

**Foodstuffs - Determination of aflatoxins B1, and the sum of B1, B2, G1 and G2 in maize, raw peanuts and peanut butter - Immunoaffinity column coupled with high performance liquid chromatography postcolumn derivatization**

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## EESTI STANDARDI EESSÕNA

## NATIONAL FOREWORD

<p>Käesolev Eesti standard EVS-EN 12955:2001 sisaldab Euroopa standardi EN 12955:1999 ingliskeelset teksti.</p> <p>Käesolev dokument on jõustatud 18.06.2001 ja selle kohta on avaldatud teade Eesti standardiorganisatsiooni ametlikus väljaandes.</p> <p>Standard on kättesaadav Eesti standardiorganisatsioonist.</p>	<p>This Estonian standard EVS-EN 12955:2001 consists of the English text of the European standard EN 12955:1999.</p> <p>This document is endorsed on 18.06.2001 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><b>Käsitlusala:</b> This draft European Standard specifies a method for the determination of aflatoxins contents of greater than 8 µg/kg.</p>	<p><b>Scope:</b> This draft European Standard specifies a method for the determination of aflatoxins contents of greater than 8 µg/kg.</p>
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**ICS** 67.060, 67.080.10, 67.200.10

**Võtmesõnad:**

**English version**

Foodstuffs

**Determination of aflatoxin B<sub>1</sub> and the sum of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in cereals, shell-fruits and derived products**  
High-performance liquid chromatographic method with post column derivatization and immunoaffinity column clean-up

Produits alimentaires – Dosage de l'aflatoxine B<sub>1</sub> et de la somme des aflatoxines B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> et G<sub>2</sub> dans les céréales, les fruits à coque et les produits dérivés – Méthode de chromatographie en phase liquide haute performance avec dérivation post-colonne et purification en colonne d'immuno-affinité

Lebensmittel – Bestimmung von Aflatoxin B<sub>1</sub> und der Summe von Aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> und G<sub>2</sub> in Getreiden, Schalenfrüchten und verwandten Produkten – Hochleistungsflüssigchromatographisches Verfahren mit Nachsäulenderivatisierung und Immunoaffinitätssäulen-Reinigung

This European Standard was approved by CEN on 1999-06-07.

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

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## Foreword

This European Standard 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, and conflicting national standards withdrawn, by January 2000 at the latest.

In accordance with the CEN/CENELEC Internal Regulations, the national standards organizations of 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.

NOTE: Existing and developing legislation (national or EU) in this area will require a method with lower levels of detection. Such a method is currently being developed as an EU SMT project.

## 1 Scope

This European Standard specifies a method for the determination of aflatoxin contents of greater than 8 µg/kg.

The method has been successfully validated in an interlaboratory study according to ISO 5725:1986 on maize containing 24,5 µg/kg, peanut butter containing 8,4 µg/kg and raw peanuts containing 16 µg/kg of total aflatoxins.

Some laboratory experiences have shown that this method can be used to several types of cereals, oilseed products, shell-fruits, dried fruits and derived products, after in-house validation.

## 2 Normative reference(s)

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 European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

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

## 3 Principle

The test sample is extracted with a mixture of methanol and water. The sample extract is filtered, diluted with water, and applied to an affinity column containing antibodies specific for aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. The aflatoxins are isolated, purified and concentrated on the column then removed from the antibodies with methanol. The aflatoxins are quantified by reverse-phase high performance liquid chromatography (HPLC) with fluorescence detection and postcolumn iodine derivatization.

**WARNING** - The method described requires the use of solutions of aflatoxins. Aflatoxins are carcinogenic to humans. Attention is drawn to the statement made by the International Agency for Research on Cancer (WHO) [1], [2].

## 4 Reagents

### 4.1 General

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only water according to grade 1 of EN ISO 3696.

### 4.2 Sodium chloride

### 4.3 Iodine, crystalline

### 4.4 Aflatoxin, in crystal form or as a film in ampoules.

**WARNING:** Protect the laboratory, where the analyses are done, adequately from daylight. This can be achieved effectively by using Ultraviolet (UV) absorbing foil on the windows in combination with subdued light (no direct sunlight) or curtains or blinds in combination with artificial light (fluorescent tubes are acceptable).

Protect Aflatoxin containing solutions from light as much as possible (keep in the dark, use aluminium foil or amber-coloured glassware).

### 4.5 Acetonitrile, for HPLC

### 4.6 Methanol, for analysis

### 4.7 Methanol, for HPLC

### 4.8 Toluene