
**Krma - Določanje vsebnosti aflatoksina B₁ (prevzet standard
ISO 6651:1987 z metodo platnice)**

Animal feeding stuffs - Determination of aflatoxin B₁ content

Aliments des animaux - Dosage de l'aflatoxine B₁

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SIST ISO 6651:1995

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UVOD

Standard SIST ISO 6651, Krma - Določanje vsebnosti aflatoksina B₁, prva izdaja, 1995, ima status slovenskega standarda in je z metodo platnice prevzet mednarodni standard ISO 6651, Animal feeding stuffs - Determination of aflatoxin B₁ content, second edition, 1987-08-15.

PREDGOVOR

Mednarodni standard ISO 6651:1987 je pripravil tehnični odbor Mednarodne organizacije za standardizacijo ISO/TC 34 Kmetijski pridelki in živilski proizvodi.

Odločitev za prevzem tega standarda po metodi platnice je sprejela delovna skupina WG 10 Analitika krme v okviru tehničnega odbora USM/TC KŽP Kmetijski pridelki in živilski proizvodi.

Ta slovenski standard je dne 1995-05-08 odobril direktor USM.

ZVEZA S STANDARDI

S prevzemom tega mednarodnega standarda veljajo naslednje zveze:

SIST ISO 6654:1995 (en)	Krma - Določanje vsebnosti sečnine
SIST ISO 6866:1995 (en)	Krma - Določanje vsebnosti prostega in skupnega gosipola
SIST ISO 6870:1995 (en)	Krma - Določanje vsebnosti zearalenona
SIST ISO 5498:1995 (en)	Kmetijski pridelki in živilski proizvodi - Določanje vsebnosti surove vlaknine - Splošna metoda
SIST ISO 5983:1995 (en)	Krma - Določanje vsebnosti dušika in izračun vsebnosti surovih beljakovin
SIST ISO 5984:1995 (en)	Krma - Določanje surovega pepela
SIST ISO 5985:1995 (en)	Krma - Določanje pepela, netopnega v klorovodikovi kislini
SIST ISO 6490-1:1995 (en)	Krma - Določanje vsebnosti kalcija - 1. del: Titrimetrična metoda
SIST ISO 6490-2:1995 (en)	Krma - Določanje vsebnosti kalcija - 2. del: Metoda atomske absorpcijske spektrometrije
SIST ISO 6491:1995 (en)	Krma - Določanje vsebnosti skupnega fosforja - Spektrofotometrična metoda
SIST ISO 6495:1995 (en)	Krma - Določanje vsebnosti v vodi topnih kloridov
SIST ISO 6496:1995 (en)	Krma - Določanje vsebnosti vlage
SIST ISO 5506:1995 (en)	Sojini proizvodi - Določanje ureazne aktivnosti
SIST ISO 6541:1995 (en)	Kmetijski pridelki in živilski proizvodi - Določanje vsebnosti surove vlaknine - Modificirana Scharrerjeva metoda

OSNOVA ZA IZDAJO STANDARDA

- Prevzem standarda ISO 6651:1987

OPOMBI

- Povsod, kjer se v besedilu standarda uporablja izraz mednarodni standard , to pomeni v SIST ISO 6651:1995 slovenski standard .
- Uvod in predgovor nista sestavni del standarda.

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INTERNATIONAL STANDARD

ISO
6651Second edition
1987-08-15

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION
ORGANISATION INTERNATIONALE DE NORMALISATION
МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ

Animal feeding stuffs — Determination of aflatoxin B₁ content*Aliments des animaux**Dosage de l'aflatoxine B₁***ITeH STANDARD PREVIEW**
(standards.iteh.ai)SIST ISO 6651:1995

<https://standards.iteh.ai/catalog/standards/sist/5ed16d25-4bb5-4713-8fd5-4eae2172c81f/sist-iso-6651-1995>

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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 6651 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

This second edition cancels and replaces the first edition (ISO 6651 : 1983), subclause 5.14.1 of which has been technically revised.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

Animal feeding stuffs — Determination of aflatoxin B₁ content

1 Scope

This International Standard specifies two methods for the determination of the aflatoxin B₁ content of animal feeding stuffs.

2 Field of application

2.1 Method A is applicable to the following simple feeding stuffs :

- oilseeds and oilseed residues, and in particular ground-nut, copra, linseed, soya, sesame, babassu palm;
- manioc meal;
- maize germ expeller;
- cereals and cereal products;
- pea meal;
- potato pulp and flour.

In the presence of substances interfering with the determination by method A, it is recommended that the determination be carried out in accordance with method B.

2.2 Method B is applicable to mixed feeding stuffs and to simple feeding stuffs not mentioned in 2.1.

This method is not applicable to feeding stuffs containing citrus pulp.

2.3 The lower limit of detection of aflatoxin B₁ is 0,01 mg/kg.

3 Reference

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

4 Principle

Extraction of the test portion with chloroform, filtration, and purification of an aliquot portion on a silica gel column.

Evaporation of the eluate and dissolution of the residue in a specified volume of chloroform or mixture of benzene and acetonitrile.

Thin-layer chromatography, one-dimensional for method A and two-dimensional for method B, of an aliquot portion of this solution.

Determination of the aflatoxin B₁ content, either visually or by fluorodensitometry, by examination of the chromatogram under ultraviolet light and comparison with known quantities of standard aflatoxin B₁ applied to the same plate as the test portion extract.

Confirmation of the identity of aflatoxin B₁ by formation of the hemiacetal derivative.

5 Reagents

All reagents shall be of recognized analytical quality. The water used shall be distilled water or water of at least equivalent purity.

5.1 Chloroform, stabilized with 0,5 to 1,0 % of 96 % (V/V) ethanol.

5.2 *n*-Hexane.

5.3 Diethyl ether, anhydrous, free from peroxides.

5.4 Benzene/acetonitrile, (98 + 2) mixture.

Mix 98 volumes of benzene with 2 volumes of acetonitrile.

5.5 Chloroform/methanol, (97 + 3) mixture.

Mix 97 volumes of chloroform with 3 volumes of methanol.

5.6 Developing solvents.¹⁾

5.6.1 Chloroform/acetone, (90 + 10) mixture.

Mix 90 volumes of chloroform with 10 volumes of acetone, in an unsaturated tank.

¹⁾ The solvents should be used in covered tanks. When saturated tanks are specified, this is achieved by lining the tanks with absorbent paper and allowing the interiors to become saturated with solvent vapour.

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5.6.2 Diethyl ether/methanol/water, (96 + 3 + 1) mixture.

Mix 96 volumes of diethyl ether, 3 volumes of methanol and 1 volume of water, in an unsaturated tank.

5.6.3 Diethyl ether/methanol/water, (94 + 4,5 + 1,5) mixture.

Mix 94 volumes of diethyl ether with 4,5 volumes of methanol and 1,5 volumes of water, in a saturated tank.

5.6.4 Chloroform/methanol, (94 + 6) mixture.

Mix 94 volumes of chloroform with 6 volumes of methanol, in a saturated tank.

5.6.5 Chloroform/methanol, (97 + 3) mixture.

Mix 97 volumes of chloroform with 3 volumes of methanol, in a saturated tank.

5.7 Silica gel, for column chromatography, of particle size 0,05 to 0,20 mm.**5.8 Silica gel**, G-HR or equivalent, for thin-layer chromatography.**5.9 Diatomaceous earth** (Hyflosupercel), acid-washed.**5.10 Sodium sulfate**, anhydrous granules.**5.11 Trifluoroacetic acid**.**5.12 Inert gas**, for example nitrogen.**5.13 Sulfuric acid**, 50 % (V/V) solution.**5.14 Aflatoxin B₁**, standard solution containing about 0,1 µg of aflatoxin B₁ per millilitre, in the chloroform (5.1) or in the benzene/acetonitrile mixture (5.4).

WARNING — Aflatoxins are highly carcinogenic and must be handled with great care.

Prepare and check the solution as follows.

5.14.1 Preparation of stock solution and determination of concentration

Prepare a solution of aflatoxin B₁ in the chloroform (5.1) or the benzene/acetonitrile mixture (5.4) such that the concentration is between 8 and 10 µg/ml. Determine the absorption spectrum between 330 and 370 nm by means of the spectrophotometer (6.9).

Measure the absorbance (A) at 363 nm in the case of the chloroform solution, or at 348 nm in the case of the benzene/acetonitrile mixture solution.

Calculate the concentration of aflatoxin B₁, in micrograms per millilitre of solution, from the formulae :

a) for the chloroform solution :

$$\frac{312 \times A \times 1\,000}{22\,300}$$

b) for the solution in the benzene/acetonitrile mixture :

$$\frac{312 \times A \times 1\,000}{19\,800}$$

5.14.2 Dilution

Dilute the stock solution (5.14.1), as appropriate, away from daylight, to obtain a standard solution with a concentration of aflatoxin B₁ of about 0,1 µg/ml.

If kept in a refrigerator at 4 °C, this solution is stable for 2 weeks.

5.14.3 Testing of chromatographic purity of the solution

Onto a plate (6.7), apply a spot of 5 µl of the standard aflatoxin B₁ solution of concentration 8 to 10 µg/ml (5.14.1). Develop the chromatogram as indicated in 8.5.1. Under ultraviolet light, the chromatogram shall show only one spot and no fluorescence shall be perceptible in the original deposition zone.

5.15 Aflatoxin B₁ and B₂ (see the warning in 5.14), solutions for qualitative testing, containing about 0,1 µg of aflatoxin B₁ and B₂ per millilitre, in the chloroform (5.1) or in the benzene/acetonitrile mixture (5.4).

These concentrations are given as a guide. They shall be adjusted so as to obtain the same intensity of fluorescence for both aflatoxins (see 8.5.1).

6 Apparatus

Usual laboratory equipment, and in particular :

6.1 Grinder/mixer.**6.2 Sieve**, of aperture size 1,0 mm.¹⁾**6.3 Shaking apparatus or magnetic stirrer**.

1) See ISO 565, *Test sieves — Woven metal wire cloth, perforated plate and electroformed sheet — Nominal sizes of openings*.

6.4 Chromatographic tubes, made of glass (internal diameter 22 mm, length 300 mm), with a polytetrafluoroethylene tap and a 250 ml reservoir, plugged at the bottom end with cotton or glass wool.

6.5 Rotary vacuum evaporator, with a 500 ml round-bottomed flask.

6.6 Apparatus for thin-layer chromatography (TLC), i.e. that necessary for the preparation of the plates (6.7) and application of spots (capillary pipettes or microsyringes), a developing tank, and spraying apparatus for applying the sulfuric acid (5.13) to the plates.

6.7 Glass TLC plates, 200 mm × 200 mm, prepared as follows (the quantities indicated are sufficient to cover five plates).

Place 30 g of the silica gel (5.8) in a conical flask, add 60 ml of water, stopper and shake for 1 min. Spread the suspension on the plates so as to obtain a uniform layer 0,25 mm thick. Leave to dry in the air and then store in a desiccator containing silica gel. At the time of use, activate the plates by keeping them in an oven at 110 °C for 1 h.

Ready-to-use plates are suitable if they give results similar to those obtained with the plates prepared as indicated above.

6.8 Long-wavelength (360 nm) ultraviolet lamp.

The intensity of irradiation shall make it possible for a spot of 1,0 ng of aflatoxin B₁ to be clearly distinguished on a TLC plate at a distance of 10 cm from the lamp.

WARNING — Ultraviolet light is dangerous to the eyes. Protective goggles shall be worn.

6.9 Spectrophotometer, suitable for making measurements in the ultraviolet region of the spectrum.

6.10 Fluorodensitometer (optional).

6.11 Fluted filter paper.

6.12 Graduated tube, of capacity 10,0 ml, with a polyethylene stopper.

6.13 Conical flask, of capacity 500 ml, with a ground glass stopper.

6.14 Pipette, of capacity 50 ml.

6.15 Balance.

7 Sampling

Take the laboratory sample from the material to be sampled in accordance with the International Standard for the material concerned unless sampling for the determination of aflatoxin is excluded from its field of application. If no appropriate Inter-

national Standard exists, agreement shall be reached between the parties concerned, taking into account the characteristics of the material being sampled.

8 Procedure

8.1 Preparation of the test sample

8.1.1 If the sample contains more than 5 % of fat, it shall be defatted with light petroleum before grinding.

In such cases, the analytical results shall be expressed in terms of the mass of the non-defatted sample.

8.1.2 Grind the laboratory sample so that it completely passes through the sieve (6.2). Mix thoroughly. (See ISO 6498.)

8.2 Test portion

Weigh, to the nearest 0,01 g, 50 g of the prepared test sample into the conical flask (6.13).

8.3 Extraction

Add to the test portion (8.2) 25 g of the diatomaceous earth (5.9), 25 ml of water, and 250 ml of the chloroform (5.1) accurately measured from a measuring cylinder. Stopper the flask, and shake or stir for 30 min using the apparatus (6.3). Filter through the fluted filter paper (6.11), taking care to discard the first 10 ml of the filtrate, and subsequently collect at least 50 ml of the filtrate.

8.4 Column clean-up

8.4.1 Preparation of the column

Fill two-thirds of the chromatographic tube (6.4) with the chloroform (5.1) and add 5 g of the sodium sulfate (5.10). Check that the upper surface of the sodium sulfate layer is flat, then add 10 g, in small portions, of the silica gel (5.7). Stir carefully after each addition to eliminate air bubbles. Leave to stand for 15 min and then carefully add 10 g of the sodium sulfate (5.10). Open the tap and allow the liquid to flow until it is just above the upper surface of the sodium sulfate layer. Close the tap.

8.4.2 Purification

Transfer, by means of the pipette (6.14), 50 ml of the filtrate collected in 8.3 to a 250 ml conical flask, and add 100 ml of the *n*-hexane (5.2). Mix and quantitatively transfer the mixture to the column, rinsing the flask with the *n*-hexane. Open the tap and allow the liquid to flow at a rate of 8 to 12 ml/min until it is level with the upper surface of the sodium sulfate layer. Close the tap. Discard the liquid collected and pour 100 ml of the diethyl ether (5.3) into the column. Again open the tap and allow the liquid to flow until it is level with the upper surface of the sodium sulfate layer. During these operations, ensure that the column does not run dry.