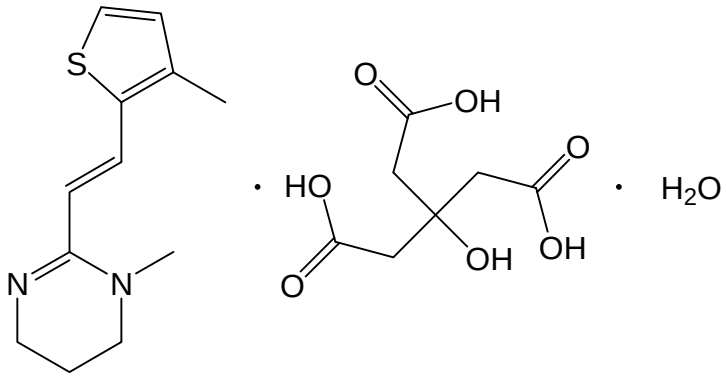
7 Morantel citrate



1-methyl-2-[(*E*)-2-(3-methylthiophen-2-yl)ethenyl]-5,6-dihydro-4*H*-pyrimidine
2-hydroxypropane-1,2,3-tricarboxylic acid salt monohydrate
$C_{18}H_{26}N_2O_8S$ MW: 430.47 CAS No.: 69525-81-1

【Outline of morantel citrate】

Morantel citrate is light yellow to yellow crystalline powder, slightly bitter with special odor, and soluble in methanol, slightly soluble in water or ethanol, and practically insoluble in ethyl acetate or benzene.

This is a chemically synthesized antiparasitic agent, having characteristic to block the transition of young ascarids in the body of domestic animals. In Japan, this agent has been designated to one of feed additives to promote the effective use of nutrient components in feeds, being approved for adding to feeds for starting piglets and growing piglets at 30 g/t.

This agent has no antibacterial potential; however, classified conveniently in synthetic antibacterials.

《Cautions for analytical processing of morantel citrate》

Quantification processing is to be performed in a dark place protected from light. Where unable to protect from light, it is effective to cover the container etc. with an aluminum foil.

When morantel citrate standard solution (0.5 μg/mL) is stored at room temperature in a transparent glass container, the level of morantel citrate decreases to approximately 10% by 2 hr after preparation; to approximately 50% by 6 hr after preparation when stored in a brown glass container. On the other hand, when both containers were covered with an aluminum foil, it still stable at 72 hr after preparation. Furthermore, when morantel citrate standard solution is stored in a brown glass container covered with an aluminum foil in a refrigerator, it is stable as long as 3 months.

【Methods listed in the Feed Analysis Standards】

1 Quantitative test methods

1.1 Liquid chromatography

1.1.1 Premix [Note 1] 〔Feed Analysis Standards Chapter 8, Section 1, 7.1.1-(1)〕

A. Reagent preparation

Morantel citrate standard solution: Place 25 mg of morantel citrate〔$C_{18}H_{26}N_2O_8S$〕exactly measured in a 250 mL brown volumetric flask, add methanol for dissolving, further add the solvent up to the gauge line to prepare the morantel citrate standard stock solution（1 mL of this solution contains an amount of morantel citrate equivalent to 0.1 mg）.

At the time of use, exactly dilute a definite amount of the standard stock solution with methanol [1] to prepare several morantel citrate standard solutions containing amounts of morantel citrate equivalent to 0.5-7 μg per mL.

B. Quantification

Extraction: Place 3-5 g of analysis sample (equivalent to 6-60 mg morantel citrate) exactly measured in a stoppered 200 mL brown Erlenmeyer flask, add 100 mL of methanol, stir it for 30 min for extraction. Place the extracted solution in a stoppered brown centrifuging tube, centrifuge at 1,500×g for 5 min, and exactly dilute a definite amount of the supernatant with methanol [1]. Filter this solution through a membrane filter（pore diameter: 0.5 μm or less）[Note 2] to obtain a sample solution for liquid chromatography.

Liquid chromatography: Inject respective 20 μL of the sample solution and each morantel citrate standard solution into the liquid chromatograph to obtain the chromatogram.

Measurement conditions (example)

Detector: Ultraviolet spectrophotometer (measurement wavelength: 320 nm)

Column: Octadecylsilylated silica-gel column (internal diameter: 4.6 mm, length: 250 mm, particle diameter: 5 μm) [Note3][2]

Eluent: Phosphate buffer solution [Note4]-acetonitrile (4:1) [3]

Flow rate: 1.0mL/min

Column temperature: 40℃

Calculation: Obtain the peak area from the chromatogram [4] to prepare the calibration curve, and calculate the morantel citrate amount in the sample.

Note 1. Process the quantification in a dark room, covering the glass container, etc., with an aluminum foil or the like.

2. Use morantel citrate without adsorbability such as made of PTFE.

3. Shodex C_{18}-5B (Showa Denko) or an equivalent one.

4. Dissolve 6.8 g of potassium dihydrogen phosphate up to make 1 L, and adjust the pH to 3.3 with phosphoric acid (1:10).

《Summary of analysis method》

This method is intended to determine the amount of morantel citrate in a premix by extracting with methanol, diluting with the solvent, and quantifying using a liquid chromatograph with an ultraviolet spectrophotometer.

Reference: Tetsuo Chihara, Masakazu Horikiri：Research Report of Animal Feed, 19, 92 (1994)

History in the Feed Analysis Standards: 【8】new【16】revision

《Validation of analysis method》

・Recovery rate and repeat accuracy

Type of sample	Concentration (mg/kg)	Repeat	Recovery rate (%)	Repeat accuracy RSD（% or less）
Premix for pig 1	3~30	3	99.9-102.4	11.5
Premix for pig 2	3~30	3	101.9-103.0	6.4
Premix fot pig 3	3~30	3	98.6-100.6	10.8

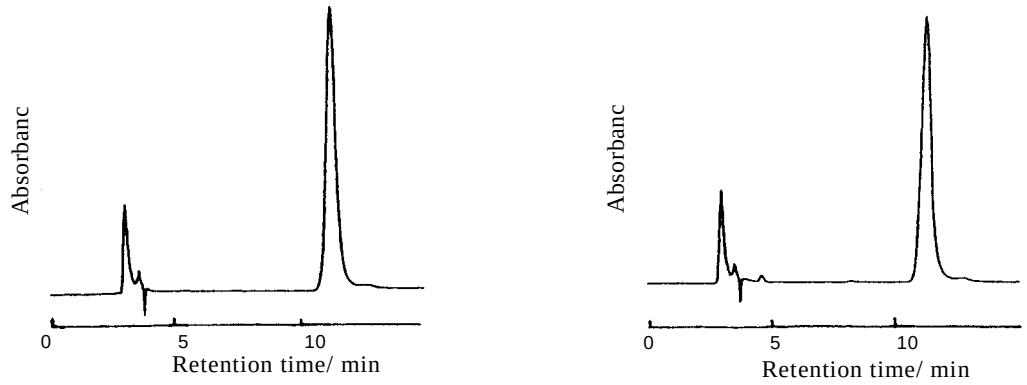
《Notes and precautions》

[1] When extracted solution is diluted with methanol-water (17:3), the calibration curve for formula feed can be used.

[2] Any column with an equivalent end-capped packing material is applicable.

[3] Since the eluent contains buffer solution, LC apparatus and columns used should be washed enough, and the eluent should be replaced entirely with methanol, acetonitrile or others before storing. At the time of use, give eluent after replacing with water.

[4] An example of chromatogram of morantel citrate is shown in Fig. 8.1.7-1.



standard solution（60 ng） Premix for pig

Fig. 8.1.7-1 A chromatogram of morantel citrate (example)

（The arrow indicates the peak of morantel）

Measurement conditions

Detector: Measurement wavelength: 320 nm

Column: Shodex C18-5B

Eluent: Phosphate buffer solution-acetonitrile（4:1）

Flow rate: 1.0 mL/min

Column temperature: 40 °C

[5] Refer to《Cautions for analytical processing of morantel citrate》

[6] Filters other than made of polytetrafluoroethylene (PTFE) possibly adsorb morantel citrate; therefore, when use a membrane filter, confirm before use that morantel citrate should not be adsorbed. A supernatant obtained by using a high-speed centrifuge at 5,000×*g* (10,000 rpm) for 3 min can be used as a sample solution.

1.1.2 Formula feed [Note1]

〔Feed Analysis Standards Chapter 8, Section 1, 7.1.1-(2)〕

A. Reagent preparation

1) Extraction solvent: Water-methanol-acetic acid (15:4:1)

2) Morantel citrate standard solution: Place 25 mg of morantel citrate〔$C_{18}H_{26}N_2O_8S$〕exactly measured in a 250 mL brown volumetric flask, add methanol for dissolving, further add the solvent up to the gauge line to prepare the morantel citrate standard stock solution（1 mL of this solution contains an amount of morantel citrate equivalent to 0.1 mg）.

At the time of use, exactly dilute a definite amount of the standard stock solution with water-methanol (7:3) to prepare several morantel citrate standard solutions containing amounts of morantel citrate equivalent to 0.2-5.0 μg per mL.

B. Quantification

Extraction: Measure 10.0 g of analysis sample, place it in a stoppered 200 mL brown Erlenmeyer flask, add 100 mL of the solvent for extraction, and stir for 30 min for extraction. Place the extracted solution in a stoppered brown centrifuging tube, and centrifuge at 1,800×g for 5 min to obtain the supernatant as a sample solution for column treatment.

Column treatment: Wash an octadecylsilylated gel minicolumn (360 mg) sequentially with 4 mL of methanol and 4 mL of water.

Place 5 mL of the sample solution in the minicolumn, and effuse the solution with pressure injection at a flow rate of approximately 1 mL/min until the fluid level reaches the top of the packing material. Place a 10 mL volumetric flask under the minicolumn, to which add 10 mL of water-methanol (7:3), and elute morantel citrate with pressure injection at a flow rate of approximately 1 mL/min. Then, add the solvent up to the gauge line of the volumetric flask. Place an appropriate amount of this solution in a stoppered plastic centrifuging tube (1.5 mL in volume) , and centrifuge at 5,000×g for 3 min to obtain the supernatant as a sample solution for liquid chromatography.

Liquid chromatography: Inject respective 20 μL of the sample solution and each morantel citrate standard solution into the liquid chromatograph to obtain the chromatogram.

Measurement conditions (example)

Detector: Ultraviolet spectrophotometer (measurement wavelength: 320 nm)

Column: Octadecylsilylated silica-gel column (internal diameter: 6.0 mm, length: 150

mm, particle diameter: 5 μm) Note 2[1]

Eluent: Phosphate buffer solution Note 3—acetonitrile (4:1)[2]

Flow rate: 1.0 mL/min

Column temperature: 40°C

Calculation: Obtain the peak area from the chromatogram [3] to prepare the calibration curve and calculate the morantel citrate amount in the sample.

Note 1. Process the quantification in a dark room, and cover the glass container, etc., with an aluminum foil or the like [4].

2. YMC-Pack ODS-AM (YMC) or an equivalent one.

3. Dissolve 6.8 g of potassium dihydrogen phosphate to make a total amount of 1 L, adjust the pH to 3.3 with phosphoric acid (1:10).

《Summary of analysis method》

This method is intended to determine the amount of morantel citrate in a formula feed by extracting with water-methanol-acetic acid (15:4:1), purifying with a C_{18} minicolumn and quantifying with a liquid chromatograph with an ultraviolet spectrophotometer.

Reference: Katsumi Yamamoto: Research Report of Animal Feed, 31, 98 (2006)

History in the Feed Analysis Standards: 【8】new,【16】revision,【19】revision,【29】revision

《Validation of analysis method》

・Recovery rate and repeat accuracy

Type of sample	Concentration (mg/kg)	Repeat	Recovery rate (%)	Repeat accuracy RSD (% or less)
Formula feed for suckling pig	15-45	3	94.3-100.9	4.8
Formula feed for growing piglet 1	15-45	3	91.6-96.4	2.3
Formula feed for growing piglet 2	15-45	3	92.9-103.5	4.2

・Cooperative testing

Type of sample	No. of labs	Indicated amoun (mg/kg)	Recovery rate (%)	Repeat accuracy in room RSD_r (%)	Reproducibility RSD_R (%)	HorRat
Formula feed for suckling pig (mush)	9	30	99.2	3.5	4.6	0.48
Formula feed for suckling pig (crumble)	9	30	87.5	1.5	4.5	0.46

《Notes and precautions》

[1] Any column with an equivalent end-capped packing material is applicable.

[2] Since the eluent contains buffer solution, LC apparatus and columns used should be washed enough, and the eluent should be replaced entirely with methanol, acetonitrile or others before storing. At the time of use, give eluent after replacing with water.

[3] An example of chromatogram of morantel citrate is shown in Fig. 8.1.7-2.

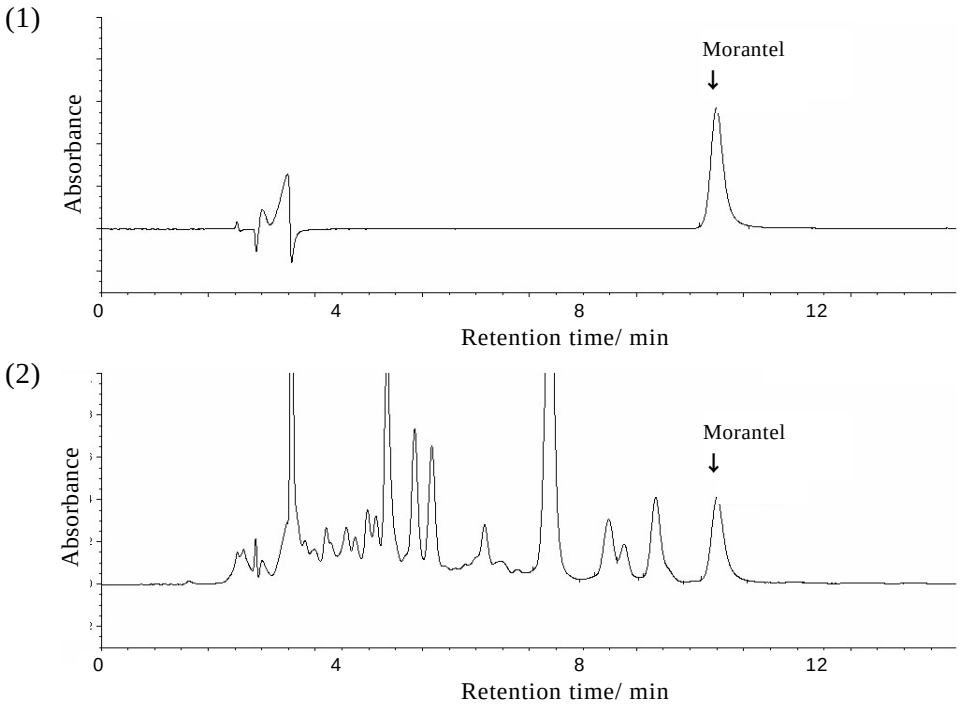


Fig. 8.1.7-2 A chromatogram of morantel citrate

(1) Standard solution (40 ng), (2) Formula feed for piglets (30 g/t)

[4] Refer to《Cautions for analytical processing of morantel citrate》

2 Microquantitative test method

2.1 Liquid chromatography [Note1] 〔Feed Analysis Standards Chapter 8, Section 1, 7.2.1〕

A. Reagent preparation

1) Morantel citrate standard solution: Place 25 mg of morantel citrate〔$C_{18}H_{26}N_2O_8S$〕exactly measured in a 250 mL brown volumetric flask, add methanol for dissolving, further add the solvent up to the gauge line to prepare the morantel citrate standard stock solution（1 mL of this solution contains an amount of morantel citrate equivalent to 0.1 mg）.

At the time of use, exactly dilute a definite amount of the standard stock solution with methanol to prepare several morantel citrate standard solutions containing amounts of morantel citrate equivalent to 0.05-1 μg per mL.

2) Basic alumina: Dry basic alumina (particle diameter: 63-200 μm (230-70 mesh) [Note 2] for a column chromatograph at 130 °C for 2 hr.

3) Carbonic acid buffer solution: Dissolve 7.6 g of sodium hydrogen carbonate and 0.5 g of sodium carbonate in water to make a total amount of 1 L, and adjust the pH to 9.0 with sodium hydroxide solution (1 mol/L).

B. Quantification

Extraction: Measure 10.0 g of analysis sample, place it in a stoppered 200 mL brown Erlenmeyer flask, add 100 mL of methanol, and stir it for 30 min for extraction. Place the extracted solution in a stoppered brown centrifuging tube, and centrifuge at 1,500×g for 5 min to obtain the supernatant

as a sample solution for column treatment I.

Column treatment I: Pack 5 g of basic alumina in a column tube (internal diameter: 10 mm) with dry processing to prepare the column.

Place the sample solution in the column, and discard 5 mL of the first effluent. Place exactly 10 mL of subsequent effluent in a 50 mL recovery flask, and concentrate it under reduced pressure in water bath at 50 °C until dry out.

Dissolve the residues by adding 5 mL of methanol-carbonic acid buffer solution (1:1) to obtain the sample solution for column treatment II.

Column treatment II [1]: Wash an octadecylsilylated silica-gel minicolumn (360 mg) sequentially with 10 mL of methanol and 10 mL of carbonic acid buffer solution.

Place the sample solution in a minicolumn, and effuse it with pressure injection Note 3. Wash the recovery flask which previously contained sample solution with 5 mL of methanol-carbonic acid buffer solution（1:1）[2] and process in a similar way. Place a 50 mL recovery flask under the minicolumn to which add 10 mL of methanol-acetic acid (200:1) to elute morantel citrate with pressure injection Note3. Concentrate the eluent under reduced pressure in a water bath at 50 °C to almost dry out, and then send nitrogen gas to obtain the dry matter.

Dissolve the residues by exactly adding 2 mL of methanol, and filter this solution through a membrane filter Note 4 (pore diameter: 0.5 μm or less) to obtain the sample solution for liquid chromatography.

Liquid chromatography: Inject respective 20 μL of the sample solution and each morantel citrate standard solution into a liquid chromatograph to obtain the chromatogram.

Measurement conditions (example)

Detector: Ultraviolet spectrophotometer (measurement wavelength: 320 nm)

Column: Octadecylsilylated silica-gel column (internal diameter: 4.6 mm, length: 250 mm, particle diameter: 5 μm) note 3[3]

Eluent: Phosphate buffer solution note 4—acetonitrile（4:1）[4]

Flow rate: 1.0 mL/min

Column temperature: 40°C

Calculation: Obtain the peak area from the chromatogram [5] to prepare the calibration curve, and calculate the morantel citrate amount in the sample.

Note 1. Process the quantification in a dark room, and cover the glass container, etc., with an aluminum foil or the like [6].

2. Aluminiumoxid 90 activ basisch Art. 1076 (Merck) or an equivalent one.
3. Flow rate is approximately 2 mL/min
4. Use morantel citrate without adsorbability such as made of PTFE [7].

《Summary of analysis method》

This method has been developed to quantify a minute amount of morantel citrate remaining in formula feeds caused by carrying over, etc. and is intended to determine the amount of morantel

citrate in feeds by extracting with methanol, purifying with a basic alumina minicolumn and a C_{18} minicolumn and quantifying using a liquid chromatograph with an ultraviolet spectrophotometer.

Reference: Tetsuo Chihara, Soichiro Matsumura, Akira Hashimoto, Masakazu Horikiri: Research Report of Animal Feed, 18, 73 (1993)

History in the Feed Analysis Standards: 【15】new

《Validation of analysis method》

Recovery rate and repeat accuracy

Type of sample	Concentration (mg/kg)	Repeat	Recovery rate (%)	Repeat accuracy RSD (% or less)
Formula feed for adult chicken	0.1-1.0	3	99.0-103.3	3.0
Formula feed for growing pig	0.1-1.0	3	98.7-105.0	4.2
Formula feed for growing beef cattle	0.1-1.0	3	99.7-104.7	3.9

・Cooperative testing

Type of sample	No. of labs	Concentration (mg/kg)	Recovery rate (%)	Repeat precision in room RSD_r (%)	Reproducibility RSD_R (%)	HorRat
Formula feed for growing beef cattle	6	0.5	98.9	1.8	6.3	0.50

《Notes and precautions》

[1] Note that mixed air in a cartridge has effects on elution and others of morantel citrate.

[2] Note that the use of methanol-carbonic acid buffer solution (1:1) 5mL or more to wash containers possibly cause elution of a part of morantel citrate kept in a minicolumn. If possible, wash them twice using each 2.5 mL of methanol-carbonic acid buffer solution (1:1).

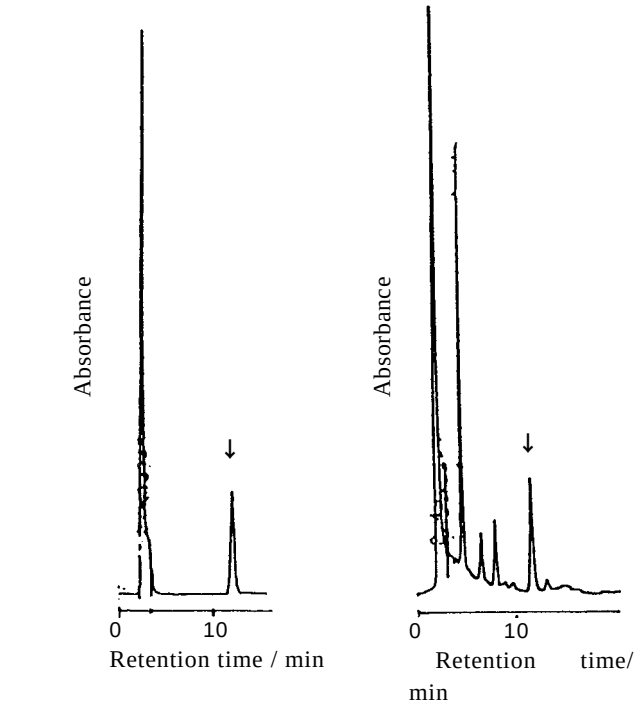
[3] Any column with an equivalent end-capped packing material is applicable.

[4] Since the eluent contains buffer solution, LC apparatus and columns used should be washed enough, and the eluent should be replaced entirely with methanol, acetonitrile or others before storing. At the time of use, give eluent after replacing with water.

[5] An example of chromatogram is shown in Fig. 8.1.7-3.

[6] Refer to《Cautions for analytical processing of morantel citrate》

[7] Since filters other than made of polytetrafluoroethylene（PTFE）possibly cause adsorption of morantel citrate, confirm that morantel citrate is never adsorbed to the filter before use. Supernatant obtained by a high-speed centrifuge at 5,000×*g* (10,000 rpm) for 3 min can be used as a sample solution.



Measurement conditions
Detector: Measurement wavelength: 320 nm
Column: Shodex C_{18}-5B
Eluent: Phosphate buffer solution-acetonitrile (4:1)
Flow rate: 1.0 mL/min
Column temperature: 40 °C

Fig. 8.1.7-3 A chromatogram of morantel citrate
(The arrow indicates the peak of morantel)

【Other analysis method by chromatograph】

3 Quantitative test method by liquid chromatograph (Formula feed Note1)

A. Reagent preparation

Morantel citrate standard solution: Place 25 mg of morantel citrate〔$C_{18}H_{26}N_2O_8S$〕exactly measured in a 250 mL brown volumetric flask, add methanol for dissolving, further add the solvent up to the gauge line to prepare the morantel citrate standard stock solution（1 mL of this solution contains an amount of morantel citrate equivalent to 0.1 mg）.

At the time of use, exactly dilute a definite amount of the standard stock solution with methanol-water (17:3) to prepare respective morantel citrate standard solutions containing 1.5 μg, 3 μg and 4.5 μg of morantel citrate per mL.

2) Basic alumina: Dry basic alumina (particle diameter 63-200 μm (230-70 mesh) Note 2 for a column chromatograph at 130 °C for2 hr.

B. Quantification

Extraction: Measure 10.0 g of analysis sample [1], place it in a stoppered 200 mL brown Erlenmeyer flask, add 100 mL of methanol-water (17:3), and stir for 30 min for extraction. Place

the extracted solution in a stoppered brown centrifuging tube, and centrifuge at 1,500×g for 5 min to obtain the supernatant as a sample solution for column treatment.

Column treatment: Pack 5 g of basic alumina in a column tube (internal diameter: 10 mm) with dry processing to prepare the column for clean up.

Place the sample solution in the column, and discard 5 mL of the first effluent. Filter 5 mL of subsequent effluent through a membrane filter（pore diameter: 0.5 μm or less）Note 3 to obtain a sample solution for liquid chromatography.

Liquid chromatography: Inject respective 20 μL of the sample solution and each morantel citrate standard solution into a liquid chromatograph to obtain the chromatogram.

Measurement conditions (example)

Detector: Ultraviolet spectrophotometer (measurement wavelength: 320 nm)

Column: Octadecylsilylated silica-gel column (internal diameter: 4.6 mm, length: 250 mm, particle diameter: 5 μm) Note 2[2]

Eluent: Phosphate buffer solution Note 4—acetonitrile（4:1）[3]

Flow rate: 1.0 mL/min

Column temperature: 40°C

Calculation: Obtain the peak area from the chromatogram [4] to prepare the calibration curve, and calculate the morantel citrate amount in the sample.

Note 1. Process the quantification in a dark room, and cover the glass container, etc., with an aluminum foil or the like.

2. Aluminiumoxid 90 aktiv basisch Art. 1076 (Merck) or an equivalent one.

3. Use one not adsorbing morantel citrate [6].

4. Dissolve 6.8 g of potassium dihydrogen phosphate to make a total amount of 1 L, and adjust the pH to 3.3 with phosphoric acid (1:10).

《Summary of analysis method》

This method is intended to determine the amount of morantel citrate in formula feeds by extracting with methanol-water (17:3), purifying with a basic alumina column, and quantifying using a liquid chromatograph with an ultraviolet spectrophotometer.

This method had been temporarily listed in Feed Analysis Standards; however, abolished at the time of establishment of the current standards.

Reference: Soichiro Matsumura, Yuji Fukumoto, Atsushi Kito, Yuzo Ono, Tetsuo Chihara: Research Report of Animal Feed, 22, 77 ((1997)

History in the Feed Analysis Standards: 【19】revision,【29】Replaced by the current standards.

《Notes and precautions》

[1] In quantification, morantel citrate can be underestimated for expanded feed. In that case, it may be improved by using water-methanol-acetic acid (15:4:1) as the extraction solvent.

Reference: Eiich Ishiguro: Research Report of Animal Feed, 11, 75 (1986)

[2] Any column with an equivalent end-capped packing material is applicable. **The column**

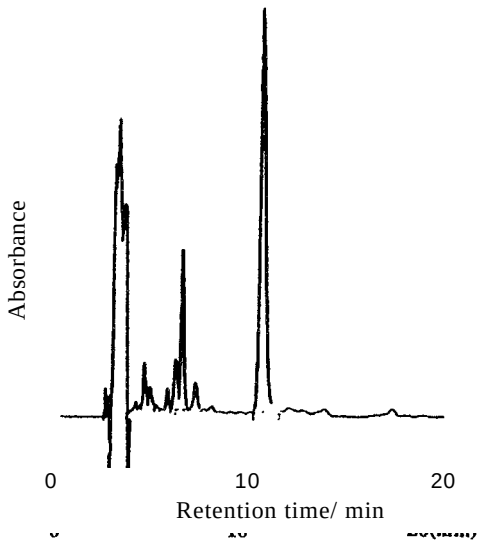
used at the time of discussing about development of this analysis method was Shodex C_{18}-5B**.**

[3] Since the eluent contains buffer solution, LC apparatus and columns used should be washed enough, and the eluent should be replaced entirely with methanol, acetonitrile or others before storing. At the time of use, give eluent after replacing with water.

[4] An example of chromatogram is shown in Fig. 8.1.7-4. Since some samples may show peaks of foreign substances after 40 min or later, the analysis time of LC should be set not to effect the next injection.

[5] Refer to **《Cautions for analytical processing of morantel citrate》**

[6] Filters other than made of polytetrafluoroethylene (PTFE) possibly adsorb morantel citrate; therefore, when use a membrane filter, confirm before use that morantel citrate should not be adsorbed. A supernatant obtained by using a high-speed centrifuge at 5,000×*g* (10,000 rpm) for 3 min can be used as a sample solution.



Measurement conditions

Detector: Measurement wavelength: 320 nm

Column: Shodex C_{18}-5B

Eluent: Phosphate buffer solution-acetonitrile (4:1)

Flow rate: 1.0 mL/min

Column temperature: 40 °C

Fig. 8.1.7-4 A chromatogram of a formula feed for piglets containing 30 g/t of morantel citrate