

**Foodstuffs - Determination of ochratoxin A
in cereals and cereal products - Part 2:
High performance liquid chromatographic
method with bicarbonate clean up**

Foodstuffs - Determination of ochratoxin A in
cereals and cereal products - Part 2: High
performance liquid chromatographic method with
bicarbonate clean up

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

<p>Käesolev Eesti standard EVS-EN ISO 15141-2:2003 sisaldab Euroopa standardi EN ISO 15141-2:1998 ingliskeelset teksti.</p> <p>Käesolev dokument on jõustatud 14.08.2003 ja selle kohta on avaldatud teade Eesti standardiorganisatsiooni ametlikus väljaandes.</p> <p>Standard on kättesaadav Eesti standardiorganisatsioonist.</p>	<p>This Estonian standard EVS-EN ISO 15141-2:2003 consists of the English text of the European standard EN ISO 15141-2:1998.</p> <p>This document is endorsed on 14.08.2003 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>Käsitlusala: This European Standard specifies a method for the determination of ochratoxin A (OTA) at levels greater than 3 µg/kg</p>	<p>Scope: This European Standard specifies a method for the determination of ochratoxin A (OTA) at levels greater than 3 µg/kg</p>
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ICS 67.050

Võtmesõnad: carbonates, cereal products, chemical analysis and testing, determination, food inspection, food products, grain crops, hplc, inter-laboratory tests, liquid chromatography, maize, maize .gb, mathematical calculations, methods of analysis, ochratoxin, wheat bran

ICS 67.060

Descriptors: Food, cereals, ochratoxin content, testing.

English version

Foodstuffs

Determination of ochratoxin A in cereals and cereal products

**Part 2: High-performance liquid chromatographic method
with bicarbonate clean-up
(ISO 15141-2 : 1998)**

Produits alimentaires – Dosage de l'ochratoxine A dans les céréales et produits dérivés – Partie 2: Méthode par chromatographie liquide haute performance comprenant une étape d'extraction par une solution de bicarbonate (ISO 15141-2 : 1998)

Lebensmittel – Bestimmung von Ochratoxin A in Getreide und Getreideerzeugnissen – Teil 2: Hochleistungsflüssigchromatographisches Verfahren mit Bicarbonatreinigung (ISO 15141-2 : 1998)

This European Standard was approved by CEN on 1998-07-01.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

The European Standards exist 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 Central Secretariat has the same status as the official versions.

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart 36, B-1050 Brussels

Foreword

International Standard

ISO 15141-2 : 1998 Foodstuffs – Determination of ochratoxin A in cereals and cereal products – Part 2: High-performance liquid chromatographic method with bicarbonate clean-up,

which was prepared by ISO/TC 34 ‘Agricultural food products’ of the International Organization for Standardization, has been adopted by Technical Committee CEN/TC 275 ‘Food analysis – Horizontal methods’, the Secretariat of which is held by DIN, as a European Standard.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, and conflicting national standards withdrawn, by April 1999 at the latest.

In accordance with the CEN/CENELEC Internal Regulations, the following countries are bound to implement this European Standard:

Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, the Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 15141-2 : 1998 was approved by CEN as a European Standard without any modification.

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1 Scope

This standard specifies a method for the determination of ochratoxin A (OTA) at levels greater than 3 µg/kg.

The method has been successfully validated in interlaboratory studies according to ISO 5725:1986 [1] on whole barley containing 2,9 µg/kg, 3,0 µg/kg, 7,4 µg/kg and 14,4 µg/kg of ochratoxin A, on whole maize containing 8,2 µg/kg and 16,3 µg/kg of ochratoxin A as well as on wheat bran containing 3,8 µg/kg and 4,5 µg/kg of ochratoxin A.

NOTE: Numerous laboratory experiences have shown that this method is also applicable to wheat flour.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions 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 draft European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

ISO 3696 Water for analytical laboratory use - Specification and test methods

3 Principle

Ochratoxin A is extracted from grains with chloroform-aqueous phosphoric acid and isolated by liquid-liquid partitioning into aqueous bicarbonate solution. The solution is applied to a C₁₈ cartridge, and ochratoxin A is eluted with ethyl acetate-methanol-acetic acid. Ochratoxin A is separated by reversed phase HPLC and identified and quantified by fluorescence. Chromatography of ochratoxin A methyl ester derivative confirms the identification [2] to [5].

WARNING: Ochratoxin A causes kidney and liver damage and is a probable carcinogen. Observe appropriate safety precautions [6] for handling such compounds and in particular avoid handling in dry form as the electrostatic nature can result in dispersion and inhalation. Glassware can be decontaminated with 4 % sodium hypochlorite solution. Attention is drawn to the statement made by the International Agency for Research on Cancer (WHO) [7], [8].

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of grade 1 according to ISO 3696. Solvent shall be of quality for HPLC analysis.

4.1 Chloroform, stabilized with for example 2-methyl-2-butene or ethanol

4.2 Phosphoric acid, $c(\text{H}_3\text{PO}_4) \approx 0,1 \text{ mol/l}$

4.3 Diatomaceous earth

Soak about 900 g of acid-washed diatomaceous earth e.g. Celite® 545¹⁾ overnight in methanol (4.7). Filter through a double layer of paper in a Buchner funnel (5.6), wash with 8 l of water and dry for 12 h at 150 °C.

¹⁾ Celite® 545 is an example of a suitable product available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of these products.