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Utgåva 1

Foodstuffs – Determination of ochratoxin A in barley and roasted coffee – HPLC method with immunoaffinity column clean-up

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EUROPEAN STANDARD

NORME EUROPÉENNE

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EN 14132

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ICS 67.140.20

English version

Foodstuffs - Determination of ochratoxin A in barley and roasted coffee - HPLC method with immunoaffinity column clean-up

Produits alimentaires - Dosage de l'ochratoxine A présente dans l'orge et dans le café torréfié - Méthode par CLHP et par purification en colonne d'immunoaffinité

Lebensmittel - Bestimmung von Ochratoxin A in Gerste und Röstkaffee - HPLC-Verfahren mit Reinigung an einer Immunoaffinitätssäule

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Foreword

This document (EN 14132:2003) 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of an identical text or by endorsement, at the latest by November 2003, and conflicting national standards shall be withdrawn at the latest by November 2003.

Annex A is informative.

WARNING — Ochratoxin A is a potent nephrotoxin and liver toxin and has been reported to have immunosuppressant properties. It is classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (Group 2B). Acetonitrile is hazardous. Toluene is highly flammable and harmful. Observe appropriate safety precautions for handling such compounds.

Gloves and safety glasses shall be worn at all times and all standard and sample preparation stages shall be carried out in a fume cupboard. Operation outside the fume cupboard, such us measurement of standards by UV spectrophotometer, shall be performed with the standard in closed containers.

Decontamination procedures for laboratory wastes have been reported by the International Agency for Research on Cancer (IARC), see [1].

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, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and the United Kingdom.

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1 Scope

This European Standard specifies a method for the determination of ochratoxin A content in barley and roasted coffee using immunoaffinity column clean up and high performance liquid chromatography (HPLC). This method has been validated for ochratoxin A contents in barley in the range from 0,1 µg/kg up to 4,5 µg/kg and for roasted coffee in the range from 0,2 µg/kg up to 5,5 µg/kg.

2 Normative reference

This European Standard incorporates by dated or undated reference, provision from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

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

3 Principle

Ochratoxin A is extracted from barley by blending with aqueous acetonitrile. The extract is purified by passing through an immunoaffinity column. Ochratoxin A is extracted from ground roasted coffee by blending with methanol and sodium hydrogen carbonate. The extract is cleaned up by passing first through a phenyl silane column and then through an immunoaffinity column. Ochratoxin A is separated by reverse-phase HPLC and determined by fluorescence.

4 Reagents

4.1 General

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of grade 1 as defined in EN ISO 3696. Solvents shall be of quality for HPLC analysis.

Commercially available reagents with equivalent properties to the ones listed may be used.

4.2 Sodium chloride

4.3 Disodium hydrogen phosphate

4.4 Potassium dihydrogen phosphate

4.5 Potassium chloride

4.6 Sodium hydroxide solution, ρ(NaOH) = 8,0 g/l

Dissolve 8 g of sodium hydroxide in 900 ml of water, then dilute to 1 l with water.

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4.7 Phosphate buffered saline (PBS)

Dissolve 8 g of sodium chloride (4.2), 1,2 g of disodium hydrogen phosphate (4.3), 0,2 g of potassium dihydrogen phosphate (4.4) and 0,2 g of potassium chloride (4.5) in 900 ml of distilled water. Adjust the pH to 7,4 with sodium hydroxide solution (4.6) then dilute to 1 l with water.

Commercially available phosphate buffered saline tablets with equivalent properties may be used.

4.8 Sodium hydrogen carbonate solution, $\rho(NaHCO_3)$ = 30 g/l

In a 1-l-volumetric flask dissolve 30 g sodium hydrogen carbonate in 900 ml of water. Dilute to volume with water.

4.9 Glacial acetic acid, $\varphi(CH_3COOH)$ = 98 %

4.10 Methanol

4.11 Acetonitrile

4.12 Toluene

4.13 Solvent mixture of toluene and glacial acetic acid

Mix 99 parts per volume of toluene (4.12) with 1 part per volume of glacial acetic acid (4.9).

4.14 Barley extraction solvent mixture

Mix 6 parts per volume acetonitrile (4.11) with 4 parts per volume of water.

4.15 Roasted coffee extraction solvent mixture

Mix 1 part per volume of methanol (4.10) with 1 part per volume of sodium hydrogen carbonate solution (4.8).

4.16 Injection solvent

Mix 30 parts per volume of methanol (4.10) with 70 parts per volume of water and 1 part per volume of glacial acetic acid (4.9).

4.17 Mobile phase

Mix 102 parts per volume of water with 96 parts per volume of acetonitrile (4.11) and 2 parts per volume of glacial acetic acid (4.9), filter through a 0,2 μm filter (5.12) and degas with for example helium before use.

4.18 Phenyl silane column wash reagent 1

Mix 20 parts per volume of methanol (4.10) with 80 parts per volume of sodium hydrogen carbonate solution (4.8).

4.19 Phenyl silane column wash reagent 2, $\rho(NaHCO_3)$ = 1 g/100 ml

In a 100 ml volumetric flask dissolve 1 g of sodium hydrogen carbonate in 90 ml water. Dilute to volume with water.

4.20 Phenyl silane column elution reagent

Mix 7 parts per volume methanol (4.10) with 93 parts per volume of water.

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4.21 Ochratoxin A stock solution

Dissolve 1 mg of the ochratoxin A (in crystal form) or the contents of 1 ampoule (if ochratoxin A has been obtained as a film) in solvent mixture (4.13) 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 5 nm steps in 1 cm quartz cells (5.14) and solvent mixture (4.13) as reference. Identify the wavelength for maximum absorption and calculate the mass concentration of ochratoxin A, ρ_{ota}, in micrograms per millilitre, using equation (1):

$$\rho_{ota} = \frac{A_{max} \times M \times 1000}{\varepsilon \times \delta} \qquad (1)$$

where:

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

M is the relative molecular mass of ochratoxin A (M = 403,8 g/mol);

ε is the relative molar absorption coefficient of ochratoxin A in the solvent mixture (4.13), (here: 5440 m^2/mol);

δ is the path length of the quartz cell in centimetres.

This solution is stable at –18°C for at least four years.

4.22 Ochratoxin A standard solution

Dilute the stock solution (4.21) with the solvent mixture (4.13) to obtain a standard solution with a mass concentration of ochratoxin A of 10 μg/ml. Store this solution in a refrigerator at approximately 4°C and check its stability.

4.23 Ochratoxin A calibration solution

Pipette 200 µl of the 10 µg/ml ochratoxin A standard solution (4.22) into a glass vial and dilute to 1 ml with 800 µl of solvent mixture (4.13). This gives 2 µg/ml ochratoxin A solution. Pipette 100 µl of the 2 µg/ml ochratoxin A solution into a glass vial (5.1). Evaporate the solvent under a stream of nitrogen. Redissolve in 10 ml injection solvent (4.16) which has been filtered through a 0,2 µm filter (5.12). This gives a calibration solution containing 20 ng/ml.

Prepare the calibration solutions at the beginning of every day of the analysis.

4.24 Spiking solution

Pipette 100 µl of the 10 µg/ml ochratoxin A standard solution (4.22) into a glass vial. Dilute to 2 ml with 1,9 ml of the mixture of toluene and acetic acid (4.13). This gives a mass concentration of 500 ng/ml ochratoxin A.

4.25 Immunoaffinity column

The immunoaffinity column contains antibodies raised against ochratoxin A. The column shall have a total binding capacity of not less than 100 ng of ochratoxin A. The performance of the column should be checked by applying a solution of 100 ng ochratoxin A in a solvent mixture of the same composition as the sample extract (6.1.3) to be applied. This shall give a recovery of not less than 85 %.

4.26 Phenyl silane solid phase extraction columns

500 mg sorbent weight and 3 ml reservoir volume (to ensure adequate column bed depth and prevent analyte breakthrough). The column should have a total capacity of not less than 100 ng ochratoxin A and should give a recovery of not less than 85 % when applied in a standard solution of ochratoxin A in the roasted coffee extraction solvent (4.15) containing 100 ng of ochratoxin A.