

Foodstuffs — Determination of ochratoxin A in cereal based foods for infants and young children — HPLC method with immunoaffinity column cleanup and fluorescence detection

ICS 67.050; 67.230

National foreword

This British Standard is the UK implementation of EN 15835:2010.

The UK participation in its preparation was entrusted to Technical Committee AW/-/3, Food analysis - Horizontal methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 28 February 2010
© BSI 2010

ISBN 978 0 580 63016 3

Amendments/corrigenda issued since publication

Date	Comments

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 15835

February 2010

ICS 67.050; 67.230

English Version

**Foodstuffs - Determination of ochratoxin A in cereal based foods
for infants and young children - HPLC method with
immunoaffinity column cleanup and fluorescence detection**

Produits alimentaires - Dosage de l'ochratoxine A dans les
aliments à base de céréales pour nourrissons et jeunes
enfants - Méthode CLHP avec purification sur colonne
d'immuno-affinité et détection par fluorescence

Lebensmittel - Bestimmung von Ochratoxin A in Säuglings-
und Kleinkindernahrung auf Getreidebasis - HPLC-
Verfahren mit Reinigung an einer Immunoaffinitätssäule
und Fluoreszenzdetektion

This European Standard was approved by CEN on 25 December 2009.

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 CEN Management Centre or to any CEN member.

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 CEN Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, 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 United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents

Page

Foreword.....	3
1 Scope	4
2 Normative references	4
3 Principle	4
4 Reagents	4
5 Apparatus	7
6 Procedure	9
7 HPLC analysis	10
8 Calculation	11
9 Precision	12
10 Test report	13
Annex A (informative) Typical chromatograms	14
Annex B (informative) Precision data.....	15
Annex C (informative) Data on the in-house study	16
Bibliography	18

Foreword

This document (EN 15835:2010) 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.

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 has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

Annexes A, B and C are informative.

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, Croatia, 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.

1 Scope

This European Standard specifies a method for the determination of ochratoxin A in cereal based foods for infants and young children by high performance liquid chromatography (HPLC) with immunoaffinity column 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 0,050 µg/kg to 0,217 µg/kg. For further information on the validation see Clause 8 and Annex B. Additional studies have shown that this method is applicable to cereal based baby foods containing 8 different types of cereals, honey and cocoa, at levels up to 3,540 µg/kg, see Annex C and [6].

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 tert-butyl methyl ether after addition of 0,5 mol/l phosphoric acid / 2 mol/l sodium chloride solution. The extract is evaporated and redissolved in methanol and phosphate buffered saline (PBS) solution. After removal of lipophilic compounds with hexane, the extract is applied to an immunoaffinity column containing antibodies specific to ochratoxin A. The toxin is eluted from the column with methanol. Ochratoxin A is determined by HPLC with enhanced fluorescence detection involving post column reaction with ammonia.

NOTE Some investigations indicate that HPLC can be also performed without the use of ammonia although this results in at least a two-fold decrease of the response for ochratoxin A. In this case, the fluorescence detection conditions need to be changed (excitation wavelength = 333 nm, emission wavelength = 460 nm).

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 [4].

4.2 Helium purified compressed gas

4.3 Nitrogen

4.4 Disodium hydrogen phosphate, Na_2HPO_4 anhydrous or $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$

4.5 Potassium chloride

4.6 Potassium dihydrogen phosphate

4.7 Sodium chloride

4.8 Sodium hydroxide

4.9 Ammonium hydroxide solution, the mass fraction $w(\text{NH}_4\text{OH}) = 25 \%$ in water (post column reagent)

Degas the solution with a degasser (5.21.7).

4.10 Hydrochloric acid solution, $w(\text{HCl})$ is 37 % (acidimetric)

4.11 Phosphoric acid solution, $w(\text{H}_3\text{PO}_4) = 85 \%$

4.12 Hydrochloric acid solution, $c(\text{HCl}) = 0,1 \text{ mol/l}$

Dilute 8,28 ml of hydrochloric acid solution (4.10) to 1 l of water.

4.13 Sodium hydroxide solution, $c(\text{NaOH}) = 0,1 \text{ mol/l}$

Dissolve 4 g of sodium hydroxide (4.8) in 1 l of water.

4.14 Phosphate buffered saline (PBS) solution, $c(\text{NaCl}) = 120 \text{ mmol/l}$, $c(\text{KCl}) = 2,7 \text{ mmol/l}$,
 $c(\text{phosphate buffer}) = 10 \text{ mmol/l}$, $\text{pH} = 7,4$

Dissolve 8,0 g of sodium chloride (4.7), 1,2 g of anhydrous disodium hydrogen phosphate or 2,9 g of $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ (4.4), 0,2 g of potassium dihydrogen phosphate (4.6) and 0,2 g of potassium chloride (4.5) in 900 ml of water.

After dissolution, adjust the pH to 7,4 with hydrochloric acid solution (4.12) or sodium hydroxide solution (4.13) as appropriate, then dilute to 1 l with water. Alternatively, a PBS solution with equivalent properties can be prepared from commercially available PBS material.

4.15 Mixture of phosphoric acid solution and sodium chloride solution, $c(\text{H}_3\text{PO}_4) = 0,5 \text{ mol/l}$,
 $c(\text{NaCl}) = 2 \text{ mol/l}$

Dissolve 118 g of sodium chloride (4.7) in approximately 900 ml of water. Add 33 ml of phosphoric acid (4.11) and make up to 1,0 l with water.

4.16 Glacial acetic acid, the mass fraction $\geq 99,7 \%$

4.17 Acetic acid solution, the volume fraction is 9 %

Add 90 ml of glacial acetic acid (4.16) and 910 ml of water.

4.18 Hexane

WARNING — Hexane is highly flammable. Operations involving this solvent shall be performed in a fume cupboard. Serious health problems can be derived from prolonged exposure to this reagent.