criteria of trueness and precision in respective concentration levels to evaluate test methods excluding Chromatography are shown in Table 1. The criteria for Chromatography [(1)] are shown in Table 2.

The target of trueness (recovery) is generally within the criteria of Table 1 and Table 2. As for the precision, although the target is recommended to be within the criteria of Table 1 and Table 2, the permissible level is up to 1.5 times as much as the criteria.

Note (1) Gas chromatography, Gas Chromatography/Mass Spectrometry, High Performance Liquid Chromatography, High Performance Liquid Chromatography/Tandem Mass Spectrometry, Ion Chromatography, etc.

Table 1 Criteria of trueness and precision in respective concentration levels (Test methods excluding Chromatography)

Concentration level	Trueness Recovery (%)	Precision		
		Reproducibility relative standard deviation (%)	Intermediate relative standard deviation (%)	Repeatability relative standard deviation (%)
≧25 %	98～102	2.5	2	1
≧10 %	97～103	3	2.5	1.5
≧1 %	96～104	4	3.5	2
≧0.1 %	94～106	6	4.5	3
≧100 mg/kg	92～108	8	6.5	4
≧10 mg/kg	90～110	11	9	6
≧1 mg/kg	85～115	16	13	8
≧100 μg/kg	85～115	22	18	11
≧10 μg/kg	80～120	22	18	11
<10 μg/kg	75～125	22	18	11

Table 2 Criteria of trueness and precision in respective concentration levels (Chromatography)

Concentration level	Trueness Recovery (%)	Precision		
		Reproducibility relative standard deviation (%)	Intermediate relative standard deviation (%)	Repeatability relative standard deviation (%)
≧10 %	90～108	8	6.5	4
≧1 %	85～110	8	6.5	4
≧0.1 %	85～110	8	6.5	4
≧100 mg/kg	80～115	8	6.5	4
≧10 mg/kg	70～120	11	9	6
≧1 mg/kg	70～120	16	13	8
≧100 μg/kg	70～120	22	18	11
≧10 μg/kg	70～120	22	18	11
<10 μg/kg	60～125	22	18	11