SIST

SLOVENSKI STANDARD
SIST EN 15851:2010

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Živila - Določevanje aflatoksina B1 v žitnih kašicah za dojenčke in majhne otroke - Metoda HPLC z imunoafinitetnim kolonskim čiščenjem in fluorescenčno detekcijo

Foodstuffs - Determination of aflatoxin B1 in cereal based foods for infants and young children - HPLC method with immunoaffinity column cleanup and fluorescence detection

Lebensmittel - Bestimmung von Aflatoxin B1 in Säuglings- und Kleinkindernahrung auf Getreidebasis - HPLC-Verfahren mit Reinigung an einer Immunoaffinitätssäule und Fluoreszenzdetektion

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Produits alimentaires - Dosage de l'aflatoxine B1 dans les produits pour nourrissons et jeunes enfants à base de céréales - Méthode de chromatographie liquide haute performance avec purification sur colonne d'immunoaffinité et détection par fluorescence

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ICS:

67.060	Žita, stročnice in proizvodi iz njih	Cereals, pulses and derived products
67.230	Predpakirana in pripravljena hrana	Prepackaged and prepared foods

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EUROPEAN STANDARD

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Foodstuffs - Determination of aflatoxin B_1 in cereal based foods for infants and young children - HPLC method with immunoaffinity column cleanup and fluorescence detection

Produits alimentaires - Dosage de l'aflatoxine B_1 dans les produits pour nourrissons et jeunes enfants à base de céréales - Méthode de chromatographie liquide haute performance avec purification sur colonne d'immunoaffinité et détection par fluorescence

Lebensmittel - Bestimmung von Aflatoxin B_1 in Säuglings- und Kleinkindernahrung auf Getreidebasis - HPLC-Verfahren mit Reinigung an einer Immunoaffinitätssäule und Fluoreszenzdetektion

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Foreword

This document (EN 15851:2010) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by October 2010, and conflicting national standards shall be withdrawn at the latest by October 2010.

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1 Scope

This European Standard specifies a method for the determination of aflatoxin B_1 in baby food by high performance liquid chromatography (HPLC) with immunoaffinity cleanup and fluorescence detection. This method has been validated in an interlaboratory study via the analysis of both naturally contaminated and spiked samples ranging from 0,07 µg/kg to 0,18 µg/kg.

For further information on the validation, see Clause 9 and Annex B.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 3696:1995, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

3 Principle

A test portion is extracted with a mixture of methanol and water. The extract is filtered, diluted with phosphate buffered saline (PBS) to a specified solvent concentration, and applied to an immunoaffinity column containing antibodies specific to aflatoxin B_1. Aflatoxin B_1 is purified and concentrated on the column and removed from the antibodies using methanol as eluent. Aflatoxin B_1 is quantified by reverse-phase high performance liquid chromatography (RP-HPLC) with post column derivatization (PCD) involving bromination followed by fluorescence detection.

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The post column derivatization is achieved with either electrochemically generated bromine or with pyridinium hydrobromide perbromide (PBPB).

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4 Reagents

4.1 General

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696:1995, unless otherwise specified. Solvents shall be of quality for HPLC analysis, unless otherwise specified. Commercially available solutions with equivalent properties to those listed may be used.

WARNING — Dispose of waste solvents according to applicable environmental rules and regulations. Decontamination procedures for laboratory wastes have been reported by the International Agency for Research on Cancer (IARC), see [4].

4.2 Helium purified compressed gas.

4.3 Nitrogen.

4.4 Disodium hydrogen phosphate, Na_2HPO_4 anhydrous or $Na_2HPO_4 \cdot 12\ H_2O$.

4.5 Potassium bromide.

4.6 Potassium chloride.

4.7 Potassium dihydrogen phosphate, KH_2PO_4.

4.8 Sodium chloride.

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4.9 Sodium hydroxide.

4.10 Hydrochloric acid solution, mass fraction *w*(HCl) = 37 % in water.

4.11 Hydrochloric acid solution, substance concentration *c*(HCl) = 0,1 mol/l.

Dilute 8,28 ml of hydrochloric acid solution (4.10) to 1 l with water.

4.12 Sodium hydroxide solution, *c*(NaOH) = 0,1 mol/l.

Dissolve 4 g of sodium hydroxide (4.9) in 1 l of water.

4.13 Phosphate buffered saline (PBS) solution, *c*(NaCl) = 120 mmol/l, *c*(KCl) = 2,7 mmol/l, *c*(phosphate buffer) = 10 mmol/l, pH = 7,4.

Dissolve 8,0 g of sodium chloride (4.8), 1,2 g of anhydrous disodium hydrogen phosphate or 2,9 g of $Na_2HPO_4 \cdot 12\ H_2O$ (4.2), 0,2 g of potassium dihydrogen phosphate (4.7) and 0,2 g of potassium chloride (4.6) in 900 ml of water. After dissolution, adjust the pH to 7,4 with hydrochloric acid solution (4.11) or sodium hydroxide solution (4.12) as appropriate, then dilute to 1 l with water.

Alternatively, a PBS solution with equivalent properties can be prepared from commercially available PBS material.

4.14 Pyridinium hydrobromide perbromide (PBPB), [CAS: 39416-48-3].

4.15 Acetonitrile.

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WARNING — Acetonitrile is hazardous and samples shall be blended using an explosion proof blender which is housed within a fume cupboard. After blending, samples shall be filtered inside a fume cupboard.

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4.16 Methanol, HPLC grade.

4.17 Methanol, technical grade.

4.18 Toluene.

4.19 Extraction solvent.

Mix eight parts per volume of methanol (4.17) with two parts per volume of water.

4.20 Nitric acid, *c*(HNO_3) = 4 mol/l.

4.21 HPLC mobile phase A, for use with PBPB.

Mix six parts per volume of water with two parts per volume of acetonitrile (4.15) and three parts per volume of methanol (4.16). Degas mobile phase A with for example helium (4.2).

4.22 HPLC mobile phase B, for use with electrochemically generated bromine.

Mix six parts per volume of water with two parts per volume of acetonitrile (4.15) and three parts per volume of methanol (4.16). Add 120 mg of potassium bromide (4.5) and 350 µl of nitric acid (4.20) per litre of mobile phase. Degas mobile phase B with for example helium (4.2).

4.23 Post-column reagent.

Dissolve 50 mg of PBPB (4.14) in 1 l of water. To be used with mobile phase solvent A (4.21). The solution may be used up to four days if stored in a dark place at room temperature.

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4.24 Mixture of toluene and acetonitrile.

Mix nine parts per volume of toluene (4.18) with one part per volume of acetonitrile (4.15).

4.25 Immunoaffinity column.

The immunoaffinity column shall contain antibodies raised against aflatoxin B_1. The column shall have a capacity of not less than 100 ng of aflatoxin B_1 and shall give a recovery of not less than 80 % when 5 ng of aflatoxin B_1 are applied as a standard solution in a mixture of ten parts per volume of methanol and 90 parts per volume of water.

4.26 Aflatoxin B_1, in crystal form or as a film in ampoules or in form of commercially available aflatoxin B_1 solution.

WARNING — Aflatoxins are subject to light degradation. Protect the laboratory, where the analyses are done, adequately from daylight. This can be achieved effectively by using ultraviolet (UV) absorbing foil on the windows in combination with subdued light (no direct sunlight) or curtains or blinds in combination with artificial light (fluorescent tubes are acceptable).

Protect aflatoxin containing solutions from light as much as possible (keep in the dark, use aluminium foil or amber-coloured glassware).

4.27 Aflatoxin B_1 stock solution, $c \approx 10$ µg/ml.

Prepare a solution of aflatoxin B_1 in the mixture of toluene and acetonitrile (4.24) to give a solution with a mass concentration of approximately 10 µg/ml.

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To determine the exact mass concentration, record the absorption curve between 330 nm and 370 nm in 1 cm quartz cells in a spectrometer (5.14) with the mixture of toluene and acetonitrile (4.24) as reference. Identify the wavelength for maximum absorption (between 330 nm and 370 nm). Calculate the mass concentration of aflatoxin B_1, ρ_{afl}, in µg/ml, using Equation (1):

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$$\rho_{afl} = \frac{A_{max} \times M \times 100}{\varepsilon \times b} \quad (1)$$

where

A_{max} is the absorption determined at the maximum of the absorption curve (between 330 nm and 370 nm);

M is the molar mass, in g/mol, of aflatoxin B_1 (M = 312 g/mol);

ε is the molar absorption coefficient, in square metres per mole, of aflatoxin B_1 in the mixture of toluene and acetonitrile (4.24) (1 930 m^2/mol, see [5]);

b is the optical path length, in centimetres, of the quartz cell.

Store this solution in a freezer at approximately - 18 °C. Allow to reach room temperature before opening. A solution stored in this way is usually stable for 12 months. Confirm the concentration of the solution if it is older than 12 months.

4.28 Aflatoxin B_1 standard solution, ρ = 5,00 ng/ml.

Pipette a volume of aflatoxin B_1 stock solution (4.27) containing exactly 1,00 µg aflatoxin B_1 into a 200 ml calibrated volumetric flask and dilute to the mark with the mixture of toluene and acetonitrile (4.24). This solution contains 5,00 ng/ml aflatoxin B_1.

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Wrap the flask tightly in aluminium foil and store it at less than 4 °C. Before use, do not remove the aluminium foil until the contents have reached room temperature to avoid incorporation of water by condensation. A solution stored in this way is stable for at least four weeks.

4.29 Aflatoxin B_1 spiking solution, ρ = 2 µg/ml.

Pipette a volume of aflatoxin B_1 stock solution (4.27) containing exactly 20 µg aflatoxin B_1 into a 10 ml calibrated volumetric flask. Evaporate the mixture of toluene and acetonitrile solution just to dryness under a stream of nitrogen at room temperature. Make up to volume with methanol (4.16) and shake well. The concentration of this spiking solution is 2 µg/ml for aflatoxin B_1.

Wrap the flask tightly in aluminium foil and store it at less than 4 °C. Before use, do not remove the aluminium foil until the contents have reached room temperature to avoid incorporation of water by condensation. A solution stored in this way is stable for at least three months.

5 Apparatus

WARNING — All glassware coming into contact with aqueous solutions of aflatoxins shall be washed with acid solution before use. Many laboratory washing machines do this as part of the washing programme. Otherwise soak laboratory glassware coming into contact with aqueous solutions of aflatoxins in sulfuric acid (*c* = 2 mol/l) for several hours (e.g. 15 h overnight), then rinse well (e.g. at least three times) with water to remove all traces of acid. Check the absence of acid with pH paper.

This treatment is necessary, because the use of non-acid washed glassware can cause losses of aflatoxins. In practice, the treatment is necessary for round bottomed flasks, volumetric flasks, measuring cylinders, vials or tubes used for calibration solutions and final extracts (particularly autosampler vials), and Pasteur pipettes, if these are used to transfer calibration solutions or extracts.

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Usual laboratory glassware and equipment and, in particular, the following.

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5.1 Analytical balance, capable of weighing to 0,000 1 g.

5.2 Laboratory balance, capable of weighing to 0,01 g.

5.3 Adjustable vertical or horizontal shaker.

5.4 Filter paper, e.g. 24 cm diameter, prefolded.

5.5 Conical flask, with screw top or glass stopper of 500 ml capacity.

5.6 Glass microfibre filter, retention size 1,6 µm or smaller.

5.7 Reservoir, of 75 ml capacity with luer tip connector and attachments for immunoaffinity column (IAC).

5.8 Hand pump, 20 ml syringe with luer lock or rubber stopper for IAC.

5.9 Volumetric flasks, of 5 ml, 10 ml, 20 ml, 150 ml, and 200 ml capacity with an accuracy of at least 0,5 %.

5.10 Disposable syringe filter unit, with pore size of 0,45 µm.

Prior to usage, verify that no aflatoxin losses occur during filtration (recovery testing).

NOTE There is a possibility that various filter materials retain aflatoxins.

5.11 Volumetric pipettes, with 2 ml and 10 ml capacity.

5.12 Calibrated microlitre syringe(s) or microlitre pipette(s), with 25 µl to 500 µl capacity.