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პრෆღუძტი პმების. ფუროზამინ B1, B2 — ის რაოღენობის ბანსაზღმრა მაისის პმების პროღუძტებში. HPLC მეთოღი იმუნოაფინური სმეტის ბასუფთავებით

საქართველოს სტანდარტების, ტექნიკური რეგლამენტების და მეტროლოგიის ეროვნული სააგენტო თგილისი სსტ ენ 14352 : 2010

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#### 4 30ᲠᲕᲔᲚᲐᲓ

**5 რმბისტრირმბშლია** საქართველოს სტანდარტების, ტექნიკური რეგლამენტების და მეტროლოგიის ეროვნული სააგენტოს რეესტრში: 2010 წლის 15 თებერვალი №268-1.3-3824

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## EUROPEAN STANDARD NORME EUROPÉENNE EUROPÄISCHE NORM

EN 14352

July 2004

ICS 67.060

#### **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-Vefahren mit Immunoaffinitätssäulen-Reinigung

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

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.

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 Central Secretariat has the same status as the official versions.

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



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

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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 — Fumonisins are 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 hypchlorite. 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.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

#### 1 Scope

This document specifies a method for the determination of fumonisin  $B_1$  (FB<sub>1</sub>) and fumonisin  $B_2$  (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  $\mu$ g/kg to 1414  $\mu$ g/kg FB<sub>1</sub> and 90  $\mu$ g/kg to 558  $\mu$ g/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

Fumonisins are 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-phthaldialdehyde-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  $\varphi(H_3PO_4) = 85 \%$
- 4.5 o-phthaldialdehyde (OPA)
- 4.6 2-mercaptoethanol (MCE)
- **4.7** Sodium dihydrogen phosphate solution, substance concentration  $c(NaH_2PO_4 \cdot 2H_2O) = 0.1 \text{ mol/l}$

Dissolve 15,6 g of NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O in 1 I of distilled water.