SLOVENSKI STANDARD
SIST EN 15781:2009

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Krma - Določanje amonijevega maduramicina s tekočinsko kromatografijo visoke ločljivosti s postkolonsko derivatizacijo

Animal feeding stuffs - Determination of maduramicin-ammonium by reversed-phase HPLC using post-column derivatisation

Futtermittel - Bestimmung von Maduramicin-Ammonium durch Umkehrphasen HPLC-Verfahren mittels Nachsäulenderivatisierung

Aliments des animaux - Détermination de la maduramicine ammonium par HPLC en phase inverse à l'aide de la dérivation post-colonne

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN 15781:2009) 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of an identical text or by endorsement, at the latest by February 2010, and conflicting national standards shall be withdrawn at the latest by February 2010.

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1 Scope

This European Standard specifies a high performance liquid chromatography (HPLC) method for the determination of the content of maduramicin in feeding stuffs and premixtures.

The usual concentration of maduramicin in feedstuffs is 5 mg/kg, in premixtures 500 mg/kg. The limit of quantification is 2 mg/kg. The limit of detection is 0,5 mg/kg.

NOTE A lower limit of quantification may be achievable but shall be validated by the user.

2 Normative reference(s)

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

EN ISO 3696, *Water for analytical laboratory use - Specification and test methods* (ISO 3696:1987)

3 Principle

The sample is extracted with methanol. Maduramicin is determined by reversed-phase HPLC using post-column derivatization with vanillin and detection at 520 nm.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

WARNING — Use all solvents and solutions in a fume hood. Wear safety glasses, protective clothing and avoid skin contact.

4.1 Water, complying with EN-ISO 3696, grade 1

4.2 Methanol (CH_3OH), HPLC grade

4.3 1,5-Dimethylhexylamine ($CH_3(CH_2)_4CH_2N(CH_3)_{2)}$

4.4 Sulfuric acid (H_2SO_4), purity 95% to 97% by volume

4.5 Ortho-phosphoric acid, (H_3PO_4), purity approximately 85% by volume

4.5.1 Diluted o-phosphoric acid

Dissolve 10 ml of ortho-phosphoric acid (4.5) to 100 ml with water (4.1).

4.6 Potassium dihydrogen phosphate (KH_2PO_4)

4.7 Phosphate buffer solution, (KH_2PO_4) = 10 mmol/l, pH 4,0

Dissolve 1,36 g of potassium dihydrogen phosphate (4.6) in 500 ml of water (4.1). Add 3,0 ml of ortho-phosphoric acid (4.5) and 10 ml of 1,5-dimethylhexylamine (4.3). Adjust the pH to 4,0 with diluted ortho-phosphoric acid (4.5.1) and fill with demineralised water to 1 000 ml. The solution can be stored for some weeks, but in case of fungal growth, prepare a new one.

4.8 Mobile phase

Dilute 100 ml of phosphate buffer solution (4.7) with methanol (4.2) to 1 000 ml.

4.9 Vanillin, 4-hydroxy-3-methoxybenzaldehyde, minimum 98% purity by volume (HPLC grade)

4.9.1 Vanillin reagent

Dissolve 10 g of vanillin (4.9) in a mixture of 250 ml of methanol (4.2) and 5,0 ml of sulphuric acid (4.4). Mix well and sonicate for some minutes under vacuum at room temperature. This solution has to be prepared daily prior to use and has to be cooled with ice water during use.

NOTE Dimethylaminobenzaldehyde (DMAB) is also suitable as a reagent for post-column derivatisation (details are given in Annex B) although a full validation of this reagent has not been performed.

4.10 Maduramicin

WARNING — Maduramicin is very toxic. Avoid inhalation and exposure to the toxic standard material and solutions thereof.

4.11 Standard solutions

4.11.1 Stock-standard-solution, 100 µg/ml

Accurately weigh 10 mg to the nearest 0,1 mg maduramicin (4.10) into a 100 ml volumetric flask. Dissolve in methanol (4.2) and dilute to volume. Store below 4°C. Prepare fresh every month.

4.11.2 Standard solution, 10 µg/ml

Dilute 10 ml of the stock-standard-solution (4.11.1) to 100 ml with methanol (4.2) in a 100 ml volumetric flask. Store below 4°C. Prepare fresh every week.

4.11.3 Calibration solutions

The interlaboratory study was performed with 8 calibration solutions. Into a series of 50 ml volumetric flasks transfer 1,0 ml, 2,0 ml, 3,0 ml, 4,0 ml, 5,0 ml, 6,0 ml, 8,0 ml and 10,0 ml of the intermediate standard solution (4.11.2). Dilute to volume with methanol (4.2) and mix. These solutions correspond to 0,2 µg, 0,4 µg, 0,6 µg, 0,8 µg, 1,0 µg, 1,2 µg, 1,6 µg, and 2,0 µg of maduramicin per ml respectively. Alternatively, you may use 5 calibration solutions with maduramicin concentrations of 0,4 µg, 0,7 µg, 1,0 µg, 1,5 µg and 2,0 µg per ml respectively. Calibration solutions should be prepared on the day of analysis.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Centrifuge

5.2 Ultrasonic bath

5.3 HPLC system consisting of the following

5.3.1 Pump, pulse free, flow capacity 0,4 ml/min

5.3.2 Injection system, manual or autosampler, with loop suitable for 50 µl injection

5.3.3 UV/VIS detector, suitable for measurements at 520 nm

NOTE Noise preferably should be < $1 \cdot 10^{-5}$ AU (250 nm, 600 nm)

5.3.4 Integrator, or computer data system.

5.3.5 Post column reactor consisting of the following

NOTE The use of stainless steel tubing in the post-column reactor and detector should be avoided.

5.3.5.1 PEEK mixing chamber

5.3.5.2 PTFE reaction coil, 1,5 ml to 2,0 ml reaction coil, for operating at 95°C

The coil may be a commercially available knitted coil or it may be made using 7,5 m to 10 m of 316 SS tubing, 0,5 mm ID, coiled in a format to fit the reactor heating chamber (a suggestion is to wrap the coil in enough aluminium foil to make it fit snugly in the heater and to provide good heat transfer to the coil). A knitted coil is preferable. To ensure effective mixing of reagent and column effluent, use a vortex or static mixing tee (not a regular tee) before the reaction coil.

NOTE 1 The length of the polytetrafluoroethylene (PTFE) tube (e.g. 1 m ID 0,25 mm) between reagent-pump and mixing chamber and the length of the Teflon tube (e.g. 3 m ID 0,17 mm) between reactor and detector should be optimized if there are problems with bubbles.

NOTE 2 A temperature of 92°C to 98°C is possible, high stability (1°C) should be guaranteed.

5.3.5.3 Reactor oven or water bath for the PTFE-reaction coil, suitable for operating at 95°C

5.3.6 Post column reagent pump, pulse free, flow capacity 0,4 ml/min

5.3.7 Liquid chromatographic column, 250 mm x 4,6 mm, 5 µm material, Hypersil BDS C18 or equivalent

5.3.8 Column oven, suitable for operating at 40°C

5.4 Shaker, rotary or wrist-action shaker

5.5 Freezer

5.6 Membrane-filter, PTFE, pore size within the range of 0,20 µm – 0,45 µm

6 Sampling

It is important that the laboratory receives a sample that is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this European Standard. A recommended sampling method is given in EN ISO 6497.

7 Preparation of test sample

Sample preparation is not part of the method specified in this European Standard. A recommended method that can be used for sample preparation is given in ISO 6498.

8 Procedure

8.1 Preparation of quality control sample

8.1.1 Blank feed

For the performance of the recovery test (8.1.2) a blank feed should be analyzed to check that neither maduramicin nor interfering substances are present. The blank feed should be similar in type to that of the sample and maduramicin or interfering substances should not be detected.

8.1.2 Recovery test

A recovery test should be carried out by analyzing the blank feed which has been fortified by the addition of a quantity of maduramicin, similar to that present in the sample. To fortify at a level of 5 mg/kg, transfer 500 µl stock-standard solution (4.11.1) to a flask. Add 10 g of the blank feed, mix thoroughly and leave for 10 min, mixing again several times before proceeding with the extraction step (8.2)

Alternatively, if a blank feed similar in type to that of the sample is not available (8.1.1), a recovery test can be performed by means of the standard addition method. In this case, the sample to be analyzed is fortified with a quantity of maduramicin similar to that already present in the sample. This sample is analyzed together with the unfortified sample and the recovery can be calculated by subtraction.

Acceptable recovery is between 90% and 110%.

8.2 Extraction

8.2.1 Feeding stuffs

Accurately weigh 10 g to the nearest 0,01 g of the ground sample (with particles of ≤ 1mm) into a 250 ml volumetric flask and add 50 ml methanol (4.2). Close the flask with a suitable method, and place it in an ultrasonic bath (5.2) at 50°C for 20 min. Shake vigorously (5.4), store and cool down to room temperature in approximately 15 min, decant the clear supernatant and place in a freezer (5.5) for 2 h to 3 h to settle down fat. Then centrifuge an aliquot for 1 min to 2 min. After membrane (5.6) filtration, 50 µl of this solution is injected into the HPLC-apparatus.

8.2.2 Premixes

Accurately weigh 1 g to the nearest 0,01 g of the ground sample (with particles of ≤ 0,5 mm) into a 250 ml volumetric flask and add 50 ml methanol (4.2). Close the flask with a suitable method, and place in an ultrasonic bath (5.2) at 50°C for 20 min. Cool down to room temperature, shake vigorously (5.4), store some min and dilute an aliquot of the clear supernatant 1:10 with methanol and place in a freezer (5.5) for 2 h to 3 h to settle down fat. Then centrifuge an aliquot for 1 min to 2 min. After membrane filtration (5.6), 50 µl of this solution is injected into the HPLC-apparatus.

NOTE A larger sample amount may be used, but shall be validated by the user. In the case a larger sample amount is used the volume of the extraction solvent has to be adjusted accordingly.