SLOVENSKI STANDARD
SIST EN ISO 14939:2001

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Krma - Določ evanje karbadoksa - Metoda s tekoč insko kromatografijo visoke loč ljivosti (ISO 14939:2001)

Animal feeding stuffs - Determination of carbadox content - Method using high-performance liquid chromatography (ISO 14939:2001)

Futtermittel - Bestimmung des Carbadoxgehaltes - Hochleistungs-flüssigchromatographisches Verfahren (ISO 14939:2001)

Aliments des animaux - Détermination de la teneur en carbadox - Méthode par chromatographie liquide a haute performance (ISO 14939:2001)

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65.120	Krmila	Animal feeding stuffs

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Animal feeding stuffs - Determination of carbadox content - Method using high-performance liquid chromatography (ISO 14939:2001)

Aliments des animaux - Détermination de la teneur en carbadox - Méthode par chromatographie liquide à haute performance (ISO 14939:2001)

Futtermittel - Bestimmung des Carbadoxgehaltes - Hochleistungs-flüssigchromatographisches Verfahren (ISO 14939:2001)

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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 Management Centre has the same status as the official versions.

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

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Foreword

The text of the International Standard ISO 14939:2001 has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with 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of an identical text or by endorsement, at the latest by February 2002, and conflicting national standards shall be withdrawn at the latest by February 2002.

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

Endorsement notice

The text of the International Standard ISO 14939:2001 was approved by CEN as a European Standard without any modification.

INTERNATIONAL STANDARD

ISO 14939

First edition
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Animal feeding stuffs — Determination of carbadox content — Method using high-performance liquid chromatography

Aliments des animaux — Détermination de la teneur en carbadox — Méthode par chromatographie liquide à haute performance

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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 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.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 14939 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

Annexes A and B of this International Standard are for information only.

Animal feeding stuffs — Determination of carbadox content — Method using high-performance liquid chromatography

1 Scope

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

The method is applicable to animal feeding stuffs with a mass fraction of carbadox of 0,5 mg/kg (limit of quantification) to 100 mg/kg, and to premixtures with a mass fraction of carbadox up to 10 %.

The lower limit of detection is 0,1 mg/kg.

NOTE 1 For animal feeding stuffs the mass fraction of carbadox is expressed in milligrams per kilogram, and for premixtures as a percentage by mass.

NOTE 2 Carbadox is a chemotherapeuticum belonging to the quinoxaline group. Carbadox is used as a growth-promoting feed additive for piglets.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of IEC and ISO maintain registers of currently valid International Standards.

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

3 Principle

Carbadox is extracted from the sample with a mixture of acetonitrile and methanol. Animal feeds are prewetted with water. The extract of animal feeds is purified through a short aluminium oxide column. The extract of premixtures is directly diluted with a mixture of water, acetonitrile and methanol. The final extract is analysed by reverse-phase HPLC with UV detection at a wavelength of 365 nm (see references [1] to [3]).

The presence of dimetridazole, nitrofurazone or sulfadimidine sodium can interfere with the determination of carbadox.

Alternatively, carbadox may be determined after post-column derivatization with sodium hydroxide with detection at a wavelength of 420 nm.

4 Reagents

Use only reagents of recognized analytical grade.

4.1 Water, demineralized or deionized, with resistivity of at least 10 MΩ·cm, or water of at least equivalent purity.

4.2 Extraction solvent: mixture of acetonitrile and methanol (1:1 by volume).

Combine equal volumes of acetonitrile and methanol. Mix well and allow to adjust to room temperature before use.

4.3 Dilution solvent: mixture of extraction solvent (4.2) and water (4.1) (70:30 by volume).

Mix 70 ml of extraction solvent (4.2) with 30 ml of water (4.1).

4.4 Acetic acid, volume fraction, $w(CH_3CO_2H)$ = 10 %.

Dilute 10 ml of glacial acetic acid to 100 ml with water.

4.5 Sodium acetate solution, $c(C_2H_3NaO_2)$ = 0,01 mol/l, pH = 6,0.

Weigh 0,82 g of water-free sodium acetate into a 1 000 ml one-mark volumetric flask. Dissolve in 700 ml of water. Adjust the pH to pH = 6,0 with acetic acid (4.4). Dilute to the mark with water and mix.

4.6 Mobile phase for HPLC.

Combine 825 ml of sodium acetate solution (4.5) and 175 ml of acetonitrile and mix. Filter the eluent through a 0,22 µm filter using a solvent filtration system (5.2), and degas for 10 min in an ultrasonic bath (5.3) before use.

4.7 Carbadox standard material, 3-(2-quinoxalinyl methylene) carbazic acid methy ester *N*, *N′* -dioxide (CAS number 6804-07-5).

WARNING — Because of the sensitivity of carbadox to light, conduct all operations in the absence of daylight or artificial white light. Avoid inhalation of and exposure to the toxic carbadox standard material and solutions thereof. Work in a fume cupboard when handling the solvents and solutions. Wear safety glasses and protective clothing.

4.8 Carbadox stock solution (approximately 100 µg/ml).

Weigh 10 mg ± 1 mg of carbadox (4.7), to the nearest 0,1 mg, into a 100 ml one-mark volumetric flask. Dissolve in extraction solvent (4. 2), dilute to the mark and mix. Calculate the concentration taking into account the purity of the standard material. Prepare fresh every month. Store in the dark at 0 °C to 8 °C.

4.9 Carbadox working solutions (approximately 2 µg/ml and 10 µg/ml).

Pipette 1,0 ml and 5,0 ml of the carbadox stock solution (4.8) into separate 50 ml one-mark volumetric flasks. Dilute to the mark with dilution solvent (4.3) and mix. Prepare fresh for each series of samples.

4.10 Carbadox working solutions (approximately 0,4 µg/ml and 2 µg/ml).

Pipette 1,0 ml of the carbadox stock solution (4.8) into a 50 ml one-mark volumetric flask, dilute to the mark with mobile phase (4.6) and mix. Pipette 10 ml of this solution (2 µg/ml) into a 50 ml one-mark volumetric flask, dilute to the mark with mobile phase (4.6) and mix. Prepare fresh for each series of samples.

4.11 Dimetridazole standard material, 1,2-dimethyl-5-nitro-1*H*-imidazole (CAS number 551-92-8).

WARNING — Because of the sensitivity of dimetridazole to light, conduct all operations in the absence of daylight or artificial white light. Avoid inhalation of and exposure to the toxic dimetridazole standard

material and solutions thereof. Work in a fume cupboard when handling the solvents and solutions. Wear safety glasses and protective clothing.

4.12 Dimetridazole stock solution (approximately 100 µg/ml).

Weigh 10 mg ± 1 mg of dimetridazole (4.11), to the nearest 0,1 mg, into a 100 ml one-mark volumetric flask. Dilute to the mark with methanol and mix. Calculate the concentration taking into account the purity of the standard material. Prepare fresh every month. Store in the dark at 0 °C to 8 °C.

4.13 Dimetridazole working solution (approximately 20 µg/ml).

Pipette 2,0 ml of the dimetridazole stock solution (4.12) into a 10 ml one-mark volumetric flask. Dilute to the mark with water and mix. Prepare fresh for each series of samples.

4.14 Sulfadimidine standard material, sodium salt of 4-amino-*N*-(4,6-dimethyl-2-pyrimidinyl) benzene sulfonamide (CAS number 1981-58-4).

WARNING — Avoid inhalation of and exposure to the toxic sulfadimidine standard material and solutions thereof. Work in a fume cupboard when handling the solvents and solutions. Wear safety glasses and protective clothing.

4.15 Sulfadimidine stock solution (approximately 200 µg/ml).

Weigh 10 mg ± 1 mg of sulfadimidine standard material (4.14), to the nearest 0,1 mg, into a 50 ml one-mark volumetric flask. Dilute to the mark with methanol and mix. Calculate the concentration taking into account the purity of the standard material. Prepare fresh every month. Store in the dark at 0 °C to 8 °C.

4.16 Sulfadimidine working solution (approximately 20 µg/ml).

Pipette 1,0 ml of sulfadimidine stock solution (4.15) into a 10 ml one-mark volumetric flask. Dilute to the mark with water and mix. Prepare fresh for each series of samples.

4.17 Nitrofurazone standard material, 5-nitro-2-furaldehyde semicarbazone (CAS number 59-87-0).

WARNING — Because of the sensitivity of nitrofurazone to light, conduct all operations in the absence of daylight or artificial white light. Avoid inhalation of and exposure to the toxic nitrofurazone standard material and solutions thereof. Work in a fume cupboard when handling the solvents and solutions. Wear safety glasses and protective clothing.

4.18 Nitrofurazone stock solution (approximately 100 µg/ml).

Weigh 10 mg ± 1 mg of nitrofurazone (4.17), to the nearest 0,1 mg, into a 100 ml one-mark volumetric flask. Dilute to the mark with methanol and mix. Calculate the concentration taking into account the purity of the standard material. Prepare fresh every month. Store in the dark at 0 °C to 8 °C.

4.19 Nitrofurazone working solution (approximately 20 µg/ml).

Pipette 2,0 ml of nitrofurazone stock solution (4.18) into a 10 ml one-mark volumetric flask. Dilute to the mark with water and mix. Prepare fresh for each series of samples.

4.20 Neutral aluminium oxide, activity 1.

For total de-activation 0 % to 1 % of water is necessary.

4.21 Sodium hydroxide solution, c(NaOH) = 0,5 mol/l.

Weigh 20 g of sodium hydroxide into a 1 litre one-mark volumetric flask and dissolve in 10 ml of water. Dilute to the mark with water and mix.