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Standards

Irish Standard
I.S. EN 17250:2020

Foodstuffs - Determination of ochratoxin A in spices, liquorice, cocoa and cocoa products by IAC clean-up and HPLC-FLD

I.S. EN 17250:2020

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National Foreword

I.S. EN 17250:2020 is the adopted Irish version of the European Document EN 17250:2020, Foodstuffs - Determination of ochratoxin A in spices, liquorice, cocoa and cocoa products by IAC clean-up and HPLC-FLD

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EUROPEAN STANDARD
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EN 17250

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ICS 67.140.30; 67.220.10

English Version

Foodstuffs - Determination of ochratoxin A in spices,
liquorice, cocoa and cocoa products by IAC clean-up and
HPLC-FLD

Produits alimentaires - Dosage de l'ochratoxine A dans
les épices, la réglisse, les produits à base de réglisse, le
cacao et les produits à base de cacao par purification
sur colonne d'immuno-affinité et CLHP-DFL

Lebensmittel - Bestimmung von Ochratoxin A in
Gewürzen, Süßholz, Kakao und Kakaoerzeugnissen
nach IAC-Reinigung mit HPLC-FLD

This European Standard was approved by CEN on 18 November 2019.

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EN 17250:2020 (E)

Contents

Page

European foreword.....	3
Introduction	4
1 Scope.....	5
2 Normative references.....	5
3 Terms and definitions	5
4 Principle	5
5 Reagents	5
6 Apparatus and equipment.....	9
7 Procedure.....	10
8 HPLC analysis.....	11
9 Calculation	13
10 Precision.....	14
11 Test report.....	16
Annex A (informative) Typical chromatograms	17
Annex B (informative) Precision data	21
Bibliography.....	27

European foreword

This document (EN 17250:2020) 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of an identical text or by endorsement, at the latest by July 2020, and conflicting national standards shall be withdrawn at the latest by July 2020.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

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EN 17250:2020 (E)**Introduction**

The mycotoxin ochratoxin A has a chemical structure comprising a dihydrocoumarin moiety linked to a molecule of L- β -phenylalanine via an amide bond. Ochratoxin A is produced by several fungal species in the *Penicillium* and *Aspergillus* genera, primarily *Penicillium verrucosum*, *Aspergillus ochraceus* and *Aspergilli* of the section *Nigri*, especially *A. carbonarius*. Cereals such as wheat are especially affected, as well as a diverse range of other foodstuffs such as dried fruit, spices, cocoa, coffee, wine, beer, liquorice and products thereof.

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 (EU) 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 — Ochratoxin A is a potent nephrotoxic agent, a carcinogen and has genotoxic properties. Ochratoxin A has been classified by IARC as Group 2B.

1 Scope

This document specifies a procedure for the determination of ochratoxin A (OTA) in chilli, paprika, black and white pepper, nutmeg, spice mix, liquorice (root and extracts), cocoa and cocoa products by high performance liquid chromatography (HPLC) with immunoaffinity column clean-up and fluorescence detection (FLD).

This method has been validated in interlaboratory studies via the analysis of both naturally contaminated and spiked samples ranging from 1,0 µg/kg to 84,9 µg/kg for spices (paprika and chili [5], black and white pepper, nutmeg and spice mix [6]), ranging from 7,7 µg/kg to 96,8 µg/kg for liquorice and liquorice products [7] and ranging from 2,1 µg/kg to 26,3 µg/kg for cocoa and cocoa products [6].

For further information on the validation, see Clause 10 and Annex B.

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/ui>

4 Principle

Spices or liquorice and liquorice products are extracted with a mixture of methanol and aqueous sodium hydrogen carbonate solution, whereas cocoa and cocoa products are extracted with aqueous methanol. The extract is filtered, diluted with phosphate buffered saline (PBS), polysorbate 20 (except for liquorice and liquorice products), and applied to an immunoaffinity column containing antibodies specific to ochratoxin A. The ochratoxin A is isolated, purified and concentrated on the column then released using methanol. The purified extract is quantified by reversed-phase high performance liquid chromatography (RP-HPLC) coupled with fluorescence detection (FLD).

5 Reagents

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696, unless otherwise specified. Commercially available solutions with equivalent properties to those listed may be used.

5.1 Nitrogen, minimum 99,95 % purity.

5.2 Methanol, technical grade.

5.3 Methanol, HPLC grade.

5.4 Acetonitrile, HPLC grade.

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