Mold Inhibitors

[Summary of mold inhibitors]

Water content of dry feed ingredients and formula feed is generally below 13 %. Therefore, when storing feed in a chilly environment at relative humidity of 70 % or so, molds are unlikely to generate. However, for a long-time storage or in the rainy or summer seasons, feed absorbs moisture, and water content in feed may exceed 15 %, depending on the storage condition. As water content in feed increases, molds in the feed activate and grow proliferously, causing loss of nutrients and deterioration of taste. Some mold species produce toxic substances such as aflatoxin and they will possibly cause adverse effects to livestock and poultry. Molds are more likely to generate at higher temperatures and on pellets and smaller-particle feed.

The substance which has a function to suppress generation of molds is called mold inhibitor. Three items are designated as the feed additive: propionic acid (propanoic acid in systematic IUPAC name), sodium propionate and calcium propionate.

These substances can be added to any type of feed and the additive amount is limited to less than 0.3 %. Mold inhibitors are often added to formula feed usually from June to September. They can also be added to silage to prevent the second fermentation and their additive amount is set less than 1 %.

The most common mold which generates in feed is *Aspergillus* genus. Table 12-1 shows temperature and humidity required for molds to grow and their species.

Table 12-1 Humidity and temperature required for main molds

Mold species	Relative humidity (%)	Water content (%)		Lowest temperature (°C)	Optimum temperature (°C)
		Corn	Milo		
Aspergillus trictus group	70	13.5 - 14.5	14.0 - 14.5	5 - 10	30 - 35
A. glaucus group	73	14 - 14.5	14.5 - 15.0	0 - 5	30 - 35
A. candidus group	80	15 - 15.5	16.0 - 16.5	10 - 15	45 - 50
A. flavus group	85	18 - 18.5	19.0 - 19.5	10 - 15	40 - 45
Penicillium group	80~90	16 - 19.0	17.0 - 19.5	-5 - 0	20 - 25

According to the report of Takashima et al., the seasonal mold generation rates in various feed ingredients are as shown in Figure 12-1.

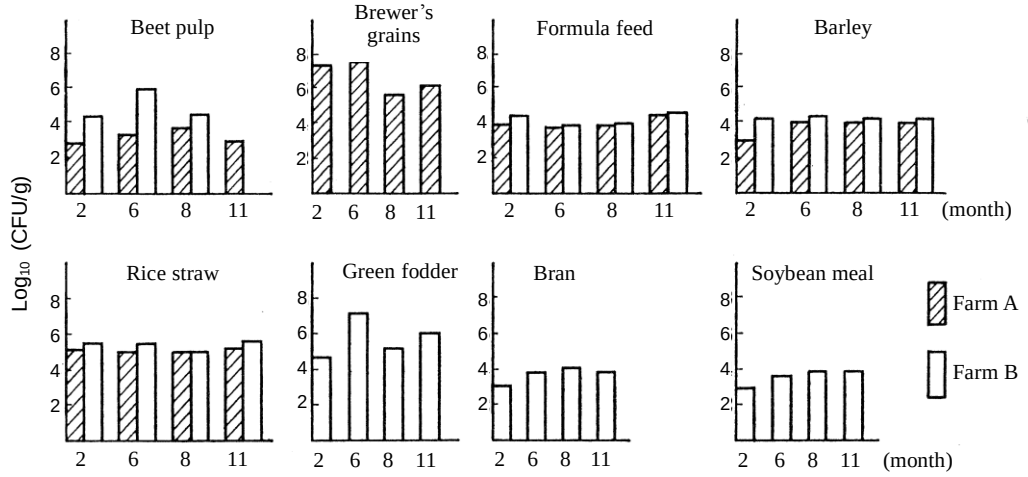
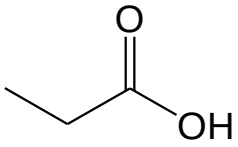


Figure 12-1 Seasonal mold generation rates in various feed ingredients

By Kousuke Takashima, Sueo Kondo: Japanese Society of Animal Science Report, 49, 578 (1978).

Section 1　Monograph

1　Propionic acid, Calcium propionate, Sodium propionate



propanoic acid

$C_3H_6O_2$　MW: 74.08　CAS No.: 79-09-4

sodium propanoate	$C_3H_5NaO_2$	MW: 96.07	CAS No.: 137-40-6
calcium propanoate	$C_6H_{10}CaO_4$	MW: 186.22	CAS No.: 4075-81-4

[Summary of propionic acid, calcium propionate, and sodium propionate]

Propionic acid and its saline grioup (calcium propionate and sodium propionate) are feed additives mainly used as mold inhibitors. (Calcium propionate was officially designated in 1976, and Sodium propionate, in 1978.) It has been proved that these substances have effects to improve deterioration of feed quality in combination with formic acid.

Japan, a hot and humid country, must pay special attentions on mold generation. Propionic acid is added to formula feed mainly around the rainy season, featuring retainment of feed preference. Its antibacterial activity is effective to molds and aerobic sporulating bacteria.

«Standards and Specifications in the Act on Safety Assurance and Quality Improvement of Feed» [Appended Table 1, 1-(1)-E, the Ministerial Ordinance Concerning the Ingredient Specifications for Feeds and Feed Additives]

Silage: 1.0 % or less as propionic acid

Other feed (excluding ingredients or materials used to produce feed): 0.3 % or less as propionic acid

[Method listed in the Analytical standards of feeds]

1　Simultaneous analysis method for organic acids by capillary electrophoresis [Analytical standards of feeds, Article 1.1, Section 1, Chapter 12]

Compounds to be analyzed: Propionic acid, formic acid, citric acid, acetic acid, tartaric acid, lactic acid, fumaric acid and malic acid (8 components)

See article 1, section 2, multicomponent analysis method.

2　Simultaneous analysis method for inorganic ions and organic acids by capillary electrophoresis [Analytical standards of feeds, Article 1.2, Seciton 1, Chapter 12]

Compounds to be analyzed: Chlorine, nitrite nitrogen, nitrate nitrogen, propionic acid (including calcium propionate and sodium propionate), formic acid, **c**itric acid, acetic acid, *iso*-valeric acid, *n*-valeric acid, *n*-hexanoic acid and butyric acid (12 components)

Scope: Silage

See article 1, section 2, chapter 4 (Inorganic constituent) .

3 Liquid chromatography method[1] [Analytical standards of feeds, Article 1.3, Section 1, Chapter 12]

Scope: Feed

A. Reagent preparation

Propionic acid standard solution. Weigh 1.257 g of calcium propionate [$C_6H_{10}CaO_4$] or 1.297 g of sodium propionate [$C_3H_5NaO_2$] into a 100 mL brown volumetric flask, add water and dissolve it. Then add water to the marked line to prepare propionic standard stock solution (1 mL of the solution contains 10 mg of propionic acid.)

In use, dilute a certain amount of the standard stock solution with water to prepare propionic acid standard solutions containing 25 to 500 µg of propionic acid per 1 mL of the solution.

B. Quantification

Distillation. Weigh accurately less than 50 g of the analysis sample (equivalent to 10 to 100 mg as propionic acid), place in a 500 mL Kjeldahl flask. Add 200 mL of water, 80 g of sodium chloride, 10 mL of phosphoric acid solution (1+9) and a few drops of silicon oil as the antifoam to the flask. Join this Kjeldahl flask to the steam distillation apparatus connected to a receiver containing 30 mL of water and distill the flask until the volume of distillate reaches around 450 mL[2].

Pour distillate with water into a 500 mL volumetric flask and add water up to the marked line. Then, filter it using a membrane filter (pore size: 0.5 µm or less.) Use the filtered liquid as the sample solution to be provided to liquid chromatography.

Liquid chromatography. Inject 20 µL of sample solution and respective propionic acid standard solution into liquid chromatograph to obtain chromatogram.

Example of measurement conditions:

Detector: UV absorbance detector (wavelength: 210nm)

Column: Strongly acidic cation-exchange column (internal diameter 8 mm, length 300 mm, average particle size 10 µm)*1

Guard Column: Strongly acidic cation-exchange column (internal diameter 8 mm, length 50 mm, average particle size 10 µm)*2

Eluent: Phosphoric acid solution (1+1,000)

Flow rate: 0.8 mL/min

Column temperature: 50 °C

Calculation. Calculate the peak area or peak height from obtained chromatogram[3] to prepare a calibration curve, and calculate the amount of propionic acid in the sample.

* 1. ULTRON PS-80H (Shinwa chemical industries) or equivalent

2. ULTRON PS-80G (Shinwa chemical industries) or equivalent

«Summary of analysis method»

This method is to distill propionic acid and its salts from the sample and quantify the distillated propionic acid using an ion-exclusion liquid chromatograph.

Figure 12. 1.1-1 shows the flow sheet of analysis method.

50 g or less of sample (10-100 mg of propionic acid)
↓
500 mL Kjeldahl flask
- Purified water; 200 mL
- Sodium chloride; 80 g
- Phosphoric acid solution (1 : 9); 10 mL
- Silicone oil; 1-2 drops

Distillation
- Distill the sample until the distillated liquid will be about 450 mL

Constant volume
- Adjust to constant volume with water in 500 mL volumetric flask

Membrane filter (with pore diameter of 0.5 μm or less)
↓
LC-UV (210 nm)

Figure 12.1.1-1 Flow sheet of analysis method for propionic acid, calcium propionate or sodium propionate in feed

References: Akira Suzuki, Yoshitsugu Tanaka and Yoshihiro Shishido: Research Report of Animal Feed, 7, 12 (1981)

Masato Shibata, Kouki Fujiwara and Toshiaki Yamata: Research Report of Animal Feed, 24, 79 (1999)

«Methods validation»

· Spike recovery and repeatability

Spiked component	Sample type	Spike concentration (%)	Replicate	Average spike recovery (%)	Repeatability RSD (% or less)
Propionic acid	Fomula feed for poultry	0.05-0.3	3	90.3-94.7	4.5
	Fomula feed for swine	0.05-0.3	3	89.7-92.3	5.0
	Fomula feed for cattle	0.05-0.3	3	89.3-92.3	6.1

· Collaborative study

Spiked component	Sample type	No. of labs	Label claim (%)	Average spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Propionic acid	Formula feed for finishing beef cattle	6	0.15	98.5	4.3	6.4	1.20

· Limit of quantification: 0.02 %

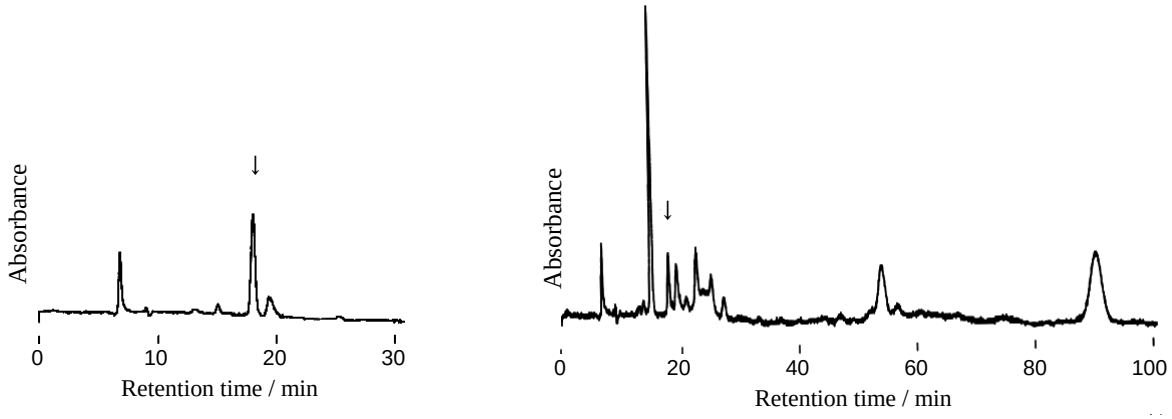
«Notes and precautions»

[1] Carbon dioxide and many organic compounds have UV absorption band around 210 nm. Use high-quality water which does not contain these compounds for eluent and dilution.

[2] During distillation, the liquid volume in the Kjeldahl-flask increases and the salting-out effect of sodium chloride deteriorates. Therefore, about 450 mL of distillate must be collected.

[3] Figure 12.1.1-2 shows examples of chromatogram.

When analyzing ingredients of animal origin such as fish meal, a large peak may appear in the chromatogram. Pay attention to this point during continuous analysis.



Formula feed for finishing beef cattle (Propionic acid is added.)

Fish meal (Propionic acid is not added.)

Figure 12.1.1-2 Chromatogram for propionic acid added feed and additive-free feed (Arrows show the peaks of propionic acid.)

Measurement conditions:		
	Detector	: UV (210 nm)
	Column	: ULTRON PS-80H
	Guard Column	: ULTRON PS-80G
	Eluent	: Phosphoric acid solution (1+1,000)
	Flow rate	: 0.8 mL/min
	Column temperature	: 50 °C

4 Gas chromatograph method [Analytical standards of feeds, Article 1.4, Section 1, Chapter 12]

Scope: Feed

A. Reagent preparation

1) Propionic acid standard solution. Weigh 1 g of propionic acid [$C_3H_6O_2$] into a 100 mL volumetric flask, add water to dissolve it. Add water to the marked line to prepare propionic acid standard stock solution. (1 mL of this solution contains 10 mg of propionic acid).

In use, dilute a quantity of the standard stock solution with water to prepare propionic acid standard solution containing 1 mg of propionic acid per 1 mL.

2) Crotonic acid standard solution[1]. Weigh 1 g of crotonic acid into a 100 mL volumetric flask, add water to dissolve it. Add water to the marked line to prepare crotonic acid standard stock solution. (1 mL of this solution contains 10 mg of crotonic acid).

In use, dilute a quantity of the standard stock solution with water to prepare crotonic acid solutions containing 1 mg of crotonic acid per 1 mL.

3) Propionic acid-crotonic acid mixture standard solution. Transfer several amounts of propionic acid standard stock solution between 5 and 30 mL into each 100 mL volumetric flask accurately, add 10 mL of crotonic acid standard stock solution to each volumetric flask. Add water up to the marked line to

prepare the propionic acid-crotonic acid mixture standard solution. (Prepare when it is needed.)

B. Quantification

Distillation. Weigh accurately less than 50 g of the analysis sample (equivalent to 10 to 100 mg as propionic acid) into a 500 mL round bottom flask. Add 200 mL[2] of water, 80g of sodium chloride, 10 mL[3] of phosphoric acid solution (1+9) and a few drops of silicone oil as the antifoam to the flask. Join this flask to the steam distillation apparatus[6] connected to a receiver[5] containing 20 mL of water[4] and distill the flask until the volume of distillate reaches around 250 mL[7].

Add 5 mL of crotonic acid standard stock solution[8] accurately to the distillate and mix it. Filter the mixed solution through a membrane filter (with pore diameter of 1 μm or less) to use the filtrate as the sample solution subject to gas chromatography.

Gas chromatography. Inject constant amount of the sample and respective propionic acid-crotonic acid mixture standard solution into the gas chromatograph and generate chromatogram.

Example of measurement conditions:

Detector: Flame ionization detector

Column tube: Glass (3 mm in internal diameter, 2 m in length)

Column packing material: Diatomaceous earth for gas chromatography (250 to 177 μm in particle size (60 to 80 mesh))[*1]

Carrier gas: N_2 (50 mL/min)

Hydrogen: 0.8 kg/cm^2

Dry air: 2.0 kg/cm^2

Column temperature: 170 °C

Injection port temperature: 200 °C

Detector temperature: 220 °C

Calculation. Calculate the peak area ratio between propionic acid and crotonic acid from the obtained chromatogram to prepare the calibration curve of weight ratio between propionic acid and crotonic acid.

Calculate the peak area ratio based on the chromatogram[9] of the sample solution and calculate[10] the amount of propionic acid in the sample.

* 1. Chromosorb 101 (Celite Corporation) or equivalent

«Summary of analysis method»

This method is to distill propionic acid in the sample under acidic condition with phosphoric acid and determine propionic acid in the distillate by using a gas chromatograph equipped with a hydrogen flame ionization detector (internal standard method).

This method enables to quantify sodium propionate and calcium propionate contained in feed as total of propionic acid.

References: Edition of Pharmaceutical Society of Japan: Standard Methods of Analysis for Hygienic Chemists with Commentary, Kanehara & Co. (1980)

Akira Suzuki, Yoshitsugu Tanaka and Yoshihiro Shishido: Research Report of Animal Feed, 7, 12 (1981)

«Method validation»

· Spike recovery rate and repeatability

Spiked component	Sample type	Spike concentration (%)	Replicate	Average spike recovery (%)	Repeatability RSD (% or less)
Propionic acid	Formula feed for broiler	0.05-0.3	9	94.7-98.1	10.2
Sodium propionate	Formula feed for broiler	0.05-0.4	2	98.5-103.5	6.4
Calcium propionate	Formula feed for broiler	0.05-0.5	2	92.5-104.0	5.1

· Collaborative study

Spiked component	Sample type	No. of labs	Label claim (%)	Average spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Propionic acid	Formula feed	6	0.2	98.5	6.2	12.3	2.40

«Notes and precautions»

[1] Used as the internal standard substance for gas chromatography.

[2] The sample may possibly harden by adding water. Be sure to shake the sample well while adding water.

[3] The pH of the solution will be about 2 to 3.

[4] In the analysis of food, propionic acid is captured with sodium hydroxide solution and passed through an ion-exchange column. Since calcium carbonate is added to formula feed, CO_2 is generated as well as propionic acid simultaneously during distillation. They react with sodium hydroxide in the receiver to generate Na_2CO_3. Through the column operation, Na_2CO_3 generates CO_2, which has been considered to affect ion exchange adversely. To avoid the problem, this method simply captures distilled propionic acid with water, passes it through a membrane filter, without using the ion-exchange column, and measures the filtrate using a gas chromatograph.

[5] Because of volatileness of propionic acid, it is preferable to use a 300 to 500 mL Erlenmeyer flask as a receiver and chill the receiver with ice.

[6] Figure 12.1.1-3 shows an example of the distillation apparatus.

[7] Most of propionic acid is distilled in the first 50 to 100 mL. Transfer the distillate into a 250 mL volumetric flask, add 5 mL of crotonic acid standard stock solution and fill up to the marked line with water.

[8] The amount when the contained amount of propionic acid in the sample is 0.1 to 0.3 %. It is preferable that the additive amount of crotonic acid correspond to the amount of propionic acid in the distillate.

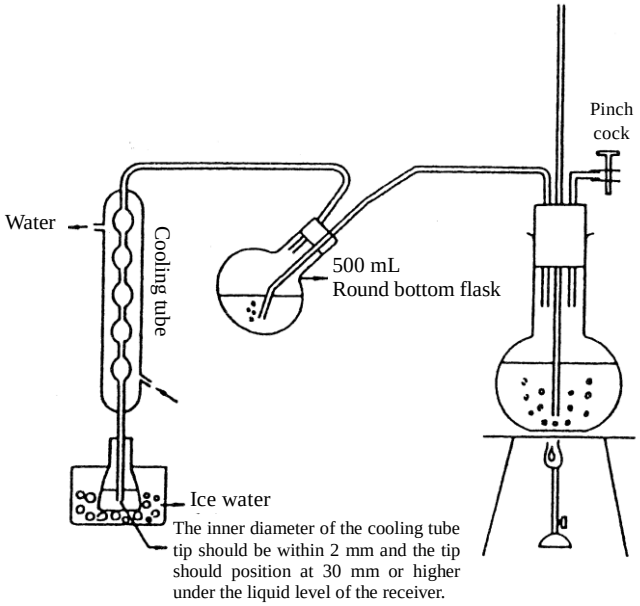
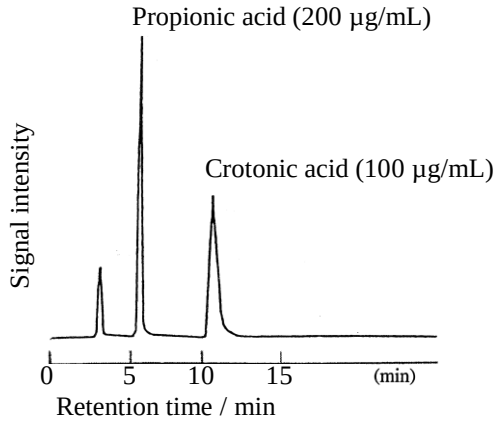


Figure 12.1.1-3 Distillation apparatus

[9] Figure 12.1.1-1 shows an example of the chromatogram.



Measurement conditions

Column tube: Glass tube, 3 mm φ, 2 m

Column filler:Chromosorb101
(60 to 80 mesh)

Carrier gas: N_2 40 to 60 mL/min

Hydrogen: 0.8 kg/cm^2

Column temperature: 170 °C

Detector temperature: 220 °C

Figure 12.1.1-4 Gas chromatogram of propionic acid

The conditions by using a capillary column are as below:

Detector: Hydrogen flame ionization detector

Column: DB-WAX (0.32 mm × 30 m, film thickness 0.5 µm)

Carrier gas: He 2.0 kg/cm^2 (initial flow rate)

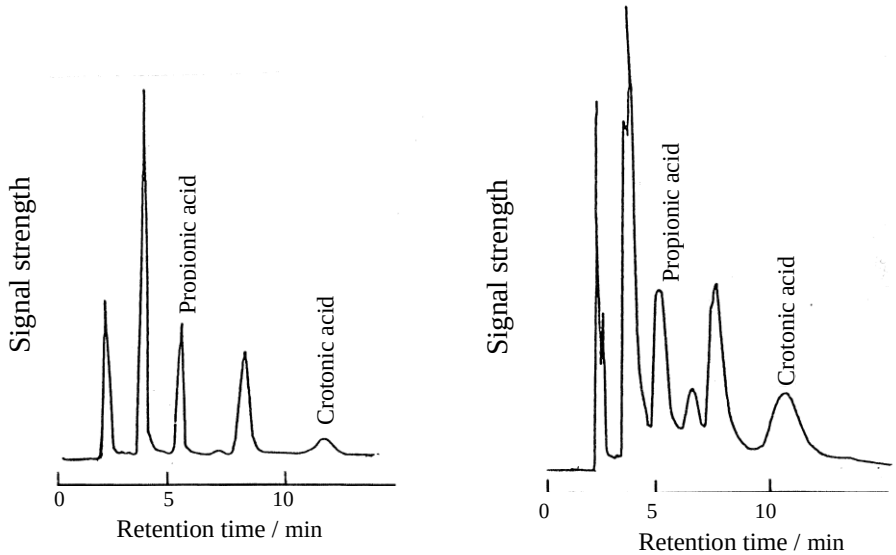
Hydrogen: 0.60 kg/cm^2

Dry air: 0.50 kg/cm^2

Column temperature: 170 °C (hold for 6 min) → ramp 20 °C/min → 200 °C (hold for 1 min)

Detector temperatire: 280 °C

Generally, animal feed contains relatively large amount of short-chain fatty acid. Fish meal contains about 0.01 to 0.06 %, and meat-and-bone meal contains about 0.03 % of propionic acid. As the commercially available aquatic formula feed contains around 40 % of fish meal, the natural contained amount is estimated to be 1/10 of the additive amount. Thus, due considerations on this influence must be taken (Figure 12.1.1-5).



Aquatic formula feed (Propionic acid is not added.)

Fish meal (Propionic acid is not added.)

Figure 12.1.1-5 Gas chromatogram of propionic acid

[10] To calculate the amount of calcium propionate or sodium propionate, multiply the amount of propionic acid by 1.257 for the former and by 1.297 for the latter.

Section 2 Multicomponent analysis method

1 Simultaneous analysis method of organic acids by capillary electrophoresis[1]*1 [Analytical standards of feeds, Article 1, Section 2, Chapter 12]

Target analyte: Propionic acid, formic acid, citric acid, acetic acid, tartaric acid, lactic acid, fumaric acid and malic acid (8 compounds)

A. Reagent preparation

1) Propionic acid standard stock solution. Weigh 1.257 g of calcium propionate [$C_6H_{10}CaO_4$] or 1.297 g of sodium propionate [$C_3H_5NaO_2$] into a 1,000 mL volumetric flask, add water to dissolve it. Then add water to the marked line to prepare propionic acid standard stock solution (1 mL of this solution contains 1 mg of the propionic acid).
2) Formic acid standard stock solution. Weigh 1.478 g of sodium formate [CHO_2Na] into a 1,000 mL volumetric flask, add water to dissolve it. Then add water to the marked line to prepare formic acid standard stock solution (1 mL of this solution contains 1 mg of formic acid).
3) Citric acid standard stock solution. Weigh 1.093 g of citric acid monohydrate[$C_6H_{10}O_8 \cdot H_2O$] into a 1,000 mL volumetric flask, add water to dissolve it. Then add water to the marked line to prepare citric acid standard stock solution (1 mL of the solution contains 1 mg of citric acid).
4) Acetic acid standard stock solution. Weigh 1.467 g of calcium acetate monohydrate [$C_4H_8O_5Ca \cdot H_2O$] into a 1,000 mL volumetric flask, add water to dissolve it. Then add water to the marked line to prepare acetic acid standard stock solution (1 mL of the solution contains 1 mg of acetic acid).
5) Tartaric acid standard stock solution. Weigh 1 g of L-tartaric acid [$C_6H_6O_6$] accurately into a 1,000 mL volumetric flask, add water to dissolve it. Then add water to the marked line to prepare tartaric acid standard stock solution (1 mL of the solution contains 1 mg of tartaric acid).
6) Lactic acid standard stock solution. Weigh 1.711 g of calcium lactate 5-hydrate [$C_6H_{10}O_6Ca \cdot 5H_2O$] into a 1,000 mL volumetric flask, add water to dissolve it. Then add water to the marked line to prepare lactic acid standard stock solution (1 mL of the solution contains 1 mg of lactic acid).
7) Fumaric acid standard stock solution. Weigh 1 g of fumaric acid [$C_4H_4O_4$] accurately into a 1,000 mL volumetric flask, add water to dissolve it. Then add water to the marked line to prepare fumaric acid standard stock solution (1 mL of the solution contains 1 mg of fumaric acid).
8) Malic acid standard stock solution. Weigh 1 g of L-malic acid [$C_4H_6O_5$] accurately into a 1,000 mL volumetric flask, add water to dissolve it. Then add water to the marked line to prepare malic acid standard stock solution (1 mL of this solution contains 1 mg of malic acid).
9) Mixed organic acids standard solution. Mix a certain amount of each organic acid standard stock solution, dilute it accurately with sodium hydroxide solution (0.02 mol/L) to prepare several mixed organic acids standard solutions containing 10 to 250 μg of each organic acid per 1 mL.

B. Quantification

Extraction. Weigh 5 g of sample accurately into a 200 mL stoppered Erlenmeyer flask. Add 100 mL of water to the flask, stir the solution for 30 minutes. Filter the extract through a filter paper (No.5A) and dilute a certain amount of filtrate accurately with sodium hydroxide solution (0.02 mol/L). Filter the

diluted solution centrifugally with 5,000×*g* for 15 minutes using a plastic filter cup[*2] (capacity: 1.5 mL) attached with an ultrafiltration membrane (cut-off molecular weight: 30,000) to obtain permeate[*3] as a sample solution subject to capillary electrophoresis.

Capillary electrophoresis. Inject sample solution and respective mixed organic acids standard solution into capillary electrophoresis apparatus and obtain electropherogram by indirect absorbance method.

Example of measurement conditions:

Column: Capillary column made of fused silica (internal diameter: 50 μm, effective length: 104 cm, overall length: 112.5 cm)[*4]

Migration buffer: Buffer solution containing 2,6-pyridinedicarboxylic acid[*5]

Voltage: −30 kV

Column temperature: 18 °C

Sample injection method: Pressure injection method (50 kPa, 6 s)

Detector: Ultraviolet absorption detector (Detection wavelength: 350 nm, reference wavelength: 275 nm)

Column cleaning: Before injecting the sample solution and mixed organic acids standard solution into the capillary electrophoresis apparatus, rinse capillary with migration buffer solution for more than 4 minutes.

Calculation. Calculate the peak area from obtained electropherogram to prepare a calibration curve, and calculate the amount of each organic acid in the sample.

* 1. Use water of an electric conductivity of less than 5.6 μS/m. (specific resistance of 18 MΩ·cm or above)

2. Microcon YM-30 (Millipore) or equivalent

3. Use supernatant of the permeate in the filter cup as the sample solution.

4. Internal diameter of the column is settled to 50 μm, and effective length is between 100 and 110 cm.

5. Capillary electrophoresis buffer for analysis of plating liquid (Agilent Technologies) or equivalent.

«Summary of analysis method»

This method is to extract eight types of organic acid added to formula feed, using water, and simultaneously determine the amounts of extracted organic acids using the capillary electrophoresis apparatus. This makes analyses quicker and easier than using the conventional gas chromatograph or liquid chromatograph.

Figure 12.2.1-1 shows the flow sheet of analysis method.

Sample 5 g
- Add 100 mL of water
- Stir for 30 min

Filtration (No.5A)
- Dilute 2-5 fold with sodium hydroxide (0.02 mol/L)

Filter through ultrafiltration membrane (cut-off molecular weight: 30,000)

Capillary electrophoresis

Figure 12.2.1-1 Flow sheet of simultaneous analysis method of organic acids

Reference: Eiichi Ishikuro, Hiroshi Hibino, Tomoyoshi Soga, Hiroko Yanai and Hirokazu Sawada: Journal of the Food Hygenic Society of Japan, 41 (4), 261 (2000)

«Method validation»

· Spike recovery and repeatability

Spiked component	Sample type	Spike concentration (%)	Replicate	Average spike recovery (%)	Repeatability RSD (% or less)
Propionic acid	Formula feed (3 types)	0.5-2	3	88.7-101.3	6.9
Formic acid	Formula feed (3 types)	0.1-0.5	3	96.5-103.7	11.5
Citric acid	Formula feed (3 types)	0.5-2	3	88.4-101.1	8.4
Acetic acid	Formula feed (3 types)	0.1-1	3	90.1-100.8	8.5
Tartaric acid	Formula feed (3 types)	0.05-0.2	3	85.1-103.5	12.4
Lactic acid	Formula feed (3 types)	0.5-2	3	96.9-107.3	12.5
Fumaric acid	Formula feed (3 types)	0.05-2	3	93.9-99.0	13.2
Malic acid	Formula feed (3 types)	0.1-0.5	3	95.2-102.0	13.6

· Collaborative study

Spiked component	Sample type	No. of labs	Spike concentration (%)	Average spike recovery (%)	Intra-lab repeatability RSD_r (%)	Inter-lab reproducibility RSD_R (%)	HorRat
Propionic acid	Formula feed for layer	3	0.5	91.4	1.8	8.6	1.91
Formic acid	Formula feed for layer	3	0.25	92.6	2.9	6.4	1.28
Citric acid	Formula feed for layer	3	1	91.6	3.0	15.6	3.85
Acetic acid	Formula feed for layer	3	0.5	90.0	2.6	9.4	2.08
Tartaric acid	Formula feed for layer	3	0.1	90.2	7.7	16.3	2.84
Lactic acid	Formula feed for layer	3	1	105.0	1.6	9.5	2.39
Fumaric acid	Formula feed for layer	3	0.2	87.3	4.5	5.6	1.08
Malic acid	Formula feed for layer	3	0.2	90.6	7.9	11.3	2.18

· Limit of quantification: 0.02 % each (Recovery rate and relative standard deviation)

«Notes and precautions»

[1] For details on capillary electrophoresis (CE), see Chapter 4, Section 1, 6 Chlorine 1 **«Notes and precautions»** [1].

[2] For sodium hydroxide, use the highly pure quality such as the capillary conditioning solution (1.0 N sodium hydroxide solution (Part No. 5062-8576) manufactured by Agilent Technologies).

[3] Figure 12.2.1-2 shows examples of electropherogram.

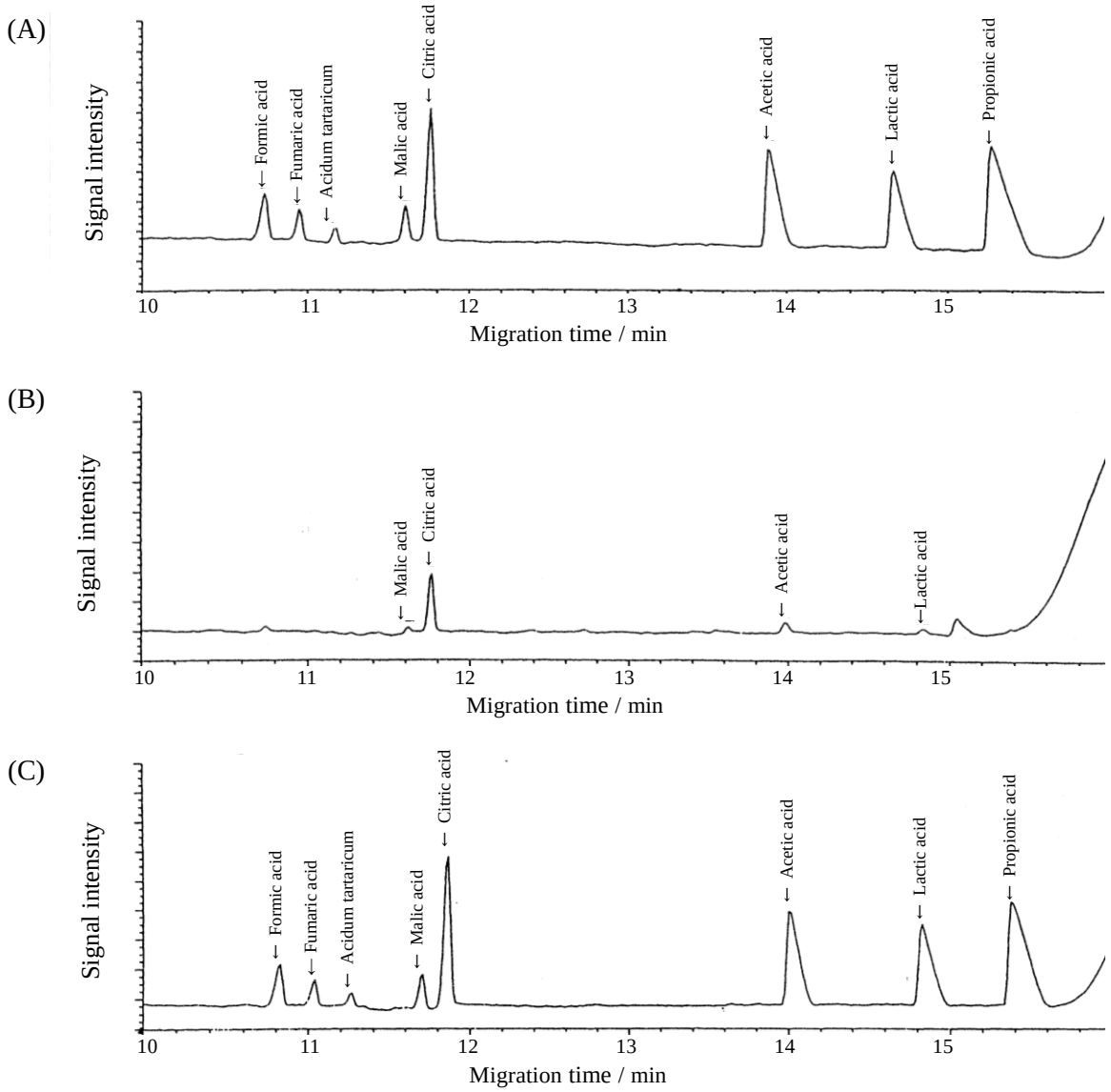


Figure 12.2.1-2 Electropherograms obtained by recovery test

(A) Standard solution

(B) Formula feed for layer (blank)

(C) Formula feed for layer (Spiked with organic acids at the levels of 0.25 % of formic acid, 0.5 % of propionic acid, 0.2 % of fumaric acid, 0.1 % of tartaric acid, 0.2 % of malic acid, 1.0 % of citric acid, 0.5 % of acetic acid and 1.0 % of lactic acid.)