
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*



INTERNATIONAL ORGANIZATION FOR STANDARDIZATION



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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.

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The main task of technical committees is to prepare International Standards. 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.

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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 the monensin, narasin and salinomycin contents of animal feeding stuffs, supplements (dry and liquid) and mineral premixtures. The method is not applicable to drug premixes (pharmaceutical products). Lasalocid and semduramicin cannot be determined by this method.

The limit of quantitation is approximately 1 mg/kg, 2 mg/kg and 2 mg/kg for monensin, salinomycin and narasin, respectively. A lower limit of quantitation can be achievable but this is to be validated by the user.

2 Normative references

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.

ISO 6498:1998, *Animal feeding stuffs — Preparation of test samples*

3 Principle

The ionophores monensin, narasin and salinomycin are extracted using methanol/water (90 + 10) with mechanical shaking for 1 h, then the extracts are filtered. 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 Water**, HPLC grade, or equivalent (e.g. Milli-Q purified water).
- 4.2 Methanol** (CH₃OH), HPLC grade.
- 4.3 Sulfuric acid** (H₂SO₄), 97 % to 98 %.
- 4.4 Acetic acid** (CH₃COOH), glacial, 97 % to 98 %.
- 4.5 Sodium hydrogen carbonate** (NaHCO₃), minimum 99 % purity.
- 4.6 Vanillin** (4-hydroxy-3-methoxybenzaldehyde), minimum 99 % purity.

4.7 Dimethylaminobenzaldehyde (DMAB), minimum 99 % purity.

4.8 Hexane [$\text{CH}_3(\text{CH}_2)_4\text{CH}_3$], distilled in glass.

4.9 Extraction solvent, methanol/water (90 + 10).

Combine 1 800 ml of methanol (4.2) and 200 ml of water (4.1) in a 2 l flask. Mix well.

4.10 Mobile phases

4.10.1 Post-column reaction system

While stirring gently, slowly add by pipette 20 ml of sulfuric acid (4.3) to 950 ml of methanol (4.2). Allow to cool, then add 30 g of vanillin (4.6) while stirring. Protect from light. Prepare fresh daily.

4.10.2 HPLC column

Use methanol (4.2)/water (4.1)/acetic acid (4.4) (940/60/1). Filter under vacuum using the equipment in 5.7.

4.11 Neutralized methanol

Add 1,0 g of sodium hydrogen carbonate (4.5) into 4 l of methanol (4.2). Mix well and filter if necessary through an 11 µm filter paper (e.g. Whatman No. 1)¹⁾. See Note to 4.13.

4.12 Reference standards

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

4.12.1 Monensin sodium²⁾

4.12.2 Narasin²⁾

4.12.3 Sodium salinomycin³⁾

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.

4.13 Ionophore stock standards, ca. 0,50 mg/ml.

Accurately weigh, to the nearest 0,1 mg, 25 mg of each standard (4.12.1 to 4.12.3) into separate 50 ml volumetric flasks. Dissolve in neutralized methanol (4.11) and dilute to volume. Prepare freshly 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.

1) This is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

2) Available from Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285, USA.

3) Available from Alpharma Inc., Animal Health Division, 1 Duggar Drive, Willow Island, WV, USA 26134-97111, and Hoechst Roussel Vet, D-65926 Frankfurt am Main, Gebaude H 790, Germany.

4.13.1 Monensin stock standard

Prepare as described in 4.13. Calculate the concentration of stock standard based on the principle component, monensin A. The minor component, monensin B, which elutes just before monensin A ^[4] is determined in test samples based on monensin A. Use the component composition identified on the reference standard profile sheet:

$$\rho_M = \frac{0,5 S_M}{100}$$

where

0,5 is the concentration of the stock standard (4.13), in milligrams per millilitre, recorded to three significant figures;

ρ_M is the concentration of the given component monensin A in the stock standard, in milligrams per millilitre;

S_M is the proportion of the given component monensin A in the reference standard according to the profile sheet, in percent.

EXAMPLE Reference standard lot RS0234 contains 93,71 % of monensin A on an “as-is” basis.

4.13.2 Salinomycin stock standard

Prepare as described in 4.13. Determine the concentration using the reference standard concentration value provided by the supplier ^[2]:

$$\rho_S = \frac{0,5 w}{1\,000}$$

where

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

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

EXAMPLE For lot WS-19B, the standard concentration is 986 µg/mg.

4.13.3 Narasin stock standard

Prepare as described in 4.13. Calculate the concentration of the stock standard based on the principle component, narasin A. The minor components (narasin D and I), which elute after narasin A ^[5], are determined in test samples based on narasin A. Use the component composition identified on the reference standard profile sheet:

$$\rho_N = \frac{0,5 S_N}{100}$$

where

ρ_N is the concentration of the component narasin A in the stock standard, in milligrams per millilitre;

S_N is the proportion of the given component narasin A in the reference standard according to the profile sheet, in percent.

EXAMPLE For reference standard lot RS0302, the percentage of each component on an “as-is” basis is:
 narasin A = 85,4 %,
 narasin D = 1,9 %,
 narasin I = 0,7 %.

4.14 Intermediate mixed standard solution, ca. 20 µg/ml, 40 µg/ml and 40 µg/ml monensin, salinomycin and narasin, respectively.

Transfer by pipette 10,0 ml, 20,0 ml and 20,0 ml of monensin, salinomycin and narasin stock standards (4.13), respectively, into a 250 ml volumetric flask. Dilute to volume with extraction solvent (4.9). Mix well. Prepare freshly every month.

4.15 Mixed HPLC standards

Prepare five mixed HPLC standard solutions by pipetting an aliquot of the mixed intermediate standard (4.14) into 100 ml low-actinic volumetric flasks and diluting to volume with extraction solvent (4.9), as specified in the Table 1. Mix well. Prepare freshly every month.

Table 1

Mixed HPLC standard identification	Amount of intermediate standard (4.14) ml	Approximate HPLC standard concentration µg/ml		
		Monensin	Salinomycin	Narasin
A	1	0,2	0,4	0,4
B	5	1	2	2
C	10	2	4	4
D	25	5	10	10
E	50	10	20	20

4.16 Single HPLC standards

4.16.1 Monensin, ca. 5 µg/ml.

Accurately pipette 1,0 ml of monensin stock standard (4.13.1) into a 100 ml low-actinic volumetric flask. Dilute to volume with extraction solvent (4.9). Mix well. Prepare freshly every month. Store in a refrigerator.

4.16.2 Salinomycin, ca. 10 µg/ml.

Accurately pipette 2,0 ml of salinomycin stock standard (4.13.2) into a 100 ml low-actinic volumetric flask. Dilute to volume with extraction solvent (4.9). Mix well. Prepare freshly every month. Store in a refrigerator.

4.16.3 Narasin, ca. 10 µg/ml.

Accurately pipette 2,0 ml of narasin stock standard (4.13.3) into a 100 ml low-actinic volumetric flask. Dilute to volume with extraction solvent (4.9). Mix well. Prepare freshly 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.