

**Toiduained. Aflatoksiin B1 sisalduse määramine
teraviljapõhises imiku- ja väikelastetoitudes
vedelikkromatograafilisel meetodil fluorestsents
detektoriga ja eelneva puhastamisega immunoaffiinsus
kolonnis**

Foodstuffs - Determination of aflatoxin B1 in cereal based
foods for infants and young children - HPLC method with
immunoaffinity column cleanup and fluorescence detection

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

Käesolev Eesti standard EVS-EN 15851:2010 sisaldab Euroopa standardi EN 15851:2010 ingliskeelset teksti.

Standard on kinnitatud Eesti Standardikeskuse 31.05.2010 käskkirjaga ja jõustub sellekohase teate avaldamisel EVS Teatajas.

Euroopa standardimisorganisatsioonide poolt rahvuslikele liikmetele Euroopa standardi teksti kättesaadavaks tegemise kuupäev on 07.04.2010.

Standard on kättesaadav Eesti standardiorganisatsioonist.

This Estonian standard EVS-EN 15851:2010 consists of the English text of the European standard EN 15851:2010.

This standard is ratified with the order of Estonian Centre for Standardisation dated 31.05.2010 and is endorsed with the notification published in the official bulletin of the Estonian national standardisation organisation.

Date of Availability of the European standard text 07.04.2010.

The standard is available from Estonian standardisation organisation.

ICS 67.060

Standardite reprodutseerimis- ja levitamiseõigus kuulub Eesti Standardikeskusele

Andmete paljundamine, taastekitamine, kopeerimine, salvestamine elektroonilisse süsteemi või edastamine ükskõik millises vormis või millisel teel on keelatud ilma Eesti Standardikeskuse poolt antud kirjaliku loata.

Kui Teil on küsimusi standardite autorikaitse kohta, palun võtke ühendust Eesti Standardikeskusega:
Aru 10 Tallinn 10317 Eesti; www.evs.ee; Telefon: 605 5050; E-post: info@evs.ee

Right to reproduce and distribute Estonian Standards belongs to the Estonian Centre for Standardisation

No part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, without permission in writing from Estonian Centre for Standardisation.

If you have any questions about standards copyright, please contact Estonian Centre for Standardisation:
Aru str 10 Tallinn 10317 Estonia; www.evs.ee; Phone: +372 605 5050; E-mail: info@evs.ee

ICS 67.060

English Version

Foodstuffs - Determination of aflatoxin B₁ in cereal based foods
for infants and young children - HPLC method with
immunoaffinity column cleanup and fluorescence detection

Produits alimentaires - Dosage de l'aflatoxine B₁ dans les
produits pour nourrissons et jeunes enfants à base de
céréales - Méthode de chromatographie liquide haute
performance avec purification sur colonne d'immunoaffinité
et détection par fluorescence

Lebensmittel - Bestimmung von Aflatoxin B₁ in Säuglings-
und Kleinkindernahrung auf Getreidebasis - HPLC-
Verfahren mit Reinigung an einer Immunoaffinitätssäule
und Fluoreszenzdetektion

This European Standard was approved by CEN on 27 February 2010.

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 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 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

Contents

Page

Foreword..... 3

1 **Scope** 4

2 **Normative references** 4

3 **Principle** 4

4 **Reagents** 4

5 **Apparatus** 7

6 **Procedure** 8

7 **HPLC analysis** 9

8 **Calculation** 11

9 **Precision** 11

10 **Test report** 12

Annex A (informative) Typical chromatogram 13

Annex B (informative) Precision data 14

Bibliography 15

Foreword

This document (EN 15851:2010) 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 October 2010, and conflicting national standards shall be withdrawn at the latest by October 2010.

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 has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

WARNING — 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, 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.

1 Scope

This European Standard specifies a method for the determination of aflatoxin B₁ in baby food by high performance liquid chromatography (HPLC) with immunoaffinity cleanup and fluorescence detection. This method has been validated in an interlaboratory study via the analysis of both naturally contaminated and spiked samples ranging from 0,07 µg/kg to 0,18 µg/kg.

For further information on the validation, see Clause 9 and Annex B.

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 3696:1995, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

3 Principle

A test portion is extracted with a mixture of methanol and water. The extract is filtered, diluted with phosphate buffered saline (PBS) to a specified solvent concentration, and applied to an immunoaffinity column containing antibodies specific to aflatoxin B₁. Aflatoxin B₁ is purified and concentrated on the column and removed from the antibodies using methanol as eluent. Aflatoxin B₁ is quantified by reverse-phase high performance liquid chromatography (RP-HPLC) with post column derivatization (PCD) involving bromination followed by fluorescence detection.

The post column derivatization is achieved with either electrochemically generated bromine or with pyridinium hydrobromide perbromide (PBPB).

4 Reagents

4.1 General

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696:1995, unless otherwise specified. Solvents shall be of quality for HPLC analysis, unless otherwise specified. Commercially available solutions with equivalent properties to those listed may be used.

WARNING — Dispose of waste solvents according to applicable environmental rules and regulations. Decontamination procedures for laboratory wastes have been reported by the International Agency for Research on Cancer (IARC), see [4].

4.2 Helium purified compressed gas.

4.3 Nitrogen.

4.4 Disodium hydrogen phosphate, Na₂HPO₄ anhydrous or Na₂HPO₄·12 H₂O.

4.5 Potassium bromide.

4.6 Potassium chloride.

4.7 Potassium dihydrogen phosphate, KH₂PO₄.

4.8 Sodium chloride.