
**Foodstuffs — Determination of
ochratoxin A in cereals and cereal
products —**

Part 2:

High performance liquid chromatographic
method with bicarbonate clean up

*Produits alimentaires — Dosage de l'ochratoxine A dans les céréales et
produits dérivés —*

*Partie 2: Méthode par chromatographie liquide haute performance
comprenant une étape d'extraction par une solution de bicarbonate*



Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 15141-2 was prepared by the European Committee for Standardization (CEN) in collaboration with ISO Technical Committee TC 34, *Agricultural food products*, Subcommittee SC 4, *Cereals and pulses*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Throughout the text of this standard, read "...this European Standard..." to mean "...this International Standard...".

ISO 15141 consists of the following parts, under the general title *Foodstuffs — Determination of ochratoxin A in cereals and cereal products*:

- *Part 1: High performance liquid chromatographic method with silica gel clean up*
- *Part 2: High performance liquid chromatographic method with bicarbonate clean up*

Annexes A and B of this part of ISO 15141 are for information only.

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Foreword

The text of EN ISO 15141-2/1998 has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 34 "Agricultural food products".

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of an identical text or by endorsement, at the latest by April 1999, and conflicting national standards shall be withdrawn at the latest by April 1999.

This European Standard „Foodstuffs - Determination of ochratoxin A in cereals and cereal products“ consists of two parts:

Part 1: High performance liquid chromatographic method with silica gel clean up

Part 2: High performance liquid chromatographic method with bicarbonate clean up

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This European Standard specifies a method for the determination of ochratoxin A (OTA) at levels greater than 3 µg/kg.

The method has been successfully validated in interlaboratory studies according to ISO 5725:1986 [1] on whole barley containing 2,9 µg/kg, 3,0 µg/kg, 7,4 µg/kg and 14,4 µg/kg of ochratoxin A, on whole maize containing 8,2 µg/kg and 16,3 µg/kg of ochratoxin A as well as on wheat bran containing 3,8 µg/kg and 4,5 µg/kg of ochratoxin A.

NOTE: Numerous laboratory experiences have shown that this method is also applicable to wheat flour.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this draft European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

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

3 Principle

Ochratoxin A is extracted from grains with chloroform-aqueous phosphoric acid and isolated by liquid-liquid partitioning into aqueous bicarbonate solution. The solution is applied to a C₁₈ cartridge, and ochratoxin A is eluted with ethyl acetate-methanol-acetic acid. Ochratoxin A is separated by reversed phase HPLC and identified and quantified by fluorescence. Chromatography of ochratoxin A methyl ester derivative confirms the identification [2] to [5].

WARNING: Ochratoxin A causes kidney and liver damage and is a probable carcinogen. Observe appropriate safety precautions [6] 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) [7], [8].

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of grade 1 according to EN ISO 3696. Solvent shall be of quality for HPLC analysis.

4.1 Chloroform, stabilized with for example 2-methyl-2-butene or ethanol

4.2 Phosphoric acid, $c(\text{H}_3\text{PO}_4) \approx 0,1 \text{ mol/l}$

4.3 Diatomaceous earth

Soak about 900 g of acid-washed diatomaceous earth e.g. Celite® 545¹⁾ overnight in methanol (4.7). Filter through a double layer of paper in a Buchner funnel (5.6), wash with 8 l of water and dry for 12 h at 150 °C.

¹⁾ Celite® 545 is an example of a suitable product available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of these products.