SLOVENSKI STANDARD
SIST EN 16158:2012

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Krma - Določevanje semduramicina - Metoda tekočinske kromatografije z "razvejanim" analitskim pristopom

Animal feeding stuffs - Determination of semduramicin content - Liquid chromatographic method using a "tree" analytical approach

Futtermittel - Bestimmung des Semduramicingehalts - Flüssigkeitschromatographisches Verfahren mit verzweigter analytischer Vorgehensweise

Aliments pour animaux - Dosage de la semduramicine - Chromatographie liquide utilisant une approche analytique en arbre

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65.120	Krmila	Animal feeding stuffs

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Contents

Page

Foreword

This document (EN 16158:2012) 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of an identical text or by endorsement, at the latest by August 2012, and conflicting national standards shall be withdrawn at the latest by August 2012.

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1 Scope

This European standard specifies a high-performance liquid chromatographic (HPLC) method for the determination of the semduramicin content at authorized level in animal feeding stuffs [2], using mass spectrometry detection or post-column derivatization and (UV)-VIS detection (hereinafter UV detection). This method is applicable to poultry feed. The limit of quantitation is 1,0 mg/kg when mass spectrometry is used for detection and 3,0 mg/kg when the detection is performed by UV with post-column derivatization. Lower limits of quantitation are achievable but this is to be validated by the user.

The method allows the discrimination of semduramicin from monensin, salinomycin, narasin, maduramicin and lasalocid.

2 Normative references

The following referenced documents are indispensable for the application of this protocol. 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

Semduramicin is extracted using acetonitrile with mechanical shaking during 30 min. The extracts are filtered through 0,2 µm Nylon filters. Semduramicin is determined by reverse-phase liquid chromatography using electrospray (ESI) single quadrupole mass spectrometry detection in single ion monitoring (SIM) mode (LC-MS) [4] or using post-column derivatization with dimethylaminobenzaldehyde (DMAB) and spectrophotometric detection at 598 nm (LC-PCD-UV) [5]. If the detection used is ESI-MS the quantitation is performed through a standard addition approach. When LC-PCD-UV is used the quantitation is performed through external standard calibration.

4 Reagents

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

4.1 LC-MS.

4.1.1 Water, HPLC grade, or equivalent (e.g. Milli-Q purified water).

4.1.2 Acetonitrile, HPLC gradient grade, minimum 99,9 % purity.

4.1.3 Methanol, HPLC grade or hypergrade LC-MS.

4.1.4 Ammonium formate, HPLC grade.

4.1.5 Mobile phase.

4.1.5.1 Ammonium formate solution, c = 20 mmol/l.

Accurately weigh 1,25 g to the nearest 0,01 g of ammonium formate (4.1.4) into a 1 000 ml volumetric flask. Dissolve in water (4.1.1) and make up to 1 000 ml of volume with water. Prepare fresh solutions monthly.

4.1.5.2 HPLC mobile phase.

Mix methanol (4.1.3) and ammonium formate solution (4.1.5.1) in proportion of 90+10 (v+v). Filter under vacuum using a solvent filtration system (5.11) and Nylon filters (5.13).

4.2 LC-PCD-UV.

In addition to the reagents 4.1.1, 4.1.2, 4.1.3 and 4.1.4:

4.2.1 Sulphuric acid, minimum 98 % purity.

4.2.2 Dimethylaminobenzaldehyde (DMAB), minimum 99 % purity.

4.2.3 Formic acid, minimum 98 % purity.

4.2.4 Mobile phase.

4.2.4.1 Post-column reaction reagent.

In a 500 ml volumetric flask (5.7) add first about 250 ml cold methanol (4.1.3) then 15 ml sulphuric acid (4.2.1). Dissolve 15 g DMAB (4.2.2) in the mixture. Cool down and make up to 500 ml with methanol (4.1.3). Filter under vacuum using the equipment in (5.11) and a membrane filter (5.12). Store in a refrigerator (from +2 °C to + 8 °C). This reagent is stable for 28 days.

NOTE The methanol used for preparing the post-column reaction reagent should be kept refrigerated (from +2 °C to +8 °C).

4.2.4.2 Ammonium formate solution, c = 100 mmol/l at pH = 3.

Accurately weigh 6,30 g to the nearest 0,01 g of ammonium formate (4.1.4) into a 1 000 ml volumetric flask (5.7). Dissolve in 900 ml water (4.1.1). Adjust the pH to 3,0 using formic acid (4.2.3) and make up to 1 000 ml with purified water. Prepare fresh monthly.

4.2.4.3 HPLC mobile phase (solvent blank).

Mix methanol (4.1.3) and ammonium formate solution (4.2.4.2) in proportion of 90+10 (v+v). Filter under a vacuum using a solvent filtration system (5.11) and Nylon filters (5.13).

4.3 Reference standards LC-PCD-UV method.

WARNING — Avoid inhalation of and exposure to the toxic standard materials and solutions thereof. Work in a fume-hood when handling the solvents and solutions. Wear safety glasses and protective clothing.

Declaration of purity is required for each lot of reference standard.

4.3.1 Semduramicin sodium standard, minimum 93 % purity expressed as semduramicin.

NOTE Available from Phibro Animal Health Corporation, Third Floor 65 Challenger Road Ridgefield Park, NJ 07660-2103 USA. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

4.4 Reference standards LC-MS method.

In addition to the reference standard 4.3.1:

4.4.1 Nigericin sodium standard, minimum 98 % purity to be used as internal standard (I.S.).

NOTE Available from Calbiochem, A Brand of EMD Biosciences, Inc. 10394 Pacific Center Court, San Diego, CA 92121 USA. This information is given for the convenience of users of this European Standard and does not constitute an

endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results."

4.5 Standard solutions.

Protect all standard solutions from daily light.

4.5.1 Semduramicin stock standard solution, ca. 1 mg/ml.

Accurately weigh 10 mg to the nearest 0,1 mg of semduramicin sodium standard (4.3.1) into a 10 ml volumetric flask (5.7). Note down the exact weight of semduramicin sodium. Dissolve in methanol (4.1.3) and make up to 10 ml with methanol. Store at -20 °C and protected from light. Prepare freshly every 3 months.

Determine the experimental concentration of the semduramicin stock solution using the reference standard purity value provided by the supplier expressed as semduramicin using Equation (1).

$$C_s = \frac{m}{10} \times P \quad (1)$$

where

C_s is the experimental concentration of semduramicin in the stock standard in mg/ml;

P is the purity of the semduramicin standard expressed as semduramicin given by the supplier in percent e.g. 0,934;

m is the weighed mass of semduramicin sodium standard (4.3.1) in mg.

4.5.2 Nigericin sodium stock standard solution (for the LC-MS method), ca. 1 mg/ml.

Accurately weigh 10 mg to the nearest 0,1 mg of nigericin sodium standard (4.4.1) into a 10 ml volumetric flask (5.7). Note down the exact weight of nigericin sodium. Dissolve in methanol (4.1.3) and make up to 10 ml with methanol. Store at -20 °C and protected from light. Prepare freshly every 3 months.

Determine the experimental concentration of the nigericin sodium stock solutions using the reference standard purity value provided by the supplier using Equation (2).

$$C_n = \frac{m}{10} \times P \quad (2)$$

where

C_n is the experimental concentration of nigericin sodium in the stock standard in mg/ml;

P is the purity of the nigericin sodium standard given by the supplier in % e.g. 0,98;

m is the weighed mass of nigericin sodium standard (4.4.1) in mg.

4.5.3 Semduramicin intermediate standard solution (for the LC-MS method), ca. 100 µg/ml.

Accurately pipette 1,0 ml of the semduramicin stock standard solution (4.5.1) into a 10 ml volumetric flask (5.7). Make up to 10 ml with acetonitrile (4.1.2). Store at -20 °C and protected from light. Prepare fresh intermediate solutions monthly.

4.5.4 Nigericin sodium intermediate standard solution (for the LC-MS method), ca. 100 µg/ml.

Accurately pipette 1,0 ml of the nigericin sodium stock standard solution (4.5.2) into a 10 ml volumetric flask (5.7). Make up to 10 ml of with acetonitrile (4.1.2). Store at -20 °C and protected from light. Prepare fresh intermediate solutions monthly.

4.5.5 Semduramicin spiking solution (for the LC-MS method), ca. 2 µg/ml.

Accurately pipette 200 µl of the semduramicin intermediate solution (4.5.3) with an appropriate automatic pipette (5.6) into a 10 ml volumetric flask (5.7). Make up to 10 ml with acetonitrile (4.1.2). Store at -20 °C and protected from light. Prepare fresh spiking solutions weekly.

4.5.6 Nigericin sodium spiking solution (for the LC-MS method), ca. 2 µg/ml.

Accurately pipette 200 µl of the nigericin sodium intermediate solution (4.5.4) with an appropriate automatic pipette (5.6) into a 10 ml volumetric flask. Make up to 10 ml with acetonitrile (4.1.2). Store at -20 °C and protected from light. Prepare fresh spiking solutions weekly.

5 Apparatus

Usual laboratory apparatus and, in particular, the following:

5.1 LC-MS method HPLC system consisting of the following:

5.1.1 Pump, pulse free, flow capacity 0,1 ml/min to 2,0 ml/min.

5.1.2 Injection system, manual or autosampler, with a loop suitable for 10 µl injections.

5.1.3 Single quadrupole mass spectrometer or triple quadrupole mass spectrometer used in the MS configuration able to operate in a mass range at least between 200 m/z to 1 000 m/z.

NOTE For the MS detector the vacuum should be as stable as possible to ensure satisfactory repeatability. The system should therefore be pumped at least 48 h before starting the measurements.

5.1.4 Computer data system.

5.1.5 Analytical column, Alltima HP C18, 5 µm ,150 mm x 2,1 mm, 190 Å, 12 % C load and 200 m^2/g or equivalent.

5.1.6 Guard column, Alltima HP C18, 5 µm, 7,5 mm x 2,1 mm, 190 Å, 12% C load and 200 m^2/g or equivalent.

5.2 LC-PCD-UV method HPLC system consisting of the following:

5.2.1 Pump, pulse free, flow capacity 0,1 ml/min to 2,0 ml/min.

5.2.2 Injection system, manual or autosampler, with a loop suitable for 100 µl injections.

5.2.3 UV/VIS detector, variable wavelength, suitable for reliable measurements at 598 nm, or UV/VIS photodiode array detector (DAD).

5.2.4 Computer data system.

5.2.5 Post-column reactor, with a 1,5 ml to 2,0 ml reaction coil, for operation at 92 °C.

The coil may be a commercially available coil or it may be made using 1/16" 316 SS tubing, 0,020" <> 0,5 mm ID (between 8 m and 10 m length) folded to fit the reactor heating chamber. To ensure effective mixing of reagent and column effluent, use a vortex or static mixing tee (not a regular tee) before the reaction coil.

5.2.6 Post column reagent pump, pulse free, flow capacity from 0,5 ml/min to 2,0 ml/min.

5.2.7 Analytical column, Inertsil ODS-3 C18, 5 µm, 150 x 4,6 mm 100 Å 15% carbon load and 450m^2/g or equivalent.

5.2.8 Guard column, Prevail C18, 5 µm, 7,5 x 4,6 mm or equivalent.

5.3 Glass vials, 1,5 ml HPLC glass vials.

5.4 Shaker, head-over-head or equivalent.

5.5 Balances, one analytical, of 10 g capacity or greater with 0,1 mg readability.

5.6 Variable-volume positive displacement piston pipettes, suitable for pipetting volumes ranged from 20 µl to 1 000 µl.

5.7 Volumetric flasks, 10 ml, 20 ml, 500 ml and 1 000 ml.

5.8 Glass graduated cylinders, 100 ml and 1 000 ml.

5.9 Polypropylene centrifuge tubes, 50 ml capacity, stoppered (Falcon® or equivalent).

5.10 Disposable syringes, 20 ml.

5.11 Solvent filtration system, all glass filter apparatus suitable for 47 mm filters.

5.12 Membrane filters, Durapore® membrane filters, PVDF, hydrophobic, of 47 mm diameter and pore size 0,45 µm or equivalent.

5.13 Nylon filters, 47 mm diameter and pore size 0,22 µm or equivalent.

5.14 Syringe filters Nylon 0,2 µm or equivalent i.e. full chemical compatibility with acetonitrile.

5.15 pH meter.

5.16 Grinding instrument.

5.17 Sieve, with 1 mm apertures.

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 [1].

7 Preparation of test sample

7.1 General

Prepare the test sample in accordance with prEN ISO 6498.

7.2 Laboratory sample

Grind the laboratory sample (usually 50 g) so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

7.3 Test sample

The test sample consists of a representative and homogenised aliquot of the ground laboratory sample of at least 20 g.

7.4 Test portion

Accurately weigh 5,0 g ± 0,1 g of the thoroughly mixed test sample into a 50 ml polypropylene stoppered tube (Falcon® tube or equivalent). Note down the mass expressed in mg (W_{tp}). Submit it to the analysis procedure (8).

8 Procedure

8.1 Preparation of positive and negative control samples

The use of quality control samples and quality control charts is recommended.

With each set of samples, include a positive control sample (PCS) and a negative control sample (NCS) on a daily basis. For the PCS, weigh 5,0 g ± 0,1 g of a poultry blank feed. Accurately pipette, with an appropriate automatic pipette (5.6), 130 µl of the semduramicin stock standard solution (4.5.1) and add to the poultry blank. Mix thoroughly to ensure maximum contact with the feed. Allow penetration for at least 1 hour before extraction. The NCS is constituted by 5,0 g ± 0,1 g of non-spiked poultry blank feed. Both PCS and NCS will be submitted to the same treatment as unknown samples.

8.2 Samples extraction

Accurately weigh 5,0 g ± 0,1 g test portion into a polypropylene centrifuge tube (5.9). Add 15 ml acetonitrile (4.1.2), close tightly the polypropylene centrifuge tube (5.9) and check that there is no solvent leak through the cap. Shake vigorously to ensure appropriate suspension of the feed into the extraction solvent. Perform a solid-liquid extraction by shaking the mixture for 30 min on the shaker (5.4).

8.3 Filtration

Filter the supernatants (8.2) through a 0,2 µm Nylon syringe filter (5.14) into a clean polypropylene tube (5.9).

8.4 HPLC analysis

8.4.1 LC-MS

8.4.1.1 Dilution

Accurately pipette with an appropriate automatic pipette (5.6), 75 µl of the filtered extract (8.3) into a 10 ml volumetric flask (5.7) Make up to 10 ml volume with acetonitrile (4.1.2).

8.4.1.2 Standard additions

Accurately pipette with an appropriate automatic pipette (5.6), four 900 µl aliquots of the diluted extract (8.4.1.1) into 1,5 ml glass vials (5.3) and label as S0, S1, S2 and S3 respectively.