SLOVENSKI STANDARD
SIST ISO 6651:2003

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Krma - Semi-kvantitativno določ evanje aflatoksina B1 - Tankoplastne kromatografske metode

Animal feeding stuffs -- Semi-quantitative determination of aflatoxin B1 -- Thin-layer chromatographic methods

Aliments des animaux -- Dosage semi-quantitatif de l'aflatoxine B1 -- Méthodes par chromatographie sur couche mince

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Animal feeding stuffs — Semi-quantitative determination of aflatoxin B_1 — Thin-layer chromatographic methods

Aliments des animaux — Dosage semi-quantitatif de l'aflatoxine B_1 — Méthodes par chromatographie sur couche mince

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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 6651 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

This third edition cancels and replaces the second edition (ISO 6651:1987), of which it constitutes a minor revision.

Annex A of this International Standard is for information only.

Animal feeding stuffs — Semi-quantitative determination of aflatoxin B_1 — Thin-layer chromatographic methods

1 Scope

1.1 This International Standard specifies two methods for the determination of aflatoxin B_1 in animal feeding stuffs. These methods can only be used for semi-quantitative determinations.

1.2 **Method A** is applicable to the following simple feeding stuffs:

— oilseeds and oilseed residues, and in particular groundnut, copra, linseed, soya, 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.

1.3 **Method B** is applicable to mixed feeding stuffs and to simple feeding stuffs not mentioned in 1.2.

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

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 edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

3 Principle

A test portion is extracted with chloroform then filtration. An aliquot portion is purified on a silica gel column.

The eluate is evaporated and the residue is dissolved in a specified volume of chloroform or a mixture of benzene and acetonitrile.

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

The aflatoxin B_1 content is determined either visually or by fluorodensitometry, by examination of the chromatogram under ultraviolet light and comparison with known quantities of standard aflatoxin B_1 applied to the same plate as the test portion extract.

The identify of aflatoxin B_1 is confirmed by formation of the hemiacetal derivative.

4 Reagents

Use only reagents of recognized analytical quality, and distilled or deonized water or water of at least equivalent purity.

4.1 Chloroform, stabilized with 0,5 % to 1,0 % of 96 % (volume fraction) ethanol.

4.2 *n*-Hexane.

4.3 Diethyl ether, anhydrous, free from peroxides.

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

Mix 98 volumes of benzene with 2 volumes of acetonitrile.

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

Mix 97 volumes of chloroform with 3 volumes of methanol.

4.6 Developing solvents.

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.

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

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

4.6.2 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.

4.6.3 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.

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

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

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

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

4.7 Silica gel, for column chromatography, of particle size 0,05 mm to 0,20 mm.

4.8 Silica gel, G-HR or equivalent, for thin-layer chromatography.

4.9 Diatomaceous earth (Hyflosupercel), acid-washed.

4.10 Sodium sulfate, anhydrous granules.

4.11 Trifluoroacetic acid.

4.12 Inert gas, for example nitrogen.

4.13 Sulfuric acid, 50 % solution (volume fraction).

4.14 Aflatoxin B_1, standard solution containing about 0,1 μg of aflatoxin B_1 per millilitre, in the chloroform (4.1) or in the benzene/acetonitrile mixture (4.4).

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

Prepare and check the solution as follows.

4.14.1 Preparation of stock solution and determination of concentration

Prepare a solution of aflatoxin B_1 in the chloroform (4.1) or the benzene/acetonitrile mixture (4.4) such that the concentration is between 8 μg/ml and 10 μg/ml. Determine the absorption spectrum between 330 nm and 370 nm by means of the spectrometer (5.9).

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

Calculate the concentration of aflatoxin B_1, 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

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

4.14.2 Dilution

Dilute the stock solution (4.14.1), as appropriate, away from daylight, to obtain a standard solution with a concentration of aflatoxin B_1 of about 0,1 μg/ml.

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

4.14.3 Testing of chromatographic purity of the solution

Onto a plate (5.7), apply a spot of 5 μl of the standard aflatoxin B_1 solution of concentration 8 μg/ml to 10 μg/ml (4.14.1). Develop the chromatogram as indicated in 7.5.1. Under ultraviolet light, the chromatogram shall show only one spot and no fluorescence shall be perceptible in the original deposition zone.

4.15 Aflatoxin B_1 and B_2 (see the warning in 4.14), solutions for qualitative testing, containing about 0,1 μg of aflatoxin B_1 and B_2 per millilitre, in the chloroform (4.1) or in the benzene/acetonitrile mixture (4.4).

These concentrations are given as a guide. They shall be adjusted so as to obtain the same intensity of fluorescence for both aflatoxins (see 7.5.1).

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Grinder/mixer.