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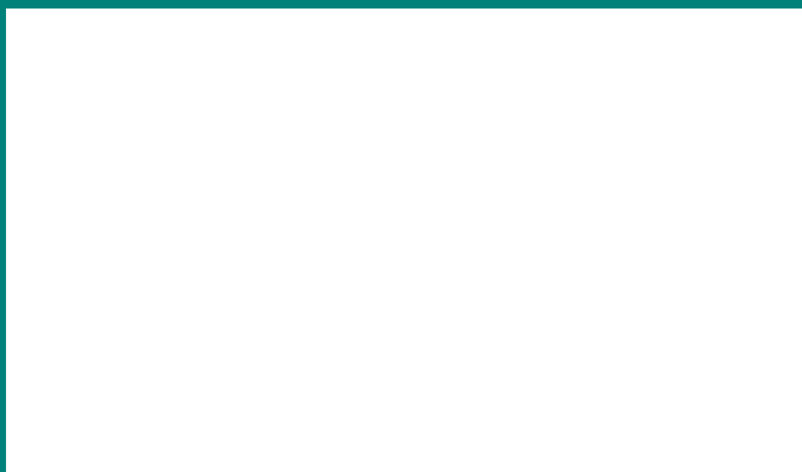
Foodstuffs – Determination of ochratoxin A in barley and roasted coffee – HPLC method with immunoaffinity column clean-up



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Denna standard ersätter SS-EN 14132, utgåva 1; SS-EN 14132/AC:2007, utgåva 1.

The European Standard EN 14132:2009 has the status of a Swedish Standard. This document contains the official English version of EN 14132:2009.

This standard supersedes the Swedish Standard SS-EN 14132, edition 1; SS-EN 14132/AC:2007, edition 1.

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 14132

May 2009

ICS 67.140.20

Supersedes EN 14132:2003

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 dans l'orge et le café torréfié - Méthode par purification sur colonne d'immuno-affinité suivie d'une analyse par chromatographie liquide haute performance (CLHP)

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

This European Standard was approved by CEN on 24 March 2009.

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Foreword

This document (EN 14132:2009) 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of an identical text or by endorsement, at the latest by November 2009, and conflicting national standards shall be withdrawn at the latest by November 2009.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document will supersede EN 14132:2003

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, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, 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

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, *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 it 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 it 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.

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 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.

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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.

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):

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

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where

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

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

ε is the molar absorption coefficient of ochratoxin A in the solvent mixture (4.13), (here: 544 m^2/mol);

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

This solution is stable at –18°C for at least 4 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 (5.2). 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 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.

5 Apparatus

Usual laboratory equipment and, in particular, the following:

5.1 Analytical balance, accurate to 0,01 mg

5.2 Glass vials, of at least 10 ml

Certain types of vials might lead to losses of ochratoxin A during evaporation. To avoid this, silanization could be applied. Prepare vials by filling them with silanizing reagent and leave this reagent in the vial for 1 min. Rinse the vial twice with appropriate solvent (toluene, acetone or hexane) followed by water (twice) and dry the vial.

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5.3 Blender, explosion proof

With 1 l capacity jar and cover and with a high speed of approximately 20 000 min^{-1}.

5.4 Displacement pipettes of 5 ml, 1 ml and 200 µl capacity with appropriate pipettes tips

5.5 Vacuum manifold to accommodate phenyl silane and immunoaffinity columns

5.6 Reservoirs and attachments to fit to immunoaffinity columns

5.7 Vacuum pump, capable of pulling a vacuum of 10 mbar and pumping 18 l/min

5.8 Wrist action shaker or similar

5.9 Cooling centrifuge capable of 1300 *g* and operating at 4 °C

5.10 Centrifuge tubes, e.g. 50 ml capacity

5.11 Filter paper, pore size 20 µm to 25 µm or similar

5.12 Disposable syringe filters, of 0,2 µm pore size and 25 mm diameter polysulfone membrane

5.13 HPLC apparatus, consisting of:

5.13.1 Injection system, a syringe-loading injection valve with 100 µl injection loop or equivalent

5.13.2 HPLC pump, isocratic, capable of maintaining a volume flow rate of 1 ml/min

5.13.3 Analytical reverse phase separating column, for example C_{18} octyldecylsilane (ODS),which ensures resolution of ochratoxin A from all other peaks. The maximum overlapping of peaks shall be less than 10 % (it could be necessary to adjust the mobile phase for a sufficient baseline resolution). A suitable pre-column should be used.

5.13.4 Fluorescence detector, fitted with a flow cell and set at 333 nm (excitation) and 460 nm (emission)

5.13.5 Data system

5.14 UV spectrometer, with suitable quartz cells.

6 Procedure

6.1 Barley

6.1.1 Extraction

Weigh, to the nearest 0,1 g, a 25 g test portion of the ground (mesh size = 0,5 mm) barley sample into a blender jar (5.3). Add 100 ml of extraction solvent mixture (4.14). Seal the jar and blend for 3 min in a high speed blender at approximately 20 000 min^{-1}. Filter the extract through a filter paper (5.11).

6.1.2 Immunoaffinity column clean-up

Prepare the immunoaffinity column according to the suppliers instructions.

Pipette 4 ml of the sample filtrate (see 6.1.1) into a 100 ml glass beaker (or similar) and dilute with 44 ml of PBS