Irish Standard
I.S. EN 16158:2012

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

I.S. EN 16158:2012

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

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

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

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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of an identical text or by endorsement, at the latest by August 2012, and conflicting national standards shall be withdrawn at the latest by August 2012.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