

# Foodstuffs — Determination of fumonisin B1 and B2 in maize based foods — HPLC method with immunoaffinity column clean up

The European Standard EN 14352:2004 has the status of a  
British Standard

ICS 67.060

# National foreword

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The UK participation in its preparation was entrusted to Technical Committee AW/-/3, Horizontal analysis, which has the responsibility to:

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## Summary of pages

This document comprises a front cover, an inside front cover, the EN title page, pages 2 to 18, an inside back cover and a back cover.

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## Amendments issued since publication

| Amd. No. | Date | Comments |
|----------|------|----------|
|          |      |          |
|          |      |          |
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|          |      |          |
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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 6 August 2004

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ISBN 0 580 44205 5

English version

**Foodstuffs - Determination of fumonisin B1 and B2 in maize based foods - HPLC method with immunoaffinity column clean up**

Produits alimentaires - Dosage des fumonisines B1 et B2 dans des aliments à base de maïs - Méthode CLHP avec purification par colonne d'immunoaffinité

Lebensmittel - Bestimmung von Fumonisin B1 und B2 in Maiserzeugnissen - HPLC-Verfahren mit Immunoaffinitätssäulen-Reinigung

This European Standard was approved by CEN on 30 April 2004.

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## Foreword

This document (EN 14352:2004) 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of an identical text or by endorsement, at the latest by January 2005, and conflicting national standards shall be withdrawn at the latest by January 2005.

**WARNING — Fumonisin is hepatotoxic, nephrotoxic and carcinogenic to rats and mice. Effects on humans are not fully known. Observe appropriate safety precautions for handling fumonisins. Any laboratory spills should be washed with 5 % solution of sodium hypochlorite. Acetonitrile is hazardous and samples shall be shaken using a shaker, which is housed within a fume cupboard.**

**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.**

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## 1 Scope

This document specifies a method for the determination of fumonisin B<sub>1</sub> (FB<sub>1</sub>) and fumonisin B<sub>2</sub> (FB<sub>2</sub>) in maize based foods using high performance liquid chromatography (HPLC) and immunoaffinity clean-up, see [1], [2], [3].

The method has been successfully validated in a collaborative study according to AOAC Guidelines for collaborative study procedures [4] to validate characteristics of a method of analysis for the determination of fumonisins in maize flour and corn flakes containing 323 µg/kg to 1414 µg/kg FB<sub>1</sub> and 90 µg/kg to 558 µg/kg FB<sub>2</sub>.

## 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, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*.

## 3 Principle

Fumonisin is extracted from the sample with a mixture of water, methanol and acetonitrile. The filtered extract is purified by immunoaffinity column and fumonisins are eluted with methanol. The extract is evaporated and the residue is redissolved in a mixture of acetonitrile and water and o-phthalaldehyde-2-mercaptoethanol (OPA-MCE) is added to form fluorescent fumonisin derivatives. The derivatives are analysed by reverse-phase high performance liquid chromatography (RP-HPLC) with fluorescence detection.

## 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 according to EN ISO 3696. Solvents shall be of quality for HPLC analysis.

### 4.2 Methanol

### 4.3 Acetonitrile

### 4.4 o-phosphoric acid, volume fraction $\varphi(\text{H}_3\text{PO}_4) = 85 \%$

### 4.5 o-phthalaldehyde (OPA)

### 4.6 2-mercaptoethanol (MCE)

### 4.7 Sodium dihydrogen phosphate solution, substance concentration $c(\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}) = 0,1 \text{ mol/l}$

Dissolve 15,6 g of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  in 1 l of distilled water.

**4.8 Disodium tetraborate solution**,  $c(\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}) = 0,1 \text{ mol/l}$ 

Dissolve 3,8 g  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  in 100 ml of distilled water.

**4.9 Sodium chloride** (NaCl)**4.10 Disodium hydrogen phosphate** ( $\text{Na}_2\text{HPO}_4$ )**4.11 Potassium dihydrogen phosphate** ( $\text{KH}_2\text{PO}_4$ )**4.12 Potassium chloride** (KCl)**4.13 Hydrochloric acid** (HCl), concentrated**4.14 Extraction solvent**

Mix 25 volume parts of acetonitrile (4.3) with 25 volume parts of methanol (4.2) and 50 volume parts of water.

**4.15 Acetonitrile-water solution**, volume fraction  $\varphi(\text{CH}_3\text{CN}) = 50 \%$ 

Mix 50 volume parts of acetonitrile (4.3) with 50 volume parts of water.

**4.16 Phosphate buffered saline (PBS)**

Dissolve 8,0 g of sodium chloride (4.9), 1,2 g of disodium hydrogen phosphate (4.10), 0,2 g of potassium dihydrogen phosphate (4.11) and 0,2 g of potassium chloride (4.12) in approximately 990 ml of water. Adjust pH to 7,0 with concentrated hydrochloric acid (4.13) and bring to 1 l with water. Phosphate buffered saline tablet or ready-to-use solutions can also be used.

**4.17 Immunoaffinity column (IAC)**

The column shall contain antibodies raised against  $\text{FB}_1$  and fumonisin  $\text{FB}_2$ . The column shall have a total capacity of not less than 5  $\mu\text{g}$  of fumonisins and shall give a recovery of not less than 90 % for  $\text{FB}_1$  and  $\text{FB}_2$  when applied as a standard solution in a mixture of methanol and PBS containing 5  $\mu\text{g}$  of fumonisins. Up to 10 volume parts of methanol or acetonitrile may be used in the mixture with PBS. The columns shall be warmed up to room temperature before use.

**4.18 HPLC mobile phase**

Mix 77 volume parts of methanol (4.2) with 23 volume parts of sodium dihydrogen phosphate solution (4.7). Adjust to pH 3,35 with o-phosphoric acid (4.4). Filter the solution through a membrane filter (5.14).

The mobile phase composition may have to be adjusted to conform to individual HPLC column characteristics.

**4.19 Derivatization reagent**

Dissolve 40 mg of OPA (4.5) in 1 ml of methanol (4.2) and dilute with 5 ml of disodium tetraborate solution (4.8). Add 50  $\mu\text{l}$  of MCE (4.6) and mix. The solution is stable for up to one week at room temperature in the dark in a capped amber vial.

**4.20 Stock solutions of  $\text{FB}_1$  and  $\text{FB}_2$** 

Prepare a stock solution of  $\text{FB}_1$  and a stock solution of  $\text{FB}_2$  in acetonitrile-water (4.15) at a mass concentration of 50  $\mu\text{g/ml}$  for each standard substance. Store the solutions at approximately 4 °C.