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Animal feeding stuffs — Determination of monensin, narasin and salinomycin contents — Liquid chromatographic method using post-column derivatization

Aliments des animaux — Détermination des teneurs en monensine, narasine et salinomycine — Méthode par chromatographie liquide utilisant la dérivatisation post-colonne

ICS 65.120

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Contents

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 14183 was prepared by Technical Committee ISO/TC 34, *Agricultural Food products*, Subcommittee SC 10, *Animal Feeding Stuffs*.

Animal feeding stuffs — Determination of monensin, narasin and salinomycin contents — Liquid chromatographic method using post-column derivatization

1 Scope

This International Standard specifies a high-performance liquid chromatographic (HPLC) method for the determination of monensin, narasin and salinomycin content of premixtures and animal feeding stuffs.

This method is applicable to all types of feed, and aqueous feed samples and water. The limit of quantitation is 0,5 mg/kg, 1 mg/kg and 1 mg/kg for monensin, salinomycin and narasin respectively. Lasalocid cannot be determined by this method.

2 Normative Reference

ISO 6498:1996, *Animal feeding stuffs - Preparation of test samples.*

3 Principle

The ionophores monensin, salinomycin and narasin are extracted using methanol/water (90+10) with mechanical shaking for 1 h. The extracts are filtered, and for low level samples, an alumina column cleanup is carried out. The ionophores are determined by reverse-phase HPLC using post-column derivatization with vanillin, and detection at 520 nm. Suspect positive trace-level samples and medicated feed samples containing unexpected ionophores are confirmed using a hexane extraction or post-column derivatization with dimethylaminobenzaldehyde (DMAB).

4 Reagents

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

4.1 **Milli-Q purified water**, or equivalent.

4.2 **Methanol**, HPLC grade.

4.3 **Sulfuric acid**, 97 - 98%

4.4 **Alumina**, basic, 80 - 200 mesh.

4.5 **Sodium bicarbonate**

4.6 **Vanillin (4-hydroxy-3-methoxybenzaldehyde),** minimum 99% purity.

4.7 **Dimethylaminobenzaldehyde** (DMAB)

4.8 Hexane, distilled in glass.

4.9 Extraction solvent, MeOH/H_20 (90 + 10).

Combine 1800 ml methanol (4.2) and 200 ml water (4.1) in a 2 litre flask. Mix well.

4.10 Mobile phases

4.10.1 Post-column reaction system: 20 g vanillin (4.6) in 500 ml cold methanol (4.2)/sulfuric acid (4.3) (1000 + 20). Keep in an ice bath, protect from light. Prepare fresh daily. Filter under vacuum using the equipment in 5.8.

4.10.2 C_{18} 5 µm HPLC column: Methanol (4.2)/acetic acid, 5% (4.11) (94 + 6). Filter under vacuum using the equipment in 5.8.

4.11 Acetic acid, 5 %

Dilute 25 ml glacial acetic acid to 500 ml with water (4.1).

4.12 Neutralized methanol

Add 1,0 g of sodium bicarbonate (4.5) into 4 l methanol. Mix well and filter if necessary through 11 µm filter paper (eg. Whatman No. 1). See Note 4.14.

4.13 Reference standards

Composition or potency is required for each lot of reference standard.

4.13.1 Monensin sodium[1]

4.13.2 Narasin[1]

4.13.3 Sodium salinomycin[2]

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

4.14 Ionophore stock standards, (ca. 0,50 mg/ml).

Accurately weigh 25 mg to the nearest 0,1 mg of each standard (4.13.1 to 4.13.3) into separate 50 ml volumetric flasks. Dissolve in neutralized methanol (4.12) and make to volume. Prepare fresh every month. Store in a refrigerator.

Protect all standard solutions from light or prepare them in low actinic flasks.

NOTE The requirement for neutralized methanol has not been verified for salinomycin. It is not required if analysing monensin only, but is required for analysis of narasin.

4.14.1 Monensin stock standard

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[1] Available from Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285, USA.

[2] Available from Roche Vitamins Inc., 45 Waterview Boulevard, Parsippany, NJ, USA. 07054-1298, Hoechst Roussel Vet Canada Inc., 240 Henderson Drive, Regina, Saskatchewan, Canada S4N 5P7, and Hoechst Roussel Vet, D-65926 Frankfurt am Main, Gebäude H 790, Germany.

Prepare as described in 4.14. Concentration of stock standard takes into account the principle component of monensin (A) and a minor component (B), which elutes just before monensin A [4]. Determine the concentration of each component using the composition identified on the reference standard profile sheet.

$$C_m = \frac{0{,}5S_m}{100}$$

where

0,5 is the concentration of the stock standard (4.14) in milligrams per millilitre, recorded to 3 significant figures,

C_m is the concentration of the given component (A or B) in the stock standard in milligrams per millilitre;

S_m is the proportion of the given component (A or B) in the reference standard according to the profile sheet in %.

m refers to component A or B.

EXAMPLE: Reference standard lot P61722 contained 94,67 % monensin A and 3,98 % monensin B.

4.14.2 Salinomycin stock standard

Prepare as described in step 4.14. Determine the concentration using the reference standard concentration value provided by the supplier [2].

$$C_s = \frac{0{,}5P}{1000}$$

where

C_s is the concentration of salinomycin in the stock standard in milligrams per millilitre;

P is the concentration of the salinomycin standard given by the supplier in micrograms per milligram.

EXAMPLE: For lot SC002A-I, the standard concentration is 950 μg/mg.

4.14.3 Narasin stock standard

Prepare as described in 4.14. Concentration of the stock standard takes into account the principle component of narasin (A) and the minor components (D and I), which elute after narasin A [5]. Determine the concentration of each component using the composition identified on the reference standard profile sheet.

$$C_n = \frac{0{,}5S_n}{100}$$

where

C_n is the concentration of the component (A, D or I) in the stock standard in milligrams per millilitre;

S_n is the proportion of the given component (A,D or I) in the reference standard according to the profile sheet in %.

n refers to component A, D or I.

EXAMPLE: For reference standard lot RS0206 the % of each component on an anhydrous basis is:

Narasin A = 94,6%

Narasin D = 0,8%

Narasin I = 0,3%

The reference standard profile defines the concentration (potency) on an anhydrous basis when corrected for moisture content after determination by the Karl Fischer method.

An alternative procedure for moisture determination is to dry the approximate required amount of standard for 2 h at 60 ^{0}C in a vacuum oven. After preparing the new standard solution, discard any remaining dried standard.

4.15 Mixed HPLC standards

4.15.1 HPLC standard A, ca. 0,2 μg/ml for monensin, ca. 0,4 μg/ml for salinomycin and narasin, respectively.

Accurately, with a 50 μl syringe, take 40 μl monensin stock standard (4.14.1) and 80 μl (with 100 μl syringe) each of salinomycin and narasin stock standard (4.14.2 and 4.14.3). Place in a 100 ml volumetric flask, and bring to volume with extraction solvent (4.9). Mix well. Prepare fresh every month. Store in a refrigerator.

4.15.2 HPLC standard B, ca. 1 μg/ml for monensin, ca. 2 μg/ml for salinomycin and narasin, respectively.

Accurately, with a syringe, take 200 μl aliquot of monensin stock standard (4.14.1) and 400 μl each of salinomycin and narasin stock standard (4.14.2 and 4.14.3). Place in a 100 ml volumetric flask and bring to volume with extraction solvent (4.9). Mix well. Prepare fresh every month. Store in a refrigerator.

4.15.3 HPLC standard C, ca. 2,5 μg/ml for monensin , ca. 5 μg/ml for salinomycin and narasin, respectively.

Accurately, pipette 0,5 ml of monensin stock standard (4.14.1) and 1,0 ml of salinomycin and narasin stock standard (4.14.2 and 4.14.3) into a 100 ml volumetric flask. Bring to volume with extraction solvent (4.9). Mix well. Prepare fresh every month. Store in a refrigerator.

4.15.4 HPLC standard D, ca. 5 μg/ml for monensin, ca. 10 μg/ml for salinomycin and narasin respectively.

Accurately pipette 1,0 ml of monensin stock standard (4.14.1) and 2,0 ml of salinomycin and narasin stock standard (4.14.2 and 4.14.3) into a 100 ml volumetric flask. Bring to volume with extraction solvent (4.9). Mix well. Prepare fresh every month. Store in a refrigerator.

4.15.5 HPLC standard E, ca. 10 μg/ml for monensin, ca. 20 μg/ml for salinomycin and narasin respectively.

Accurately pipette 2,0 ml of monensin stock standard (4.14.1) and 4,0 ml of salinomycin and narasin stock standard (4.14.2 and 4.14.3) into a 100 ml volumetric flask. Bring to volume with extraction solvent (4.9). Mix well. Prepare fresh every month. Store in a refrigerator.

4.16 Single HPLC standards

4.16.1 Monensin, ca. 5 μg/ml.

Accurately pipette 1,0 ml monensin stock standard (4.14.1) into a 100 ml low actinic volumetric flask. Bring to volume with extraction solvent (4.9). Mix well. Prepare fresh every month. Store in a refrigerator.

4.16.2 Salinomycin, ca. 10 μg/ml.

Accurately pipette 2,0 ml salinomycin stock standard (4.14.2) into a 100 ml low actinic volumetric flask. Bring to volume with extraction solvent (4.9). Mix well. Prepare fresh every month. Store in a refrigerator.

4.16.3 Narasin, ca. 10 μg/ml.

Accurately pipette 2,0 ml narasin stock standard (4.14.3) into a 100 ml low actinic volumetric flask. Bring to volume with extraction solvent (4.9). Mix well. Prepare fresh every month. Store in a refrigerator.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 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 loop suitable for 100 µl injections.

5.1.3 UV/VIS detector, variable wavelength, suitable for measurements at 520 nm and 592 nm.

5.1.4 Integrator or computer data system.

5.1.5 Post-column reactor, with a 1,5 ml to 2,0 ml reaction coil, for operation at 95 C°.

The coil may be a commercially available coil or it may be made using 7,5 m to 10 m of 316 SS tubing, 0,15 mm ID, coiled in a format to fit the reactor heating chamber (a suggestion is to wrap the coil in enough aluminum foil to make it fit snuggly in the heater. To ensure effective mixing of reagent and column effluent, use a vortex or static mixing tee (not a regular tee) before the reaction coil.

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

5.1.7 Analytical column, 5 µm C_{18}, 25 x 0,46 cm Nucleosil 120A or Partisil 5 ODS3, or equivalent.

NOTE Experience has shown the Nucleosil column to provide better resolution.

5.1.8 Guard column, C_{18}.

5.2 Syringes, of capacities 50 µl, 100 µl, 250 µl and 500 µl.

5.3 Shaker, rotary or wrist-action shaker.

5.4 Balances, one analytical, of 10 g capacity or greater with 0,1 mg readability, and one, of 100 g capacity or greater with 0,01 g readability.

5.5 Erlenmeyer flasks, of capacities 125 ml , 250 ml and 500 ml, with glass stopper.

5.6 Tubes, 15 ml, stoppered.

5.7 Filter papers, Whatman No. 41 (15 cm) or equivalent, and Whatman No. 42 (15 cm) or equivalent.

5.8 Solvent filtration system, all glass filter apparatus suitable for 47 mm filter (following item), and 47 mm diameter nylon filter of pore size 0,45 µm.

5.9 Clean-up column, glass, 25 cm length, 10 mm internal diameter, with teflon stopcock.

5.10 Sample filtration system, equipped with nylon filter of pore size 0,45 µm.

5.11 Nitrogen evaporator, for evaporation of solvents under a stream of nitrogen.

5.12 Vacuum oven, for operation at 60 ^{0}C ± 2.

5.13 Sieve, with 1 mm apertures.