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IRISH STANDARD

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67.080.10
67.200.10

# Foodstuffs - Determination Of Aflatoxin $B_1$, And The Sum Of Aflatoxins $B_1$, $B_2$, $G_1$ And $G_2$ In Cereals, Shell-fruits And Derived Products - High Performance Liquid Chromatographic Method With Post Column Derivatization And Immunoaffinity Column Clean Up

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# EUROPEAN STANDARD
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## Foodstuffs - Determination of aflatoxin $B_1$, and the sum of aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ 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 $B_1$ et de la somme des aflatoxines $B_1$, $B_2$, $G_1$ et $G_2$ 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 $B_1$ und der Summe von Aflatoxin $B_1$, $B_2$, $G_1$ und $G_2$ in Getreiden, Schalenfrüchten und verwandten Produkten - Hochleistungs-flüssigchromatographisches Verfahren mit Nachsäulenderivatisierung und Immunoaffinitätssäulen-Reinigung

This European Standard was approved by CEN on 7 June 1999.



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

**Central Secretariat: rue de Stassart, 36 B-1050 Brussels**



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## Contents



## 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of an identical text or by endorsement, at the latest by January 2000, and conflicting national standards shall be withdrawn at the latest by January 2000.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, 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 $B_1$, $B_2$, $G_1$ and $G_2$. 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 other wise stated, us 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

## 4.9 Extraction solvent

Mix 7 parts per volume of methanol (4.6) with 3 parts per volume of water.

## 4.10 Immunoaffinity column

The immuno affinity (IA) column contains antibodies raised against aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$. The column shall have a minimim binding capacity of not less than 100 ng of aflatoxin $B_1$ and shall give a recovery of not less than 80 % for aflatoxin $B_1$, $B_2$, $G_1$ and not less than 60 % for aflatoxin $G_2$ when a standard solution in 15 ml of a methanol/water-mixture (1 part per volume of methanol and 3,4 parts per volume of water) containing 5 ng of each toxin is applied on the IA column. The IA column should provide an appropriate solvent reservoir, e.g. a syringe with adapter.

## 4.11 Mobile phase

Mix 3 parts per volume of water with 1 part per volume of acetonitrile (4.5) and 1 part per volume of methanol (4.7). Degas the solution before use.

## 4.12 Postcolumn derivatization reagent

Dissolve 100 mg of iodine (4.3) in 2 ml of methanol (4.6). Add 200 ml of water, stir for 1 h, and filter through a 0,45 µm filter (5.8). Prepare the solution on the week of use and store the solution in the dark or in a bottle of brown glass. Before use stir the solution for 10 min.

## 4.13 Toluene/acetonitrile mixture

Mix 98 parts per volume of toluene (4.8) with 2 parts per volume of acetonitrile (4.5).

## 4.14 Aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ stock solutions

Dissolve aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ seperately in the toluene/acetonitrile mixture (4.13) to give seperate solutions containing 10 µg/ml.

To determine the exact concentration of aflatoxin in each stock solution, record the absorption curve between a wavelength of 330 nm and 370 nm in 1 cm quartz glass cells (5.7) in a spectrometer with toluene/acetonitrile mixture (4.13) in the reference path. Calculate the aflatoxin mass concentration of each aflatoxin, $\rho_i$, in micrograms per millilitre, using equation (1):

$$\rho_i = \frac{A_{max} \times M_i \times 100}{\epsilon_i \times d} \qquad (1)$$

where:

$A_{max}$ is the absorbance determined at the maximum of the absorption curve;
$M_i$ is the relative molecular mass of each aflatoxin, in grams per mol;
$\varepsilon_i$ is the molar absorptivity of each aflatoxin in toluene/acetonitrile (4.13), in metres squared per mol;
d is the optical path length of the cell, in centimetres.

$M_i$ and $\varepsilon_i$ are given in table 1.

**Table 1 — Relative molecular mass and molar absorptivity of aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$**

(Mixture of toluene and acetonitrile 98 + 2)

| Aflatoxin | $M_i$ g/mol | $\varepsilon_i$ m²/mol |
|---|---|---|
| $B_1$ | 312 | 1930 |
| $B_2$ | 314 | 2040 |
| $G_1$ | 328 | 1660 |
| $G_2$ | 330 | 1790 |