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I.S. EN 15835:2010

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

## I.S. EN 15835:2010

*Incorporating amendments/corrigenda issued since publication:*

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<b>NSAI</b> 1 Swift Square, Northwood, Santry Dublin 9	T +353 1 807 3800 F +353 1 807 3838 E standards@nsai.ie W NSAI.ie	<b>Sales:</b> T +353 1 857 6730 F +353 1 857 6729 W standards.ie
Údarás um Chaighdeáin Náisiúnta na hÉireann		

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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

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## **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of an identical text or by endorsement, at the latest by August 2010, and conflicting national standards shall be withdrawn at the latest by August 2010.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.

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Annexes A, B and C are informative.

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## 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

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