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Cereals and cereal products — Determination of 17 mycotoxins by ultra-high-performance liquid chromatography and tandem mass spectrometry method (UHPLC-MS/MS)

Céréales et produits céréaliers — Détermination de 17 mycotoxines par chromatographie liquide à ultra haute performance et spectrométrie de masse en tandem (CLUHP-SM/SM)

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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Cereals and cereal products — Determination of 17 mycotoxins by ultra-high-performance liquid chromatography and tandem mass spectrometry method (UHPLC-MS/MS)

1 Scope

This document specifies a method for the quantitative determination of 17 mycotoxins in cereals and cereal products (e.g. wheat, maize, husked rice, rice and their products) using ultra-high-performance liquid chromatography and tandem mass spectrometry method (UHPLC-MS/MS).

The 17 mycotoxins are aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2, deoxynivalenol, nivalenol, deoxynivalenol-3-glucoside, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, zearalenone, ochratoxin A, fumonisin B_1, fumonisin B_2, fumonisin B_3, T-2 toxin, HT-2 toxin and sterigmatocystin.

This document does not apply to foods for infants and young children.

This document is applicable to other products (e.g. nuts) provided that the method is validated for each individual case.

The calibration range of the method and the validated range during the interlaboratory study for each mycotoxin are listed in Table A.1.

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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.

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

3 Terms and definitions

No terms and definitions are listed in this document.

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle

Mycotoxins are extracted using an acetonitrile-water-acetic acid (70:29:1, v/v/v) solution. After centrifugation, the extract is diluted with water, centrifuged in low temperature and filtered. The final solution is mixed with isotope-labelled internal standard solution and subjected to UHPLC-MS/MS analysis. Quantification is performed by the isotopic dilution approach using ^{13}C isotopically labelled mycotoxins as internal standards (ISTDs).

WARNING — Mycotoxins are generally considered to be carcinogenic, neurotoxic and immunosuppressive. Observe appropriate safety precautions[1] for handling such compounds and avoid handling in dry form as the electrostatic nature can result in dispersion and inhalation.

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Glassware can be decontaminated with 4 % sodium hypochlorite solution. Attention is drawn to the statement made by the International Agency for Research on Cancer (WHO).[2][3]

5 Reagents

WARNING — This document requires handling of hazardous substances. Technical, organizational and personal safety measures shall be followed.

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of grade 1 in accordance with ISO 3696. Solvents shall be of quality for LC analysis.

5.1 Acetonitrile, LC grade or equivalent.

5.2 Methanol, LC grade or equivalent.

5.3 Ammonium acetate, American Chemical Society (ACS) grade, > 99 %.

5.4 Acetic acid, ACS grade.

5.5 Formic acid, ACS grade.

5.6 Extraction solvent: Mix 70 volume parts of acetonitrile (5.1) and 29 volume parts of water and 1 volume part of acetic acid (5.4).

5.7 Solvent of calibration solutions: Mix 50 volume parts of extraction solvent (5.6) and 50 volume parts of water.

5.8 Individual stock solutions, either prepared by dissolving neat (solid) certified standards in an appropriate solvent or from individual stock solutions purchased as such.

The mycotoxins covered in this document dissolve well in acetonitrile, except for fumonisins which are soluble in a mixture of acetonitrile and water (50:50, v/v). The information and concentration of 17 mycotoxins and the isotope-labelled internal standards is shown in Table B.1.

5.9 Mixed stock solution.

Prepare a mixed stock solution containing all individual mycotoxins at the concentration given in Table 1, using the appropriate pipettes (6.1) and dilute with deionized water. This solution can be used for six months if stored in the dark at −20 °C.

This mixed stock solution may be used for the preparation of positive control samples (see 7.5).

Table 1 — Concentration of mixed stock solution for 17 mycotoxins

Compounds	Concentration µg/ml	Compounds	Concentration µg/ml
NIV	20	FB_1	12,5
DON	15	FB_2	7,5
DON-3G	2,5	FB_3	9,0
3-AcDON	4	T-2	0,2
15-AcDON	2	HT-2	1
AFB_1	0,1	ZEN	2

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