

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

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## EESTI STANDARDI EESSÕNA

## NATIONAL FOREWORD

<p>Käesolev Eesti standard EVS-EN 14132:2003 sisaldab Euroopa standardi EN 14132:2003 + AC:2006 ingliskeelset teksti.</p> <p>Käesolev dokument on jõustatud 17.09.2003 ja selle kohta on avaldatud teade Eesti standardiorganisatsiooni ametlikus väljaandes.</p> <p>Standard on kättesaadav Eesti standardiorganisatsioonist.</p>	<p>This Estonian standard EVS-EN 14132:2003 consists of the English text of the European standard EN 14132:2003 + AC:2006.</p> <p>This document is endorsed on 17.09.2003 with the notification being published in the official publication of the Estonian national standardisation organisation.</p> <p>The standard is available from Estonian standardisation organisation.</p>
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<p><b>Käsitlusala:</b></p> <p>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</p>	<p><b>Scope:</b></p> <p>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</p>
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**Võtmesõnad:** cereal products, chemical analysis and testin, determ, determination of content, food inspection, food products, grain crops, high performance liquid chromatography, hplc, liquid chromatography, methods of analysis, mycotoxin, ochratoxin, roasted coffee, toxin

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

This European Standard was approved by CEN on 3 March 2003.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovak Republic, Spain, Sweden, Switzerland and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION  
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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 as 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.

## 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, $\rho(\text{NaOH}) = 8,0 \text{ g/l}$

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