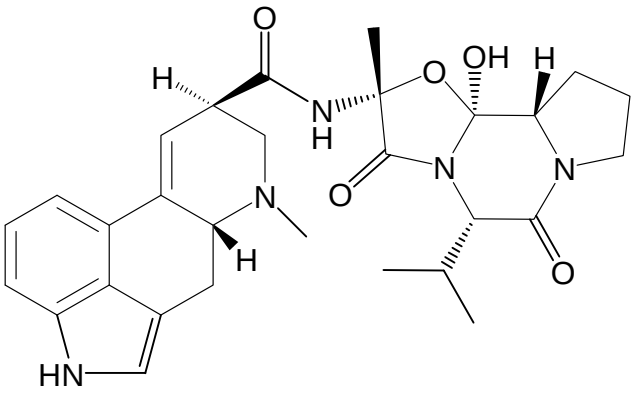
Ergovaline



$C_{29}H_{35}N_5O_5$ MW: 533.619 CAS No.: 2873-38-3

[Summary of ergovaline]
Ergovaline is a kind of ergot alkaloids produced by endophytes *Neotyphodium coenophialum* and *Neotyphodium lolli*, symbionts of the grass. There is a risk of poisoning in cattle called fesque toxicosis when its concentration in the total feed given is over 500 μg/kg. Imported hay may be contaminated with ergovaline because endophytes are widely utilized for tall fesque and perennial ryegrass in the United States and Australia.

[Methods listed in the Feed Analysis Standards]

1 Liquid chromatography [Note 1, 2][Feed Analysis Standards, Chapter 5, Section 2 1.1]

Scope of application: Hay

A. Reagent preparation

1) Dilution solvent. Dissolve 1 g of L-ascorbic acid in methanol to be 1 L.
2) Ergovaline standard solution. Weigh 11.4 mg of ergovaline tartrate $[(C_{29}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$,[Note 3][1] put in a 100-mL amber volumetric flask, dissolve by the addition of the dilution solvent, and add the same solvent up to the graduation line to prepare the ergovaline standard stock solution (1 mL of this solution contains 0.1 mg as ergovaline.).
   Before use, dilute accurately a certain amount of the standard stock solution with the dilution solvent to prepare several ergovaline standard solutions that contain 0.25-5 μg respectively as ergovaline in 1 mL.
3) Ergotamine standard solution. Weigh 11.3 mg of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$,[2] put in a 100-mL amber volumetric flask, dissolve by the addition of the dilution solvent, and add the same solvent up to the graduation line to prepare the ergotamine standard stock solution (1 mL of this solution contains 0.1 mg as ergotamine.).
   Before use, dilute accurately a certain amount of the standard stock solution

with the dilution solvent to prepare the ergotamine standard solution that contains 6 μg as ergotamine in 1 mL.

4) Mixture standard solution.[Note 1] Transfer accurately 1 mL each of the ergovaline standard solutions to 50-mL amber volumetric flasks, respectively, and add accurately 1 mL each of the ergotamine standard solution. Further add methanol-aqueous ammonia (100:0.04) up to the graduation line of each of the volumetric flasks, to prepare mixture standard solutions that contain 5-100 ng respectively as ergovaline and 120 ng as ergotamine in 1 mL (prepare before use [3].).

B. Quantification

Extraction. Weigh accurately 5 g of an analysis sample, transfer it to a 200-mL stoppered amber Erlenmeyer flask, add 100 mL of acetic acid (5:21), and extract for 2 hours by intermittently shaking for a few seconds.[Note 4] Add accurately 1 mL of the ergotamine standard solution to the extract, shake to mix, and then filter with filter paper (No. 5 A), to obtain filtrate to be a sample solution to be subjected to column treatment.

Column treatment. Wash an octadecylsilyl silica gel minicolumn (360 mg) sequentially with 1 mL of methanol and 4 mL of water.[4]

Load accurately 10 mL of the sample solution on the minicolumn, elute until the liquid level reaches the upper end of packing,[4] then add 4 mL of acetic acid (5:21) and 4 mL of water sequentially to the minicolumn and elute similarly.[4]

Place a 15-mL stoppered amber test tube under the minicolumn. Elute ergovaline, ergotamine, ergovalinine and ergotaminine by the addition of 5 mL of methanol-aqueous ammonia (100:0.04).[4] After homogenizing the eluate, filter with membrane filter (pore size 0.5 μm or less), to be a sample solution to be subjected to liquid chromatography.

Liquid chromatography. Inject 20 μL each of the sample solution and respective mixture standard solutions to a liquid chromatograph to obtain chromatograms.

Example of measurement conditions

Detector:	Fluorescence detector (excitation wavelength, 315 nm; emission wavelength, 415 nm)
Column:	Octadecylsilyl silica gel column (4.6 mm in inner diameter, 150 mm in length, particle size 5 μm)[Note 5][5]
Eluent:	Water- methanol- acetonitrile- aqueous ammonia (90:90:20:0.3) [6]
Flow rate:	1.0 mL/min
Column oven temperature:	40 °C

Calculation.[Note 6] Obtain the ratio (hereinafter referred to as "S_V/S_T") of the sum of peak areas of ergovaline and ergovalinine (hereinafter referred to as "S_V") to the sum of peak areas of ergotamine and ergotaminine (hereinafter referred to as "S_T") from the resulting chromatograms.[7] Prepare a calibration curve from S_V/S_T to the ergovaline concentration in respective standard solutions, and calculate the amount of ergovaline in the sample.

Note 1 Ergovaline and the internal standard ergotamine isomerize to ergovalinine and ergotaminine, respectively, at a constant rate during the operation of quantification. Isomerization also occurs similarly in

the mixture standard solution after preparation.

2 Conduct the quantification procedure under protection from light.

3 Auburn University (Division of Medical Chemistry)

4 Mix by shaking for a few seconds 3-4 times per hour.

5 Use a column with packing of pore size of 12 nm (CAPCELL PAK C_{18} UG120 (Shiseido) or equivalents).

6 When the ergovaline tartrate standard material that is unopened is not available, the amount of ergovaline in the sample can be approximated by the following formula: [8]

$$\text{Ergovaline content in the analysis sample (μg/kg)} = 1{,}200 \times \frac{S_V}{S_T}$$

<<Summary of analysis method>>

This method extracts ergovaline in a sample by an acetic acid solution, and after the addition of ergotamine as the internal standard, purifies with a C_{18} minicolumn, and detects by a liquid chromatograph with a fluorescence detector.

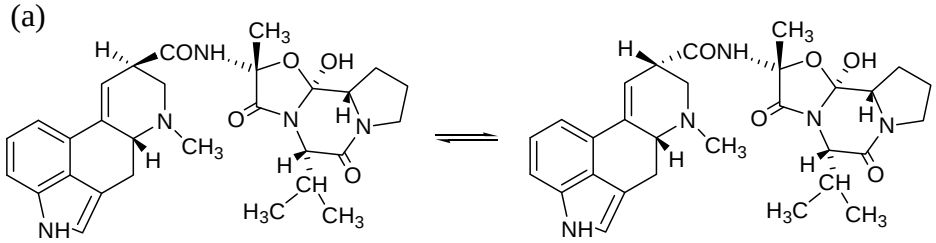
Ergovaline and ergotamine isomerize to epimers without bioactivity as shown in Figure 5.2.1-1, and thus they need to be quantitated as the total amount of ergovaline and ergovalinine, and the total amount of ergotamine and ergotaminine, respectively. For that reason, it is not taken into account if ergovaline in the analysis sample has been isomerized to ergovalinine and its toxicity has been reduced before analysis.

The flow sheet of the analysis method is shown in Figure 5.2.1-2.

References: Toshiaki Yamada and Kiyoshi Sugano: Research Report of Animal Feed, 25, 1 (2000)

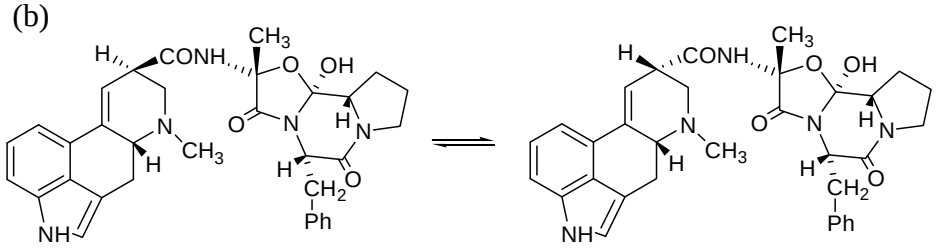
History in the Feed Analysis Standards [22] New

(a)



Ergovaline　　Ergovalinine

(b)



Ergotamine　　Ergotaminine

Figure 5.2.1-1 Epimerization formulae of ergovaline (a) and ergotamine (b)

5 g analysis sample
- 100 mL acetic acid (5:21), leave at rest for 2 hours (sometimes shake vigorously)
- Add 1 mL ergotamine internal standard solution (6 μg/mL)
- Filter (No. 5A)

Sep-Pak Plus C_{18}cartridge (wash with 1 mL methanol and 4 mL water in advance)
- Load 10 mL sample solution.
- Wash with 4 mL acetic acid (5:21) and 4 mL water.

15-mL Stoppered amber test tube
- Elute with 5 mL methanol- aqueous ammonia (100:0.04).
- Filter (membrane filter ≤0.5 μm).

LC-FL (Ex: 315 nm, Em: 415 nm)

Figure 5.2.1-2 Flow sheet of the analysis method for ergovaline

<<Analysis method validation>>

- Spike recovery and repeatability

Sample type	Spike concentration (μg/kg)	Repeat	Spike recovery (%)	Repeatability RSD (% or less)
Tall fesque	200~1,000	3	97.2~103.3	1.8
Ryegrass	200~1,000	3	93.7~104.8	1.6

• Collaborative study

Sample type	Number of laboratories	Spike concentration (μg/kg)	Spike recovery (%)	Intra-laboratory repeatability RSD_r (%)	Inter-laboratory reproducibility RSD_R (%)	HorRat
Tall fesque	7	485	100.1	2.5	8.5	0.48

- Lower limit of quantification: 50 μg/kg in a sample

<<Notes and precautions>>

[1] At the end of March 2009, ergovaline is produced in Auburn University at irregular intervals, and it may take long until the standard is available.

[2] Manufactured by Tokyo Chemical Industry. This article is a hydrate, and used without dehydration etc. Note that it is preferred to use unopened one.

[3] In order to avoid the ratio of epimers that is very different from that of the sample solution, prepare the mixture standard solution by putting the ergovaline standard solution in a volumetric flask at the start of extraction, followed by the addition of a suitable amount of methanol- aqueous ammonia (100:0.04), leaving at rest for 2 hours, and then the addition of the ergotamine standard solution.

[4] The flow rate shall be close to the rate of natural flow. When it does not flow naturally, pressurized flow can be used.

[5] Use Capcell pak C18 UG120 S-3 (Shiseido) or equivalents with endcapped packing. The packing of this octadecylsilyl silica gel column is porous spherical silica gel coated with silicone polymer thin film to which the octadecylsilyl group is bound, and has elution characteristics different from common ODS columns. When another type of ODS column is to be used, check separation etc. of the target component in advance.

[6] The pH of the eluent is outside the tolerance levels specified for the column, and is set in order to separate the contaminant that appear immediately after ergovalinine on the chromatogram of the ryegrass sample solution. Therefore, when only tall fesque is analyzed, the composition of the eluent can be water- methanol- acetonitrile- aqueous ammonia (90:90:20:0.08). Wash the column sufficiently to reduce deterioration of the column by alkali.

Prepare the eluent on the day of use because its pH and the retention time changes over time.

[7] Examples of chromatograms are shown in Figure 5.2.1-3. Care should be taken for peak detection parameters etc. because the peak width of ergotaminine is broader than the other peaks.

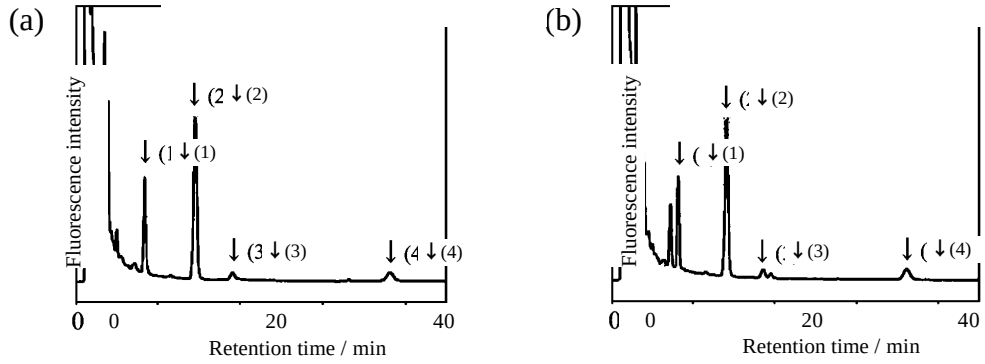


Figure 5.2.1-3 Chromatogram of hay spiked with ergovaline (500 µg/kg)
(a) Tall fesque spiked with ergovaline
(b) Ryegrass spiked with ergovaline
[(1) ergovaline, (2) ergotamine, (3) ergovalinine, (4) ergotaminine]
(the peak immediately after ergovalinine is a contaminant specific to ryegrass)

[8] This formula uses approximation of the proportionality factor as 1.0 of "the ratio of the ergovaline content in the analysis sample to the spiked amount of ergotamine" to "the ratio of S_V and S_T", and was derived considering the absolute recovery in the quantitation operation and the sensitivity factor of fluorescence detection. 1,200 is equivalent to the spiked amount of ergotamine (ng) per 1 g of the analysis sample.
When quantitation is conducted by this formula, the quantitation value is preferred to be given to two significant figures.