

Foodstuffs - Determination of aflatoxins in spices other than paprika by IAC clean-up and HPLC-FLD with post-column derivatization

## EESTI STANDARDI EESSÕNA

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ICS 67.050

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English Version

## Foodstuffs - Determination of aflatoxins in spices other than paprika by IAC clean-up and HPLC-FLD with post-column derivatization

Produits alimentaires - Dosage des aflatoxines dans les épices autres que le paprika par purification sur colonne d'immunoaffinité et CLHP-FLD avec dérivation post-colonne

Lebensmittel - Bestimmung von Aflatoxinen in Gewürzen außer Paprika mit IAC Reinigung und HPLC-FLD mit Nachsäulenderivatisierung

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## European foreword

This document (EN 17424:2020) 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of an identical text or by endorsement, at the latest by May 2021, and conflicting national standards shall be withdrawn at the latest by May 2021.

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## Introduction

Aflatoxins consist of a group of approximately twenty related fungal metabolites, although only aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> are normally found in foods. Aflatoxins B<sub>2</sub> and G<sub>2</sub> are the dihydro derivatives of the parent compounds. Aflatoxins are produced by at least three species of *Aspergillus*, *A. flavus*, *A. parasiticus* and *A. nomius*, and can occur in a wide range of important raw food commodities, including cereals, nuts, spices, figs and dried fruit.

**WARNING 1** — Suitable precaution and protection measures need to be taken when carrying out working steps with harmful chemicals. The latest version of the hazardous substances ordinance, Regulation (EC) No 1907/2006 [3], should be taken into account as well as appropriate national statements e.g. such as in [4].

**WARNING 2** — The use of this document can involve hazardous materials, operations and equipment. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

**WARNING 3** — Aflatoxins are known to have carcinogenic effects and to be both acutely and chronically toxic.

## 1 Scope

This document describes a procedure for the determination of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> and total aflatoxins (sum of B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>) in spices for which EU maximum levels are established, other than paprika, by high performance liquid chromatography (HPLC) with post-column derivatization (PCD) and fluorescence detection (FLD) after immunoaffinity column (IAC) clean-up.

The method is applicable to the spices capsicum (excluding paprika), pepper, nutmeg, ginger, turmeric and mixtures thereof.

The method has been validated for aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> and total aflatoxins in a range of test samples that comprised: ginger, pepper, nutmeg, chilli, turmeric as individual spices and mixed pepper + chilli + nutmeg (90 + 5 + 5, *m + m + m*), mixed spice+ginger (6 + 4, *m + m*) mixed spice, mixed turmeric+ginger (2 + 8, *m + m*).

The validation was carried out over the following concentration ranges: aflatoxin B<sub>1</sub> = 1 µg/kg to 16 µg/kg and total aflatoxins = 2,46 µg/kg to 36,1 µg/kg.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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)*

## 3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <https://www.iso.org/obp>

## 4 Principle

Aflatoxins are extracted from the spices with a mixture of methanol, acetonitrile and water. The sample extract is filtered, diluted with phosphate buffered saline (PBS) and applied to an immunoaffinity column containing antibodies specific to aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. The aflatoxins are eluted from the IAC and are quantified by reversed-phase HPLC with PCD followed by FLD.