



SLOVENSKI STANDARD
SIST EN 15782:2009

01-december-2009

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

Futtermittel - Bestimmung von Nicarbazin - Hochleistungsflüssigchromatographisches Verfahren

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

iTeh STANDARD PREVIEW
(standards.iteh.ai)

Ta slovenski standard je istoveten z: **EN 15782:2009**

[SIST EN 15782:2009](https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009)

[https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-](https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009)

[8869c6f1bec3/sist-en-15782-2009](https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009)

ICS:

65.120

Krmila

Animal feeding stuffs

SIST EN 15782:2009

en,fr,de

iTeh STANDARD PREVIEW
(standards.iteh.ai)

SIST EN 15782:2009

<https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009>

EUROPEAN STANDARD

EN 15782

NORME EUROPÉENNE

EUROPÄISCHE NORM

August 2009

ICS 65.120

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

This European Standard was approved by CEN on 3 July 2009.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

[SIST EN 15782:2009](https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009)

<https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009>



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents

Page

Foreword.....	3
1 Scope	4
2 Principle.....	4
3 Reagents.....	4
4 Apparatus	5
5 Sampling.....	5
6 Preparation of test sample.....	5
7 Procedure	6
8 Calculation and expression of results.....	7
9 Precision.....	8
10 Test report	8
Annex A (informative) Results of an interlaboratory study	9
Bibliography	10

iTeh STANDARD PREVIEW

(standards.iteh.ai)

SIST EN 15782:2009

<https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009>

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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

iTeh STANDARD PREVIEW (standards.iteh.ai)

[SIST EN 15782:2009](https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009)

<https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009>

EN 15782:2009 (E)

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.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

3.1 Water, resistance > 10 MOhm.cm⁻¹.

[SIST EN 15782:2009](#)

3.2 Acetonitrile (CH₃CN), HPLC grade.

[http://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009](#)

3.3 Methanol (CH₃OH), 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

absorbance measured between 340 nm and 350 nm should be within a margin of $\pm 5\%$ of the default value. The default value should be established in your own laboratory by preparing a stock standard solution in duplicate and monitoring the UV-Vis spectra as described above. The default value is the mean result of the duplicates.

NOTE 2 The susceptibility of nicarbazin to photon-degradation is well known. To avoid degradation during sample preparation and analysis, protect the samples and extracts from daylight at all times.

3.7.2 Nicarbazin working standard solutions

Prepare a range of calibration working standards containing 0 $\mu\text{g/ml}$; 0,25 $\mu\text{g/ml}$; 0,5 $\mu\text{g/ml}$; 1 $\mu\text{g/ml}$; 2 $\mu\text{g/ml}$; 5 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$ nicarbazin by diluting the stock standard solution (3.7.1) with HPLC eluent (3.5). Working standards must be prepared daily.

4 Apparatus

Usual laboratory apparatus and, in particular, the following.

4.1 High performance liquid chromatography system consisting of the following:

4.1.1 An autosampler or manual injector set to inject a volume of 20 μl .

4.1.2 A pump set to deliver a constant eluent flow rate of 1,0 ml/min.

4.1.3 HPLC column, 250 mm x 4,6 mm packed with RP C18, 5 μm material, or equivalent.

NOTE A Nova-Pak or Bonda-Pak column is recommended, but also other columns can be used provided that a satisfactory separation of DNC is achieved.

4.1.4 A detector allowing the measurement of absorbance of UV light at a wavelength of 350 nm.

4.2 Mechanical shaker (e.g. Gyrotory shaker, wrist action shaker or equivalent).

4.3 Micro filters for sample filtration, 0,2 μm to 0,5 μm .

4.4 Ultrasonic bath

4.5 Waterbath, 50°C \pm 2°C.

5 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this European Standard. A recommended sampling method is given in EN ISO 6497.

6 Preparation of test sample

6.1 General

The milling and mixing of compound feed samples prior to assay is obligatory. Grind feed samples through a

EN 15782:2009 (E)

mill equipped with a 1 mm screen. After milling, mix the entire sample thoroughly. Store the sample at room temperature in subdued light. Premix samples are not milled.

NOTE The susceptibility of nicarbazin to photon-degradation is well known. To avoid degradation during sample preparation and analysis, protect the samples and extracts from daylight at all times.

6.2 Mixing of the test samples before weighing

The container should be filled to a maximum of 50% of the total volume. Bring the container in a horizontal position and rotate bottom and top of the container in circles moving the container up and down along the virtual centre of the container for 30 sec. Put the container in an upright position and wait a few seconds for settlement of the generated dust.

6.3 Spiked feed samples; 100 mg/kg

Transfer 2,5 ml of the stock standard solution (3.7.1) in the sample tube or flask. Evaporate to a small volume (less than 0,5 ml) with a gentle stream of nitrogen, add 2,5 g blank feed, mix thoroughly and wait 10 min before starting the extraction procedure by adding water for swelling (see 7.2.2).

7 Procedure**7.1 General**

Complete each assay within one working day.

7.2 Extraction**7.2.1 Premixtures**

STANDARD PREVIEW
(standards.iteh.ai)
<https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009>

Accurately weigh 1 g to the nearest 0,01 g of the test sample directly into a volumetric flask of 200 ml.

Add 80 ml of extraction solvent (3.4), close the flask and mix manually by swirling.

Put the flasks in a waterbath of 50°C (4.5) for at least 15 min with intermediate swirling at 8 min.

Mix thoroughly for 15 min using a mechanical means (4.2).

Put the flasks in an ultrasonic bath (4.4) and sonicate for 15 min.

Cool down to room-temperature, adjust to volume with HPLC eluent (3.5) and mix.

Allow sample solids to settle (minimum 30 min).

If additional dilutions are required, dilute the samples with HPLC eluent (3.5) to a final nicarbazin concentration which falls within the standard curve levels (3.7.2).

Filter an aliquot of the final dilution through a micro filter (4.3) for analysis by HPLC.

7.2.2 Animal feeding stuffs

Accurately weigh 2,5 g to the nearest 0,01 g of the test sample in a 50 ml disposable centrifuge tube or directly in a volumetric flask of 100 ml.

Add 5 ml of water. Take care that the whole sample becomes wet.

Wait at least 10 min.

Add 35 ml of extraction solvent (3.4), close the tube or flask and mix manually by swirling.

Put the tubes or flasks in a waterbath of 50°C (4.5) for 15 min with intermediate swirling at 8 min.

Mix thoroughly for 15 min using a mechanical means (4.2).

Put the tubes or flasks in an ultrasonic bath (4.4) and sonicate for 15 min.

If using disposable centrifuge tubes, transfer the sample extract quantitatively into a 100 ml volumetric flask with HPLC eluent (3.5), adjust to volume and mix.

If additional dilutions are required, allow samples to settle (minimum 30 min) and dilute the samples with HPLC eluent (3.5) to a final nicarbazin concentration which falls within the standard curve levels.

Filter an aliquot of the final dilution through a micro filter (4.3) for analysis by HPLC.

NOTE For relatively inhomogeneous compound feed samples, the weighed sample amount should be increased to 10 gram with simultaneous up-scaling of the volume of extraction solvent used.

7.3 HPLC determination

7.3.1 HPLC conditions

The following conditions are offered for guidance, other conditions may be used provided that they give equivalent results.

analytical column	as in 4.1.3 SIST EN 15782:2009
mobile phase	as in 3.5 https://standards.iteh.ai/catalog/standards/sist/c5b4e7ae-c27f-405b-a5ed-8869c6f1bec3/sist-en-15782-2009
flow rate	1,0 ml/min
detection wavelength	350 nm
injection volume	20 µl

Check the stability of the chromatographic system, injecting several times the calibration solution (3.7.2) containing 1,0 µg/ml, until constant peak areas and retention times are achieved.

7.3.2 Calibration graph

Inject each calibration solution (3.7.2) to determine the peak area/height for each concentration. Plot a calibration graph using the peak areas/heights of the calibration solutions as the ordinate and the corresponding concentrations in µg/ml as the abscissae.

7.3.3 Sample solution

Inject the sample extract (7.2.1 or 7.2.2) at least 2 times using the same volume as taken for the calibration solutions and determine the peak area/height of the DNC peaks.

8 Calculation and expression of results

Calculate the mass fraction of nicarbazin in the test sample by the equation: