SLOVENSKI STANDARD
SIST EN 15829:2010

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Živila - Določevanje ohratoksina A v korintah, rozinah, sultaninah, mešanem sušenem sadju in sušenih figah - Metoda HPLC s čiščenjem z imunoafinitetno kolono in fluorescenčno detekcijo

Foodstuffs - Determination of ochratoxin A in currants, raisins, sultanas, mixed dried fruit and dried figs - HPLC method with immunoaffinity column cleanup and fluorescence detection

Lebensmittel - Bestimmung von Ochratoxin A in Johannisbeeren, Rosinen, Sultaninen, gemischten Trockenfrüchten und getrockneten Feigen - HPLC-Verfahren mit Reinigung an einer Immunoaffinitätssäule und Fluoreszenzdetektion

Produits alimentaires - Dosage de l'ochratoxine A dans les raisins de Corinthe, les raisins secs, les raisins secs de Smyrne, les mélanges de fruits secs et les figues sèches - Méthode CLHP avec purification sur colonne d'immuno-affinité et détection par fluorescence

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Foreword

This document (EN 15829:2010) 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of an identical text or by endorsement, at the latest by August 2010, and conflicting national standards shall be withdrawn at the latest by August 2010.

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1 Scope

This European Standard specifies a method for the determination of ochratoxin A in currants, raisins, sultanas, mixed dried fruit and dried figs 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 1,1 µg/kg to 11 µg/kg.

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

WARNING — The use of this standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

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 phosphoric acid. The extract is filtered, diluted with phosphate buffered saline, and applied to an immunoaffinity column containing antibodies specific for ochratoxin A. The ochratoxin A is isolated, purified and concentrated on the column then released with elution solvent. Ochratoxin A is quantified by reverse-phase high performance liquid chromatography (HPLC) with fluorescence detection.

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. 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 [1].

4.2 Helium purified compressed gas

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

4.4 Potassium chloride

4.5 Potassium dihydrogen phosphate

4.6 Sodium chloride

4.7 Sodium hydroxide

4.8 Ammonium hydroxide solution, substance concentration $c(NH_4OH)$ = 1,1 mol/l, for post-column pH shift

Prepare fresh when required (optional, see 7.2).

4.9 Hydrochloric acid solution, mass fraction $w(HCl)$ = 37 % in water

4.10 Phosphoric acid solution, $c(H_3PO_4)$ = 0,1 mol/l

4.11 Hydrochloric acid solution, $c(HCl)$ = 0,1 mol/l

Dilute 8,28 ml of hydrochloric acid solution (4.9) to 1 l with water.

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

Dissolve 4 g of sodium hydroxide (4.7) in 1 l of water.

4.13 Phosphate buffered saline (PBS) solution

Dissolve 8,0 g of sodium chloride (4.6), 1,2 g of anhydrous disodium hydrogen phosphate or 2,9 g of $Na_2HPO_4 \cdot 12\ H_2O$ (4.3), 0,2 g of potassium dihydrogen phosphate (4.5) and 0,2 g of potassium chloride (4.4) 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 Acetonitrile

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.

4.15 Glacial acetic acid, $w(CH_3COOH) \geq 98$ %

4.16 Methanol

4.17 Toluene

4.18 Injection solvent

Mix 80 parts per volume of water with 20 parts per volume of acetonitrile (4.14) and two parts per volume of acetic acid (4.15).

4.19 HPLC mobile phase

Mix 99 parts per volume of water with 99 parts per volume of acetonitrile (4.14) and two parts per volume of glacial acetic acid (4.15). Degas the mobile phase solvent with for example helium (4.2).

4.20 Mixture of toluene and glacial acetic acid

Mix 99 parts per volume of toluene (4.17) with one part per volume of glacial acetic acid (4.15).

4.21 Immunoaffinity column

The immunoaffinity column contains antibodies raised against ochratoxin A. The column shall have a capacity of not less than 100 ng of ochratoxin A and shall give a recovery of not less than 70 % when 5 ng of ochratoxin A is applied in a solution of five parts per volume of acetonitrile (4.14) and 95 parts per volume phosphate buffered saline (4.13).

4.22 Surface silanising fluid (optional)

Mix one part per volume of the surface silanising fluid with 19 parts per volume of toluene (4.17).

4.23 Ochratoxin A, in crystal form or as a film in ampoules

4.24 Ochratoxin A stock solution

WARNING — Ochratoxin A is a potent nephrotoxin with immunotoxic, teratogenic and potential genotoxic properties. The International Agency for Research on Cancer (IARC) has classified ochratoxin A as a possible human carcinogen (group 2B). Protective clothing, gloves and safety glasses should be worn at all times, and all standard and sample preparation stages should be carried out in a fume cupboard.

Dissolve 1 mg of the ochratoxin A or the contents of 1 ampoule (if ochratoxin A has been obtained as a film) in solvent mixture (4.20) to give a solution containing approximately 20 µg/ml to 30 µg/ml of ochratoxin A.

To determine the exact concentration, record the absorption curve between a wavelength of 300 nm and 370 nm in a 1 cm quartz cell with solvent mixture (4.20) as reference using the spectrometer (5.12). Identify the wavelength for maximum absorption. Calculate the mass concentration of ochratoxin A, ρ_{ota}, in micrograms per millilitre using Equation (1):

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

where

A_{max} is the absorption determined at the maximum of the absorption curve (here: at 333 nm);

M is the molar mass, in grams per mole, of ochratoxin A (M = 403,8 g/mol);

ε is the molar absorption coefficient, in square metres per mole, of ochratoxin A in the solvent mixture (4.20) (here: 544 m^2/mol, see [2]);

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 six months.

4.25 Ochratoxin A spiking solution

Transfer an aliquot of the stock solution (4.24) containing 12,5 µg of ochratoxin A to a 5 ml volumetric flask. Evaporate to dryness under nitrogen at no more than 50 °C. Redissolve immediately in methanol (4.16) and make up to volume. This solution contains 2,5 µg/ml ochratoxin A.

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 six months.

4.26 Ochratoxin A standard solution

Transfer 500 µl of the ochratoxin A spiking solution (4.25) to a 5 ml volumetric flask, make up to volume with methanol (4.16). This solution contains 0,25 µg/ml ochratoxin A.

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 six months.

5 Apparatus

5.1 General

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

5.2 Silanised glass vials (optional)

Prepare the vials by filling them with the silanising reagent (4.22) and leave this reagent in the vial for 1 min. Rinse the vial first with a solvent of low polarity, for example toluene (4.17) then with methanol (4.16) and dry before use.

WARNING — The use of silanised glassware may prevent ochratoxin A binding to glass during evaporation.

5.3 High speed blender or homogenizer

5.4 Analytical balance, capable of weighing to 0,000 1 g

5.5 Laboratory balance, capable of weighing to 0,1 g

5.6 Displacement pipettes, adjustable, of 10 ml, 5 ml, 1 ml and 200 µl capacity with appropriate pipette tips

5.7 Vacuum manifold, to accommodate immunoaffinity columns

5.8 Reservoirs and attachments, to fit to immunoaffinity columns

5.9 Vacuum pump, capable of pulling a vacuum of 1 kPa and pumping 18 l/min

5.10 Filter paper, with pore size of 20 µm to 25 µm

5.11 HPLC apparatus, comprising the following:

5.11.1 Injection system, capable of injecting e.g. 100 µl

5.11.2 Mobile phase pump, isocratic, pulse free, capable of maintaining a volume flow rate of 1 ml/min

5.11.3 Column oven (optional), capable of maintaining a constant temperature above any variability caused by fluctuations in the room temperature (e.g. (45 ± 1) °C, ± 0,5 °C temperature repeatability and stability).

5.11.4 Analytical reverse-phase HPLC separating column, for example C_{18} octadecylsilane (ODS), length of 25 cm, inner diameter of 4,6 mm and a particle size of 5 µm, which ensures resolution of ochratoxin A from