SLOVENSKI STANDARD
SIST EN ISO 14183:2009

01-april-2009

Krma - Določ evanje monezina, narazina in salinomicina - Metoda tekoč inske kromatografije s postkolonsko derivatizacijo (ISO 14183:2005)

Animal feeding stuffs - Determination of monensin, narasin and salinomycin contents - Liquid chromatographic method using post-column derivatization (ISO 14183:2005)

Futtermittel - Bestimmung der Gehalte an Monensin, Narasin und Salinomycin - Flüssigkeitschromatographisches Verfahren mittels Nachsäulenderivatisierung (ISO 14183:2005)

Aliments des animaux - Détermination des teneurs en monensine, narasine et salinomycine - Méthode par chromatographie liquide utilisant la dérivatisation post-colonne (ISO 14183:2005)

Ta slovenski standard je istoveten z: **EN ISO 14183:2008**

ICS:

65.120	Krmila	Animal feeding stuffs

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EUROPEAN STANDARD

NORME EUROPÉENNE

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English Version

Animal feeding stuffs - Determination of monensin, narasin and salinomycin contents - Liquid chromatographic method using post-column derivatization (ISO 14183:2005)

Aliments des animaux - Détermination des teneurs en monensine, narasine et salinomycine - Méthode par chromatographie liquide utilisant la dérivatisation post-colonne (ISO 14183:2005)

Futtermittel - Bestimmung der Gehalte an Monensin, Narasin und Salinomycin - Flüssigkeitschromatographisches Verfahren mittels Nachsäulenderivatisierung (ISO 14183:2005)

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Foreword

The text of ISO 14183:2005 has been prepared by Technical Committee ISO/TC 34 “Agricultural food products” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 14183:2008 by Technical Committee CEN/TC 327 “Animal feeding stuffs - Methods of sampling and analysis” 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of an identical text or by endorsement, at the latest by May 2009, and conflicting national standards shall be withdrawn at the latest by May 2009.

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Endorsement notice

The text of ISO 14183:2005 has been approved by CEN as a EN ISO 14183:2008 without any modification.

INTERNATIONAL STANDARD

ISO 14183

First edition
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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

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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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ISO 14183 was prepared by Technical Committee ISO/TC 34, *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 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** (CH_3OH), HPLC grade.

4.3 **Sulfuric acid** (H_2SO_4), 97 % to 98 %.

4.4 **Acetic acid** ($CH_2CH_3CO_2H$), glacial, 97 % to 98 %.

4.5 **Sodium hydrogen carbonate** ($NaHCO_3$), minimum 99 % purity.

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

4.7 Dimethylaminobenzaldehyde (DMAB), minimum 99 % purity.

4.8 Hexane [$CH_3(CH_2)_4CH_3$], 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 sodium[2)]

4.12.2 Narasin[2)]

4.12.3 Sodium salinomycin[3)]

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.

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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.