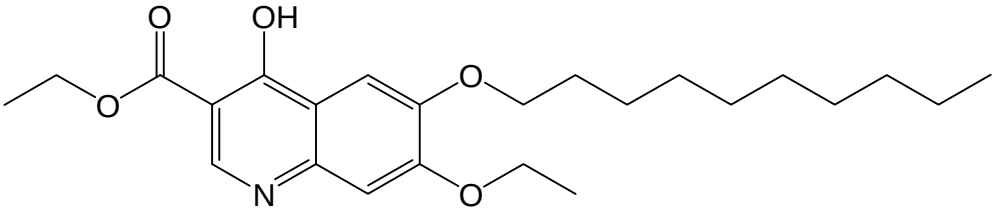
11 Decoquinate



Ethyl 6-decoxy-7-ethoxy-4-oxo-1*H*-quinoline-3-carboxylate

$C_{24}H_{35}NO_5$ MW: 417.54 CAS No.: 18507-89-6

【Outline of decoquinate】

Decoquinate is white to light brown powder without odor, slightly soluble in chloroform, and practically insoluble in water or ethanol.

This agent has been designated to one of feed additives in 1976, and is approved to add to formula feeds for starting/growing chicks and broiler chickens in the range of 20-40 g/t to promote the effective use of nutrient components in feeds.

【Methods listed in the Feed Analysis Standards】

1 Quantitative test methods

1.1 Liquid chromatography

1.1.1 Premix

〔Feed Analysis Standards Chapter 8, Section 1, 11.1.1-(1)〕

A. Reagent preparation

1) Extraction solvent: Dissolve 10 g of calcium chloride in methanol to make a total amount of 1 L.

2) Decoquinate standard solution: Place 40 mg of decoquinate〔$C_{24}H_{35}NO_5$〕exactly measured in a 100 mL brown volumetric flask, add extraction solvent for dissolving, and further add the solvent up to the gauge line to prepare the decoquinate standard stock solution (1 mL of this solution contains an amount of decoquinate equivalent to 0.4 mg).

At the time of use, exactly dilute the definite amount of the standard stock solution with methanol to prepare several decoquinate standard solutions containing amounts of decoquinate equivalent to 1-8 μg in 1 mL.

B. Quantification

Extraction: Place 2-5 g of analysis sample (20-50 mg equivalence of decoquinate) exactly measured in a stoppered 200 mL Erlenmeyer flask, add 100 mL of extraction solvent, and stir for 40 min [1] for extraction. Place the extracted solution in a stoppered centrifuging tube, centrifuge at 1,500×*g* for 5 min, and exactly dilute the definite amount of supernatant obtained [2] with ethanol. Filter this solution through a membrane filter (pore diameter: 0.5 μm or less) [3] to prepare a sample

solution for liquid chromatography.

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

Measurement conditions (Example)

Detector: Fluorescence detector (excitation wavelength: 326 nm, fluorescence wavelength: 384 nm)

Column: Octadecylsilylated silica-gel column (internal diameter: 3.9 mm, length: 300 mm, particle diameter: 10 μm) Note 1 [4]

Eluent: Dissolve 0.1 g of calcium chloride in methanol-water (9:1) to make a total amount of 1 L.

Flow rate: 1.0 mL/min

Calculation: Obtain the peak height or area from the chromatogram [5] to prepare the calibration curve, and calculate the amount of decoquinate in the sample.

Note 1: μBondapak C_{18} (Waters) or an equivalent one.

《Summary of analysis method》

This method is intended to determine the amount of decoquinate in a premix by extracting with methanol containing calcium chloride, diluting with methanol, and quantifying using a liquid chromatograph with a fluorescence detector.

Reference: Mitsuaki Kimura: Research Report of Animal Feed, 14, 16 (1989)

History in the Feed Analysis Standards: 【11】new

《Validation of analysis method》

・Recovery rate and repeat accuracy

Type of sample	Concentration (g/kg)	Repeat	Recovery rate (%)	Repeat accuracy RSD (%)
Premix for starting chick	5~20	3	103.0-110.0	3.9
Premix for growing chick	5~20	3	104.7-105.3	4.7
Premix for broiler chicken	5~20	3	100.7-102.7	3.5

《Notes and precautions》

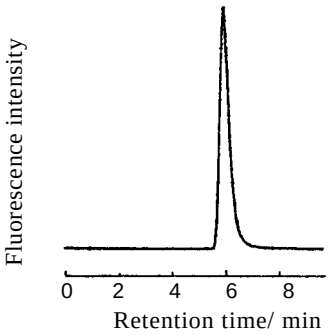
[1] Mostly extracted for approximately 5 min.

[2] Dilute to the level equivalent to approximately 2-6 μg/mL as decoquinate.

[3] Supernatant obtained by centrifuging at 5,000×*g* for approximately 3 min can be used as a sample solution for liquid chromatograph.

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

[5] An example of chromatogram is shown in Fig. 8.1.11-1.



Measurement conditions

Detector: Measurement wavelength: Em: 326 nm, Ex 384 nm

Column: μBondapak C_{18}

Eluent: Methanol-water (9:1) containing 0.1 g/L of $CaCl_2$

Flow rate: 1.0 mL/min

Fig. 8.1.11-1 A chromatogram of decoquinate added in a premix
(The arrow indicates the peak of decoquinate)

1.1.2 Formula feed

〔Feed Analysis Standards Chapter 8, Section 1, 11.1.1-(2)〕

A. Reagent preparation

1) Extraction solvent: Methanol-chloroform（9:1）

2) Decoquinate standard solution: Place 40 mg of decoquinate〔$C_{24}H_{35}NO_5$〕exactly measured in a 100 mL brown volumetric flask, add the extraction solvent for dissolving, and further add the solvent up to the gauge line to prepare the decoquinate standard stock solution (1 mL of this solution contains an amount of decoquinate equivalent to 0.4 mg).

At the time of use, dilute exactly a definite amount of the stock solution with methanol to prepare several decoquinate standard solutions containing amounts of decoquinate equivalent to 1-8 μg per mL.

B. Quantification

Extraction: Place 5-20 g of the analysis sample (containing an amount of decoquinate equivalent to 0.2-0.8 mg) exactly measured in a stoppered 200 ml Erlenmeyer flask, add 100 mL of the extraction solvent, and stir for 40 min for extraction. Place the extracted solution in a stoppered centrifuging tube, centrifuge at 1,500×*g* for 5 min, and filter the supernatant through a membrane filter (pore diameter: 0.5 μm or less) to obtain a sample solution for liquid chromatography.

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

Measurement conditions (example)

Detector: Fluorescence detector (excitation wavelength：326 nm, fluorescence wavelength: 384 nm)

Column: Octadecylsilylated silica-gel column (internal diameter: 3.9 mm, length: 300 mm, particle diameter: 10 μm) [1]

Eluent: Dissolve 0.1 g of calcium chloride in methanol-water (9:1) to make a total amount of 1 L.

Flow rate: 1.0 mL/min

Calculation: Obtain the peak height or area from the chromatogram[2] to prepare the calibration curve, and calculate the amount of decoquinate.

《Summary of analysis method》

This method is intended to determine the amount of decoquinate in a formula feed by extracting with methanol-chloroform (9:1), and quantifying using a liquid chromatograph with a fluorescence detector.

History in the Feed Analysis Standards: 【11】new

《Validation of analysis method》

・Recovery rate and repeat accuracy

Type of sample	Concentration (mg/kg)	Repeat	Recovery rate (%)	Repeat accuracy RSD (%)
Formula feed for starting chick	20-40	3	98.3-105.7	7.4
Formula feed for growing chick	20-40	3	103.0-105.7	5.1
Formula feed for broiler chicken	20-40	3	95.3-99.3	5.8

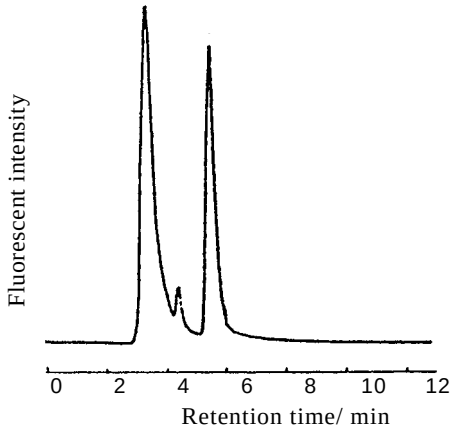
・Cooperative testing

Type of sample	No. of labs	Concentration (mg/kg)	Recovery rate (%)	Repeat accuracy in room RSD_r (%)	Reproducibility RSD_R (%)	HorRat
Formula feed for growing chick	6	40	99.7	2.4	2.4	0.36

《Notes and precautions》

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

[2] An example of chromatogram is shown in Fig. 8.1.11-2.



Measurement conditions

Detector: Measurement wavelength: Em 326 nm, Ex 384 nm

Column: μBondapak C_{18}

Eluent: 0.1 g/L $CaCl_2$ added methanol-water (9:1)

Flow rate: 1.0 mL/min

Fig. 8.1.11-2 A chromatogram of decoquinate in a formula feed
(The arrow indicates the peak of decoquinate)

1.2 Fluorometry

1.2.1 Premix

〔Feed Analysis Standards Chapter 8, Section 1, 11.1.2-(1)〕

A. Reagent preparation

1) Extraction solvent: Dissolve 10 g of calcium chloride in methanol to make a total amount of 1 L.

2) Decoquinate standard solution: Place 30 mg of decoquinate〔$C_{24}H_{35}NO_5$〕 exactly measured in a 100 mL brown volumetric flask, add the extraction solvent for dissolving, and further add the solvent up to the gauge line to prepare the decoquinate standard stock solution (1 mL of this solution contains an amount of decoquinate equivalent to 0.3 mg).

At the time of use, dilute exactly a definite amount of the standard stock solution with the extraction solvent to prepare the decoquinate standard solution containing an amount of decoquinate equivalent to 6 μg per mL.

3) Magnesium silicate: Dry synthetic magnesium silicate (particle diameter: 74-149 μm (200-100mesh)) Note1 at 120 °C for 3 hr.

B. Quantification

Extraction: Place 1-2 g of the analysis sample exactly measured in a stoppered 100 mL Erlenmeyer flask, add 50 mL of the extraction solvent, and stir for 20 min for extraction. Place the extracted solution in a stoppered centrifuging tube, centrifuge at 650×*g* for 5 min, and dilute exactly the supernatant with extraction solvent to 50 to 500-fold [1].

Place 10 mL of this diluted solution and 10 mL of chloroform exactly in a 200 mL separating funnel, add 100 mL of hydrochloric acid (1:19), shake and mix, and leave still standing for 15 min. Place the chloroform layer (lower layer) in a stoppered test tube, dehydrate with an adequate amount of sodium sulphate（anhydrous）to obtain a sample solution for column treatment.

Simultaneously, place exactly 10 mL of decoquinate standard solution in a 200 mL separating funnel, process in a similar way, to prepare the standard solution for column treatment.

Column treatment: Pack respective 0.5 g of magnesium silicate in 3 column tubes[2] (internal diameter: 7 mm) with dry processing, and prepare the column by laying respective 0.2 g of sodium sulphate (anhydrous) .

Place exactly 5 mL of the sample solution in the first column, 5 mL of the standard solution in the second column, and 5 mL of chloroform in the third column.

Effuse them until the fluid level reaches 3 mm from the top of the packing material, add respective 10 mL of methanol to each column and effuse it in a similar way.

Place a 20 mL test tube under each column, add respective 15 mL of extraction solvent to the first and second columns, and elute decoquinate to prepare the sample solution and standard solution for determination.

Simultaneously, make a blank test solution in the third column in a similar way.

Measurement: Determine the fluorescence intensity at the excitation wavelength of 330 nm and fluorescence wavelength of 390 nm for the sample solution, standard solution and blank solution, respectively.

Calculation: Calculate the amount of decoquinate in the sample solution from the fluorescence intensity obtained.

Note 1: Florisil (Floridin) or an equivalent one.

1.2.2 Formula feed

〔Feed Analysis Standards Chapter 8, Section 1, 11.1.2-(2)〕

A. Reagent preparation[3]

Prepare according to (1)-A.

B. Quantification

Extraction: Place 10.0 g of the analysis sample exactly measured in a stoppered 100 mL Erlenmeyer flask, add 50 mL of the extraction solvent, and mix while stirring for 20 min for extraction. Place the extracted solution in a stoppered centrifuging tube, and centrifuge at 650×*g* for 5 min.

Place 10 mL of the supernatant and 10 mL of chloroform exactly in a 200 mL separating funnel, add 100 mL of hydrochloric acid (1:19), mix while shaking, and leave it still standing for 15 min. Place the chloroform layer (lower layer) in a stoppered test tube, and dehydrate with an appropriate amount of sodium sulphate (anhydrous) to obtain a sample solution for column treatment.

Simultaneously, place 10 mL of decoquinate standard solution exactly in a 200 mL separating funnel, and process in a similar way to prepare the standard solution for column treatment.

Column treatment [4]: Treat according to (1)-B, Column treatment.

Measurement [4]: Measure according to (1)-B, Measurement.

Calculation [4]: Calculate according to (1)-B, Calculation

《Summary of analysis method》

This method is intended to determine the amount of decoquinate in a premix or a formula feed by extracting with methanol containing calcium chloride, purifying with liquid-liquid distribution and a magnesium silicate column, and quantifying with a fluorometer by using the characteristics of decoquinate to show strong fluorescence (Fig. 8.1.11-3). Since this method needs a slightly difficult operation, liquid chromatography is used recently.

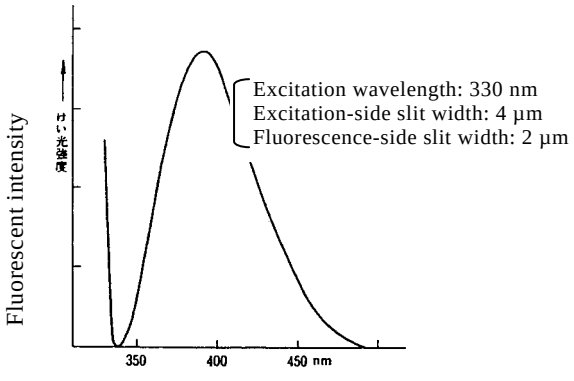


Fig. 8.1.11-3 A Fluorescence spectrum of decoquinate

References: "Official Methods of Analysis of the AOAC International", 16th Ed., 5.1.18 (1984)

Eiichi Ishiguro: Research Report of Animal Feed, 5, 100 (1979)

History in the Feed Analysis Standards: 【0】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 chicken	20-40	6	92.0-93.7	3.0

・Cooperative testing

Type of sample	No. of labs	Concentration (mg/kg)	Recovery rate (%)	Repeat accuracy in room RSD_r (%)	Reproducibility RSD_R (%)	HorRat
Formula feed	6	40	86	2.7	8.6	1.29

《Notes and precautions》

[1] Dilute to the level equivalent to approximately 6 μg as decoquinate.

[2] A column for determining Vitamin B_1 as shown in Fig. 8.1.11-4 is useful.

The packing methods of magnesium silicate include the dry processing method and wet processing method, and in the AOACI method, packing with chloroform has almost no effect on quantitative value.

In the wet processing method, column packing materials easily adhere to the chromatographic tube wall as compared with the dry processing method; therefore, the wet processing method needs more time for packing on the minus side.

Anyway, uneven packing causes difference in the separation capacity, causing enlarged analytical errors

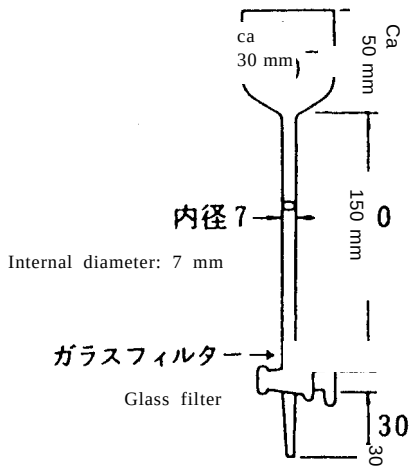


Fig. 8.1.11-4 A column to determ vitamin B_1

In the apparent quantitative data (blank test values) of materials for formula feed and feed additives (premix) obtained by this method, yeasts showed high values (approximately 15 mg/kg); however, there considered no large effect on the quantitative value of decoquinate, given the usage rate in a formula feed. Similarly, there is no large interference by other types of feed materials or feed additives

[3] The preparation methods are the same as those described in respective paragraphs under 1.2.1-A.

[4] The operation procedure is the same as that described in the paragraph under 1.2.1-B.

2 Microquantitative test methods

2.1 Liquid chromatography（premix）

〔Feed Analysis Standards Chapter 8, Section 1, 11.2.1-(1)〕

A. Reagent preparation

1) Calcium chloride-methanol solution [1] : Dissolve 10 g of calcium chloride in methanol to make a total amount of 1 L.

2) Decoquinate standard solution: Place 40 mg of decoquinate〔$C_{24}H_{35}NO_5$〕exactly measured in a 100 mL brown volumetric flask, add calcium chloride-methanol solution for dissolving, and further add the solvent up to the gauge line to prepare the decoquinate standard stock solution (1 mL of this solution contains an amount of decoquinate equivalent to 0.4 mg).

At the time of use [2], dilute a definite amount of the standard stock solution exactly with methanol to prepare several decoquinate standard solutions containing amounts of decoquinate equivalent to 5-40 ng per mL)

B. Quantification

Extraction: Place 5 g of the analysis sample [3] exactly measured in a stoppered 200 mL brown Erlenmeyer flask, add 100 mL of chloroform, and stir for 20 min for extraction. Filter the extracted solution through a paper filter on which placed an appropriate amount of sodium sulphate (anhydrous) [4] to prepare a sample solution for column treatment.

Column treatment: Wash a silica gel minicolumn (690 mg) with 10 mL of chloroform.

Place exactly 10 mL of the sample solution in the minicolumn, effuse it by pressure injection Note1 [5]. Add 10 mL of chloroform in the minicolumn, and effuse it in a similar way to wash the minicolumn. Place a 10 mL brown volumetric flask under the minicolumn, and add 8 mL of methanol to the column to elute decoquinate by pressure injection Note1 [5]. Then add methanol to the volumetric flask up to the gauge line, and filter the solution through a membrane filter (pore diameter: 0.5 μm or less) to prepare a sample solution for liquid chromatography.

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

Measurement conditions (example)

Detector: Fluorescence detector (excitation wavelength: 326 nm, fluorescence wavelength: 384 nm)

Column: Octadecylsilylated silica-gel column (internal diameter: 4.6 mm, length: 250 mm, particle diameter: 5 μm) Note2[6]

Eluent: Dissolve 1.0 g of calcium chloride in methanol-water (4:1) to make a total amount of 1L [7].

Flow rate: 1.0 mL/min

Column temperature: 40°C

Calculation: Obtain the peak height from the chromatogram[8] to prepare the calibration curve, and calculate the amount of decoquinate in the sample.

Note 1. Set the flow rate at 2-5 mL/min.

2. Wakosil 5C_{18}HG (Wako Pure Chemical) or an equivalent one.

《Summary of analysis method》

This method is intended to determine the minute amount of decoquinate remained in a premix or others caused by carry-over and the like by extracting with chloroform, purifying with a silica gel minicolumn, and quantifying using a liquid chromatograph with a fluorescence detector.

References: Toshiichi Komoriya, Noriyuki Sasaki, Haruyoshi Harada: Research Report of Animal Feed, 18, 45 (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)
Premix for finishing broiler chicken	0.2-0.8	3	92.7-100.3	4.2
Premix for growing piglet	0.2-0.8	3	98.0-106.7	8.9
Premix for cattle	0.2-0.8	3	101.3-104.0	9.2

・Cooperative testing

Type of sample	No. of labs	Concentration (mg/kg)	Recovery rate (%)	Repeat accuracy in room RSD_r (%)	Reproducibility RSD_R (%)	HorRat
Premix for adult chicken	6	0.5	97.0	6.4	7.5	0.59

《Notes and precautions》

[1] The preparation procedure is the same as that described in the paragraph under 1.2.1 fluorometry (premix), A-1).

[2] The standard solution easily changes with time under the low concentrations; therefore, prepare the solution on the day of the test, and use it as soon as possible. The degraded products elute approximately 1 min sooner than the main peak. The degradation rate is greatly varied by preparation and storing conditions.

[3] When the analysis sample is adequately fine and small amount, the amounts of the analysis sample and extraction solvent can be reduced.

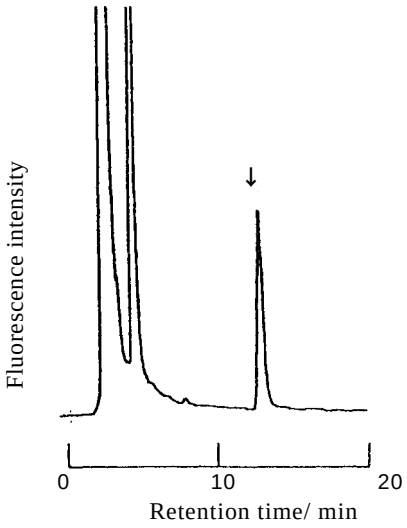
[4] Swiftly filter only the required amount to keep the concentrating due to evaporation at minimum.

[5] The rate of pressure injection may be approximately 2-5 mL/min. Carefully avoid air bubbles breaking into the cartridge.

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

[7] Prepare at the time of use.

[8] An example of chromatogram is shown in Fig. 8.1.11-5.



Measurement conditions

Detector: Measurement wavelength: Em 326 nm, Ex 384 nm

Column: Wakosil 5C18

Eluent: 1.0 g/L $CaCl_2$ added methanol-water (4:1)

Flow rate: 1.0 mL/min

Fig. 8.1.11-5 A chromatogram of decoquinate added to a premix for growing piglets (The arrow indicates the peak of decoquinate)

2.2 Liquid chromatography (formula feed)

〔Feed Analysis Standards Chapter 8, Section 1, 11.2.1-(2)〕

A. Reagent preparation

1) Calcium chloride-methanol solution: Dissolve 10 g of calcium chloride in methanol to make a total amount of 1 L.

2) Decoquinate standard solution [1]: Prepare the decoquinate standard stock solution according to (1)-A-2).

At the time of use, dilute exactly a definite amount of the standard stock solution with methanol to prepare several decoquinate standard solutions containing an amount of decoquinate equivalent to 0.025-0.4 μg per mL.

B. Quantification

Extraction: Place 50 g of the analysis sample exactly measured in a stoppered 300 mL Erlenmeyer flask, add 200 mL of chloroform, and stir for 20 min for extraction. Filter the extracted solution through a piece of paper filter on which placed an appropriate amount of sodium sulphate (anhydrous) to obtain a sample solution for column treatment.

Column treatment [2]: Treat the column according to (1)-B, Column treatment.

Liquid chromatography [2]: Process according to (1)-B, Liquid chromatography.

Calculation [2][3]: Calculate according to (1)-B, Calculation.

《Summary of analysis method》

This method is intended to determine the minute amount of decoquinate remained in a formula feed caused by carrying over, etc. Quantitative procedure is almost same as that described under 2.1, Premix.

References: Mitsuaki Kinoshita: Research Report of Animal Feed, 16, 112 (1991)

History in the Feed Analysis Standards: 【12】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	0.1-0.8	3	90.3-99.0	10.3
Formula feed for finishing broiler chicken	0.1-0.8	3	94.3-98.3	11.3
Formula feed for growing pig	0.1-0.8	3	89.7-100.7	15.2
Formula feed for dairy cattle	0.1-0.8	3	94.0-102.0	7.6

・Cooperative testing

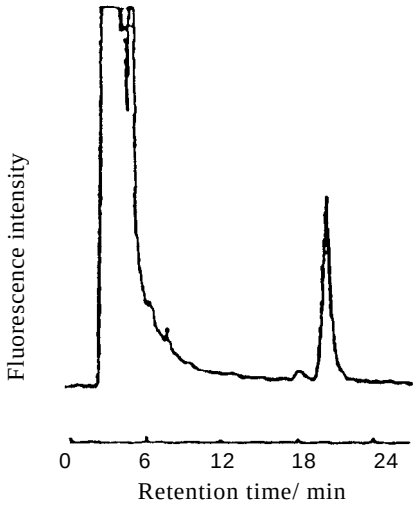
Type of sample	No. of labs	Concentration (mg/kg)	Recovery rate (%)	Repeat accuracy in room RSD_r (%)	Reproducibility RSD_R (%)	HorRat
Formula feed for finishing broiler chicken	6	0.1	96.8	5.7	10.2	0.47

《Notes and precautions》

[1] The preparation method is the same as that described in the paragraph under 2.1-A.

[2] The operation procedure is the same as that described in the paragraph under 2.1-B.

[3] An example of chromatogram is shown in Fig. 8.1.11-6.



Measurement conditions

Detector: Measurement wavelength: Em 326 nm, Ex 384 nm

Column: Wakosil 5C18

Eluent: 1.0 g/L $CaCl_2$ added methanol-water (4:1)

Flow rate: 1.2 mL/min

Fig. 8.1.11-6 A chromatogram of decoquinate added (0.1 g/t) to a formula feed for dairy cattle (The arrow indicates the peak of decoquinate)