SLOVENSKI STANDARD
SIST EN 16162:2012

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Krma - Določevanje dekokvinata s HPLC s fluorescentno detekcijo

Animal feeding stuffs - Determination of Decoquinate by HPLC with fluorescence detection

Futtermittel - Bestimmung von Decoquinat mit Hochleistungs-Flüssigchromatographie (HPLC) und Fluoreszenzdetektion

Aliments des animaux - Dosage du décoquinate par CLHP avec détection par fluorescence

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English Version

Animal feeding stuffs - Determination of decoquinate by HPLC with fluorescence detection

Aliments des animaux - Détermination du décoquinate par Chromatographie Liquide Haute Performance avec détection fluorimétrique

Futtermittel - Bestimmung von Decoquinat mit Hochleistungs-Flüssigchromatographie (HPLC) und Fluoreszenzdetektion

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Foreword

This document (EN 16162:2012) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 2012, and conflicting national standards shall be withdrawn at the latest by September 2012.

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Introduction

This European Standard has been developed to quantify decoquinate in feeding stuffs to enable the European Commission to control the content of animal feed products. However, this method can also be used to evaluate the cross contamination from medicated feed to feedstuff.

1 Scope

This European Standard specifies a method for the determination of decoquinate. This high-performance liquid chromatographic (HPLC) method with a fluorescence detection is applicable to the quantification of decoquinate content in complete and complementary compound feeds, medicated feeds, semi-liquid feeds, premixtures and feed additives.

The method was fully validated from LOQ to 60 000 mg/kg on different matrices during an international collaborative study [11], especially on complete compound feeds for poultry, at trace contamination level of 3 mg/kg and at European authorized level of 20 mg/kg to 40 mg/kg [12].

The limit of detection is between 0,1 mg/kg and 0,3 mg/kg and the limit of quantification is around 0,5 mg/kg. These limits were validated during the collaborative study [11], from results on the blank feed. Lower limits of detection or quantification could be reached but a single laboratory validation is then requested.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

prEN ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation (ISO/DIS 6498)*

3 Principle

Decoquinate is extracted from samples with a solution of 1 % calcium chloride in methanol using mechanical shaking or stirring for 60 min. After centrifugation or filtration, an aliquot is, if necessary, diluted with the extraction solvent and analysed by reversed phase HPLC with fluorescence detection. Positive trace level samples should be confirmed by HPLC analysis using an alternate excitation wavelength.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralised water or water of equivalent purity.

WARNING — This method requires the handling of hazardous substances. It is recommended to use various regulations for potentially hazardous chemicals. Organisational, technical and personal safety has to be observed.

4.1 Methanol, HPLC grade.

4.2 Methanol, technical grade.

4.3 Calcium chloride anhydrous or Calcium chloride dihydrate, each > 99 % purity.

4.4 HPLC dilution solution.

Dissolve a mass of calcium salt (4.3) equivalent to 10 g of calcium chloride anhydrous in methanol (4.1). Mix well and make up to 1 000 ml.

4.5 Extraction solvent.

Dissolve a mass of calcium salt (4.3) equivalent to 10 g of calcium chloride anhydrous in technical methanol (4.2). Mix well and make up to 1 000 ml.

4.6 Decoquinate standards.

4.6.1 Decoquinate powder reference standard with guaranteed purity.

Purity shall be certified by a certificate of analysis.

NOTE Pure reference standard is available at e.g. Alpharma®.

4.6.2 Decoquinate stock standard solution, approximately 300 µg/ml.

Accurately weigh, to the nearest 0,1 mg, 30 mg of decoquinate reference standard (4.6.1) into a 100 ml volumetric flask and dissolve in dilution solution (4.4). Use ultrasonic bath if necessary to aid dissolution. Calculate the exact concentration taking into account the purity of the standard material (4.6.1), given in the certificate. Prepare fresh monthly. Store in the dark at 0 °C to 10 °C.

4.6.3 HPLC standard solutions.

4.6.3.1 Intermediate standard solution, approximately 6 µg/ml.

Transfer by pipette 2,0 ml of stock standard solution (4.6.2) into a 100 ml volumetric flask, dilute to volume with dilution solution (4.4). Check that intermediate solution for each series of analysis. The absorbance density of intermediate solution can be evaluated at 265 nm, with dilution solvent (4.4) as reference for optical density measurement. In these conditions, the absorbance range is between 0,67 and 0,73. That intermediate solution is prepared fresh daily.

4.6.3.2 HPLC calibration standard solutions, approximately 0,15 µg/ml, 0,30 µg/ml, 0,60 µg/ml and 1,2 µg/ml.

Prepare 4 concentrations of HPLC standard solutions as it is explained in Table 1 (standards A/B/C/D). Transfer by pipette the required volume of intermediate standard (4.6.3.1) into volumetric flasks, and make to volume with dilution solution (4.4). Mix well.

Table 1 — preparation of calibration standard solutions

Standard	Parts of intermediate (4.6.3.1) solution, in ml	Volumetric flask, in ml	Dilution Factor	≅µg/ml
A	5	200	40	0,15
B	5	100	20	0,30
C	10	100	10	0,60
D	10	50	5	1,20

Evaluate precisely each exact concentration by using the exact concentration of the stock solution (4.6.2).

All solutions described here (standards A/B/C/D) are prepared fresh daily.

4.7 HPLC Mobile Phase.

Dissolve a mass of calcium salt (4.3) equivalent to 10 g calcium chloride anhydrous into 1 l of a solvent mixture of methanol (4.1) / water in proportion by volume of 825/175. Filter under vacuum (5.2) before use.

NOTE HPLC shutdown solution.

Prepare a methanol (4.1) / water solution in proportion by volume of 85/15 without calcium salt to flush the HPLC column and equipment at the end of each day of analysis.

5 Apparatus

Usual laboratory apparatus, in particular, the following:

5.1 Mechanical shaker or magnetic stirrer.

5.2 Solvent filtration system, suitable for 0,45 µm PTFE filter or equivalent.

5.3 Centrifuge and centrifuge tube (50 ml).

5.4 HPLC system consisting of the following:

5.4.1 Pump, pulse free, flow capacity of 0,2 ml/min to 5 ml/min.

5.4.2 Injection system, manual or autosampler, with loop suitable for 10 µl to 50 µl injection volumes.

5.4.3 Analytical C18 column like ACE® or Luna® or Symmetry® or Restek Ultra®; 5 µm; 4.6 mm x 250 mm or equivalent.

5.4.4 Fluorescence detector suitable for measurement using 330 nm and 260 nm excitation wavelengths and 390 nm emission wavelength.

5.4.5 Integrator or computer data system.

6 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage. Sampling is not part of the method specified in this European Standard. A recommended sampling procedure is given in EN ISO 6497 [1].

7 Sample preparation

All feed samples, with the exception of premixtures and concentrates (feed additives), are ground extraction as recommended in the guidelines prEN ISO 6498.

8 Procedure

8.1 General

Preferably, duplicate analysis is performed.

8.2 Extraction of feeds (decoquinate content between 10 mg/kg to 500 mg/kg)

NOTE For milk-replacer particular attention should be given to the addition of extraction solvent. Small or large lumps could be formed and quantitative results will be compromised. The extraction solvent addition should be done slowly under rotate shaking of the conical flask. To dissolve lumps, ultrasonic system, during approximately 5 min, is very helpful.

Accurately weigh, to the nearest 0,1 g, 10,0 g of feed in a 250 ml amber conical flask. Add 100 ml of the extraction solution (4.5). Shake or stir for 1 h on apparatus (5.1). Allow contents of flask to settle particles for about 10 min. Dilute an aliquot of the almost clear supernatant extract with dilution solution (4.4) to obtain a concentration between 0,3 µg/ml and 0,6 µg/ml. Filter the diluted solution on membrane filter (5.2) and inject it onto HPLC system (5.4). For cloudy extracts, if necessary, transfer at least 40 ml of the extract into 50 ml centrifuge tube (5.3) and centrifuge before dilution, membrane filtration and HPLC injection.

8.3 Extraction of complementary feeds, premixtures and feed additives (decoquinate content higher than 500 mg/kg)

Accurately weigh, to the nearest 0,01 g, depending on the declared concentration of decoquinate in the sample, 0,50 g to 2,00 g of sample into a 250 ml amber conical flask. Add 100 ml of the extraction solution (4.5). Shake or stir for 1 h on equipment (5.1). Allow contents of flask to settle particles for 10 min. Dilute an aliquot of the almost clear supernatant extract with dilution solution (4.4) to obtain a concentration between 0,3 µg/ml and 0,6 µg/ml. Filter the diluted solution on membrane filter (5.2) and inject it onto HPLC system (5.4). For cloudy extracts, if necessary, transfer at least 40 ml of the extract into 50 ml centrifuge tube (5.3) and centrifuge before dilution, membrane filtration and HPLC injection.

NOTE For a decoquinate declaration of 1 000 mg/kg, weigh 2 g of test portion and dilute 1 ml of clear extract in a 100 ml volumetric flask. For feed additives declared at 6 %, weigh 0,5 g and dilute by a factor of 1/400.

8.4 Extraction of trace feeds (decoquinate content lower than 10 mg/kg)

Accurately weigh, to the nearest 0,1 g, 10,0 g of feed in a 250 ml amber conical flask. Add 100 ml of the extraction solution (4.5). Shake or stir for 1 h on apparatus (5.1). Allow contents of flask to settle particles for 10 min.. Dilute an aliquot of the almost clear supernatant extract with dilution solution (4.4) to obtain a concentration between 0,3 µg/ml and 0,6 µg/ml. Filter the diluted solution on membrane filter (5.2) and inject it onto HPLC system (5.4). For cloudy extracts, if necessary, transfer at least 40 ml of the extract into 50 ml centrifuge tube (5.3) and centrifuge before dilution, membrane filtration and HPLC injection.

8.5 Quality control spiked feeds

8.5.1 Blank Feed to spike at 30 mg/kg

Accurately weigh, to the nearest 0,1 g, 10,0 g of quality control blank feed in a 250 ml amber conical flask. Add 1 ml of stock solution (4.6.2). Wait for 15 min. Then add 100 ml of the extraction solution (4.5). Proceed as described in 8.2. The recovery shall be from 80 % to 110 %.

8.5.2 Blank feed to spike at 9 mg/kg

Accurately weigh, to the nearest 0,1 g, 10,0 g of quality control blank feed in a 250 ml amber conical flask. Add 15 ml of intermediate solution (4.6.3.1). Wait for 15 min. Then add 85 ml of the extraction solution (4.5). Proceed as described in 8.4. The recovery shall be from 80 % to 120 %.

8.6 HPLC parameters

These HPLC parameters are given for guidance. Other parameters should be applicable (column, flow rate adapted to HPLC columns and optimum response of the fluorescence detector…).

— Analytical column: C18 column; 5 µm; 4.6 mm x 250 mm as described in 5.4.3;

— Column Temperature: ambient or 30 °C;

— Mobile phase (4.7): MeOH/water in proportion by volume of 825/175 with 1 % of $CaCl_2$;

— Injection Volume: 20 µl;

— Flow rate: 0,5 ml/min;

— Excitation wavelength: 330 nm;

— Emission wavelength: 390 nm.