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Djurfoder – Bestämning av nicarbazin – Vätskekromatografisk metod

Animal feeding stuffs – Determination of nicarbazin – High-performance liquid chromatographic method

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EUROPEAN STANDARD

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English Version

Animal feeding stuffs - Determination of nicarbazin - High-performance liquid chromatographic method

Aliments des animaux - Détermination de la nicarbazine - Méthode de chromatographie liquide hautes performances

Futtermittel - Bestimmung von Nicarbazin - Hochleistungsflüssigchromatographisches Verfahren

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Contents

Page

Foreword

This document (EN 15782:2009) 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of an identical text or by endorsement, at the latest by February 2010, and conflicting national standards shall be withdrawn at the latest by February 2010.

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1 Scope

This European Standard specifies a method for the determination of additive use of nicarbazin in animal feeding stuffs and premixtures (maximum concentration 2,5% nicarbazin) using high performance liquid chromatography. Nicarbazin is a 1:1 equimolar mixture of 4,4'-dinitrocarbanilide (DNC) and 4,6-dimethyl-2-pyriminol (HDP). Nicarbazin is generally determined by using DNC as the target compound. In this method the DNC moiety of nicarbazin is detected.

The limit of quantitation is 20 mg/kg. The limit of detection is 0,5 mg/kg

NOTE A lower limit of quantitation may be achievable but should be validated by the user.

2 Principle

Samples are extracted using an acetonitrile/methanol mixture. For feeding stuffs, water is added additionally. An aliquot of the extract is assayed using a reverse phase isocratic HPLC method which measures the 4,4'dinitrocarbanilide moiety at a wavelength of 350 nm.

3 Reagents

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

3.1 Water, resistance > 10 MOhm.cm$^{-1.}$

3.2 Acetonitrile (CH_3CN), HPLC grade.

3.3 Methanol (CH_3OH), HPLC grade.

3.4 Extraction solvent

Mix 500 ml of acetonitrile (3.2) with 500 ml of methanol (3.3). Mix well using a magnetic stir plate and stir bar.

3.5 Eluent for liquid chromatography

Mix 650 ml acetonitrile (3.2) with 350 ml of purified water (3.1). Mix well using a magnetic stir plate and stir bar and degas (e.g. with helium) before use.

3.6 Nicarbazin reference standard

3.7 Standard solutions

3.7.1 Nicarbazin stock standard solution, 100 µg/ml

Dissolve 10 mg, weighed to the nearest 0,1 mg, of nicarbazin reference standard (3.6) in 100 ml extraction solvent (3.4). To aid with dissolution, sonication for approximately 5 min is recommended. Mix well. This solution is stable for 24 h when stored in subdued light at ambient or refrigerated storage conditions (see remark 3.7.1).

NOTE 1 The solubility of the nicarbazin reference standard in extraction solvent is critical. The nicarbazin concentrations in the prepared stock solutions must be monitored by use of a cuvet spectrophotometer as follows. Prepare a solution of 10 µg/ml by diluting the prepared stock standard solution (3.7.1) with acetonitrile. Record a UV-Vis spectrum between 220 nm and 450 nm using a mixture of methanol/acetonitrile (5:95 v/v) as a reference solution. The maximum