
**Cereals and cereal products —
Determination of ochratoxin A — High
performance liquid chromatographic
method with immunoaffinity column
cleanup and fluorescence detection**

*Céréales et produits céréaliers — Dosage de l'ochratoxine A —
Méthode par chromatographie en phase liquide à haute performance
avec purification sur colonne d'immunoaffinité et détection par
fluorescence*





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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 documents 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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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

This first edition cancels and replaces ISO 15141-1:1998 and ISO 15141-2:1998, which have been technically revised.

The main change compared to the previous edition is that the principle of the extraction and purification has been changed.

Cereals and cereal products — Determination of ochratoxin A — High performance liquid chromatographic method with immunoaffinity column cleanup and fluorescence detection

1 Scope

This document specifies a high performance liquid chromatographic method with immunoaffinity column cleanup for the determination of ochratoxin A in cereals and cereal products.

The limit of quantification is 0,2 µg/kg. The method detection limit is dependent on the sample matrix as well as on the instrument.

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 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

Ochratoxin A (OTA) is extracted by acetonitrile-water. The extract is purified using an immunoaffinity column and ochratoxin A is determined by high performance liquid chromatography (HPLC) on a reverse-phase column and fluorescence detection. The result is verified, if required, by derivatization with boron trifluoride in methanolic solution.

WARNING — Ochratoxin A causes kidney and liver damage and is a probable carcinogen. Observe appropriate safety precautions^[1] for handling such compounds and in particular avoid handling in dry form as the electrostatic nature can result in dispersion and inhalation. 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

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 HPLC analysis.

5.1 Acetonitrile.

5.2 Methanol.

5.3 Sodium chloride (NaCl).

5.4 Glacial acetic acid, $\varphi(\text{CH}_3\text{COOH}) \geq 98 \%$.

5.5 Tween-20.

5.6 Sodium bicarbonate (NaHCO_3).

5.7 Disodium hydrogen phosphate (Na_2HPO_4).

5.8 Potassium dihydrogen phosphate (KH_2PO_4).

5.9 Potassium chloride (KCl).

5.10 Hydrochloric acid, $c(\text{HCl}) = 12 \text{ mol/l}$.

5.11 Ochratoxin A, in crystal form or as a film in ampoules.

5.12 Extraction solvent, mix 60 volume parts of acetonitrile ([5.1](#)) and 40 volume parts of water.

5.13 Phosphate buffered saline (PBS), dissolve 8 g NaCl ([5.3](#)), 1,2 g Na_2HPO_4 ([5.7](#)), 0,2 g KH_2PO_4 ([5.8](#)) and 0,2 g KCl ([5.9](#)) in about 990 ml water. Adjust pH to 7 with HCl ([5.10](#)) and dilute to 1 l with water.

5.14 Washing solution, dissolve 25 g NaCl ([5.3](#)), 5 g NaHCO_3 ([5.6](#)) and 0,1 ml Tween-20 ([5.5](#)) in 1 l water.

5.15 Mobile phase, mix 48 volume parts of acetonitrile ([5.1](#)) with 51 volume parts of water and 1 volume parts of glacial acetic acid ([5.4](#)) and degas this solution before use.

5.16 Toluene.

5.17 Solvent mixture, mix 99 volume parts of toluene ([5.16](#)) with 1 volume parts of glacial acetic acid ([5.4](#)).

5.18 Ochratoxin A stock solution.

Dissolve 1 mg of the ochratoxin A (crystals) ([5.11](#)) or the contents of 1 ampoule (if ochratoxin A has been obtained as a film) ([5.11](#)) in solvent mixture ([5.17](#)) to give a solution containing approximately 20 $\mu\text{g/ml}$ to 30 $\mu\text{g/ml}$ of ochratoxin A.

To determine the exact concentration, record the absorption curve between a wavelength of 300 nm and 370 nm in 5 nm steps in a 1 cm quartz cell ([6.12](#)) with solvent mixture ([5.17](#)) as reference. Identify the wavelength for maximum absorption by recording in 1 nm steps around the maximum as reference.