SVENSK STANDARD
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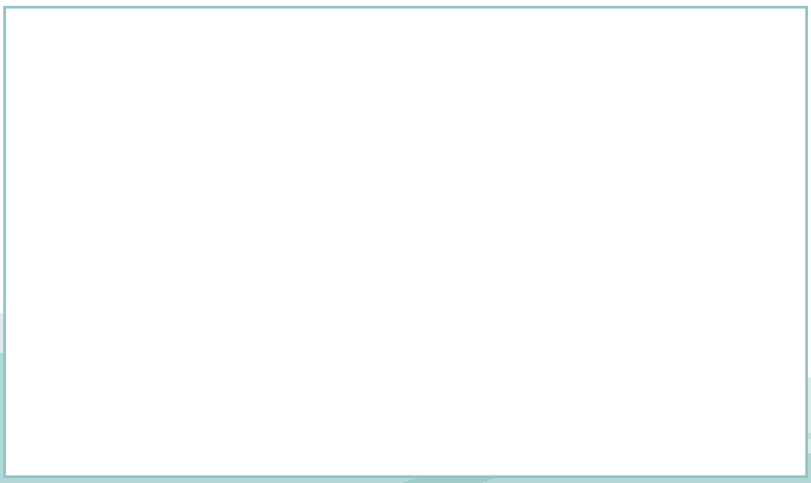
SWEDISH STANDARDS INSTITUTE

Foodstuffs – Determination of aflatoxin B1, and the total content of aflatoxins B1, B2, G1 and G2 in cereals, nuts and derived products – High-performance liquid chromatographic method (ISO 16050:2003)



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Europastandarden EN ISO 16050:2011 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN ISO 16050:2011.

Denna standard ersätter SS-EN 12955, utgåva 1.

The European Standard EN ISO 16050:2011 has the status of a Swedish Standard. This document contains the official version of EN ISO 16050:2011.

This standard supersedes the Swedish Standard SS-EN 12955, edition 1.

Upplysningar om sakinnehållet i standarden lämnas av SIS, Swedish Standards Institute, telefon 08-555 520 00. Standarder kan beställas hos SIS Förlag AB som även lämnar allmänna upplysningar om svensk och utländsk standard.

Information about the content of the standard is available from the Swedish Standards Institute (SIS), telephone +46 8 555 520 00. Standards may be ordered from SIS Förlag AB, who can also provide general information about Swedish and foreign standards.

Denna standard är framtagen av kommittén för Livsmedel och foder, SIS/TK 435.

Har du synpunkter på innehållet i den här standarden, vill du delta i ett kommande revideringsarbete eller vara med och ta fram andra standarder inom området? Gå in på www.sis.se - där hittar du mer information.

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN ISO 16050

July 2011

ICS 67.060; 67.080.10

Supersedes EN 12955:1999

English Version

Foodstuffs - Determination of aflatoxin B1, and the total content of aflatoxins B1, B2, G1 and G2 in cereals, nuts and derived products - High-performance liquid chromatographic method (ISO 16050:2003)

Produits alimentaires - Dosage de l'aflatoxine B1 et détermination de la teneur totale en aflatoxines B1, B2, G1 et G2 dans les céréales, les fruits à coque et les produits dérivés - Méthode par chromatographie liquide à haute performance (ISO 16050:2003)

Lebensmittel - Bestimmung von Aflatoxin B1 und der Summe von Aflatoxin B1, B2, G1 und G2 in Getreiden, Nüssen und verwandten Produkten - Hochleistungsflüssigchromatographisches Verfahren (ISO 16050:2003)

This European Standard was approved by CEN on 17 June 2011.

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 CEN-CENELEC Management Centre 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 CEN-CENELEC Management Centre has the same status as the official versions.

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

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

Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. EN ISO 16050:2011: E

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Foreword

The text of ISO 16050:2003 has been prepared by Technical Committee ISO/TC 34 “Food products” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 16050:2011 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 2012, and conflicting national standards shall be withdrawn at the latest by January 2012.

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.

This document supersedes EN 12955:1999.

Originally, EN 12955:1999 "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" was the basis for ISO 16050. In order to avoid having two equal standards on CEN- and ISO-level on the same topic, it was decided to take over ISO 16050 as EN ISO 16050 and to withdraw EN 12955 as soon as EN ISO 16050 is published.

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

Endorsement notice

The text of ISO 16050:2003 has been approved by CEN as a EN ISO 16050:2011 without any modification.

Foodstuffs — Determination of aflatoxin B_1, and the total content of aflatoxins B_1, B_2, G_1 and G_2 in cereals, nuts and derived products — High-performance liquid chromatographic method

WARNING — The use of this standard involves hazardous materials and operations. 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 practice and to determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard specifies a reverse-phase high-performance liquid chromatographic method, with immunoaffinity column clean-up and post-column derivatization, for the determination of aflatoxins in cereals, nuts and derived products. The limit of quantification for aflatoxin B_1, and for the sum of aflatoxins B_1, B_2, G_1 and G_2, is 8 µg/kg.

The method has been validated for maize containing 24,5 µg/kg, for peanut butter containing 8,4 µg/kg, and for raw peanuts containing 16 µg/kg of total aflatoxins. It has also been shown that this method can be used for oilseed products, dried fruits and derived products.

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.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

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 post-column derivatization.

4 Reagents

Use only reagents recognized analytical grade, unless otherwise stated.

4.1 Water, according to grade 1 of ISO 3696:1987.

4.2 Sodium chloride.

4.3 Iodine, crystalline, or as an alternative, **pyridinium hydrobromide perbromide** (PBPB)[1)].

4.4 Aflatoxin, in crystal form or as a film ampoule.

WARNING — Aflatoxins are carcinogenic to human subjects. Attention is drawn to the statement made by the International Agencies for Research on Cancer (WHO) (see [1], [2]).

Adequately protect from daylight the laboratory where the analyses are carried out. This may 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).

4.5 Acetonitrile, HPCL grade.

4.6 Methanol, analytical grade.

4.7 Methanol, HPLC grade.

4.8 Toluene, analytical grade.

WARNING — Toluene is highly flammable and harmful. Standard preparation involving this solvent shall be performed in a fume cupboard. Operations outside the fume cupboard, such as measurement of standards by UV spectrometry, shall be performed with the standards in closed containers.

4.9 Toluene/acetonitrile mixture

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

4.10 Extraction solvent

Mix 7 parts per volume of methanol (4.6) with 3 parts per volume of water (4.1).

Other extraction solvent mixtures which are compatible with the mobile phase may also be used if proved to be more effective or recommended by the manufacturer of the immunoaffinity (IA) column.

4.11 Mobile phase

Mix 3 parts per volume of water (4.1) 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 Post-column derivatization reagent

Dissolve 100 mg of iodine (4.3) in 2 ml of methanol (4.6). Add 200 ml of water (4.1), stir for 1 h, then filter through a 0,45 µm membrane filter (5.8). Prepare the solution the week of use and store the solution in the dark or in a brown glass bottle. Before use, stir the solution for 10 min.

As an alternative, dissolve 50 mg of PBPB (4.3) in 1 000 ml of water. This solution may be used for up to 4 days if stored in a dark place at room temperature.

4.13 Aflatoxin B_1, B_2, G_1 and G_2 stock solutions

WARNING — Protect solutions containing aflatoxin from light as far as possible (keep in the dark, use aluminium foil or amber-coloured glassware).

1) CAS: 39416-48-3 (CAS = Chemical Abstract Service).

Dissolve aflatoxin B_1, B_2, G_1 and G_2 separately in the toluene/acetonitrile mixture (4.9) to give separate solutions containing 10 µg/ml.

To determine the exact concentration of aflatoxin in each stock solution, record the absorption curve at a wavelength between 330 nm and 370 nm in 1 cm quartz glass cells (5.7) using a spectrometer (5.6) with a toluene/acetonitrile mixture (4.9) as reference. Calculate the aflatoxin concentration of each aflatoxin, ρ_i, in micrograms per millilitre, using Equation (1):

$$\rho_i = \frac{A_{\max} \times M_i \times 1000}{\varepsilon_i \times d} \quad (1)$$

where

$A_{\max}$ is the absorbance determined at the maximum of the absorption curve;

M_i is the molecular mass of each aflatoxin, in grams;

ε_i is the molar absorption coefficient of each aflatoxin in toluene/acetonitrile;

NOTE This value is determined in a solution that contains c = 1 mol/l of aflatoxin and in a cell with the optical pathlength d = 1 cm. The molar absorption coefficient (ε) is usually given without a unit of measurement, but from the equation $A = \varepsilon \times c \times d$, the following unit can be derived for it: $l \cdot mol^{-1} \cdot cm^{-1}$.

d is the optical pathlength of the cell, in centimetres.

M_i and ε_i are given in Table 1.

Table 1 — Molecular mass and molar absorption coefficient of aflatoxins B_1, B_2, G_1 and G_2

Aflatoxin	M_i	ε_i
B_1	312	19 300
B_2	314	20 400
G_1	328	16 600
G_2	330	17 900
NOTE A mixture of toluene and acetonitrile (98 + 2) is used as solvent.		

4.14 Stock solution of mixed aflatoxins

Prepare a stock solution containing 500 ng/ml of aflatoxin B_1, 125 ng/ml of aflatoxin B_2, 250 ng/ml of aflatoxin G_1 and 125 ng/ml of aflatoxin G_2 in toluene/acetonitrile (4.9). If the solution has to be stored, weigh the flask before storage. Wrap the flask tightly in aluminium foil and store it at approximately 4 °C. Immediately before use, reweigh the flask and record any change in mass after storage.

NOTE Normal exposure to UV light during absorbance measurement results in no observable conversion to photoproducts.

4.15 Standard solution of mixed aflatoxins

Transfer each quantity, as specified in Table 2, of mixed aflatoxin stock solution (4.14) into a series of four 2 ml volumetric flasks (5.5). Evaporate the solutions just to dryness under a stream of nitrogen at room temperature. To each flask, add 1 ml of methanol (4.6). Dissolve the dry residue in it, dilute the solution to the mark with water (4.1) and mix. Prepare the solution freshly on the day of use.