ILNAS

Institut luxembourgeois de la normalisation
de l'accréditation, de la sécurité et qualité
des produits et services

ILNAS-EN 17203:2018

Determination of citrinin in food by liquid chromatography tandem mass spectrometry (LC-MS/MS)

Lebensmittel - Bestimmung von Citrinin in Lebensmitteln mit Flüssigchromatographie und Tandem-Massenspektrometrie (LC-MS/MS)

Produits alimentaires - Dosage de la citrinine dans les produits alimentaires par chromatographie liquide couplée à une spectrométrie de masse en tandem

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National Foreword

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English Version

Foodstuffs - Determination of citrinin in food by liquid chromatography tandem mass spectrometry (LC-MS/MS)

Produits alimentaires - Dosage de la citrinine dans les produits alimentaires par chromatographie liquide couplée à une spectrométrie de masse en tandem (CL-SM/SM)

Lebensmittel - Bestimmung von Citrinin in Lebensmitteln mit Flüssigchromatographie und Tandem-Massenspektrometrie (LC-MS/MS)

This European Standard was approved by CEN on 9 November 2018.

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European foreword

This document (EN 17203:2018) 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of an identical text or by endorsement, at the latest by June 2019, and conflicting national standards shall be withdrawn at the latest by June 2019.

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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Introduction

The mycotoxin citrinin is a polyketide secondary metabolite produced mainly post-harvest in food and feed by several fungi of the genera *Penicillium (e.g. P. citrinum), Aspergillus (e.g. A. candidus)*, and *Monascus (e.g. M. purpureus)*. Citrinin occurs mainly in stored grains like rice, maize, wheat, barley, oats, and rye. Citrinin can be found as a contaminant in red fermented rice with *Monascus purpureus* and its formulated dietary supplements.

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 [5] should be taken into account as well as appropriate National statements.

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 — Citrinin is known to have nephrotoxic properties, damaging the proximal tubules of the kidney [6].

1 Scope

This document describes a procedure for the determination of the citrinin content in food (cereals, red yeast rice (RYR)), herbs and food supplements by liquid chromatography tandem mass spectrometry (LC-MS/MS).

This method has been validated for citrinin in red yeast rice and in the formulated food supplements in the range of 2,5 µg/kg to 3000 µg/kg and in wheat flour in the range of 2,5 µg/kg to 100 µg/kg.

Laboratory experiences have shown that this method is also applicable to white rice, herbs such as a powder of *ginkgo biloba* leaves and the formulated food supplements in the range of 2,5 µg/kg to 50 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 http://www.iso.org/obp

4 Principle

A test portion is humidified with a hydrochloric acid aqueous solution and extracted with ethyl acetate/acetonitrile/glacial acetic acid mixture for 60 min. Magnesium sulfate and sodium chloride are added to the extract, agitated and centrifuged in order to expel water and allow phase separation from the mixture. An aliquot of supernatant is collected, filtered, isotopic labelled internal standard (ISTD) solution is added and analysed by reversed phase LC-MS/MS. Quantification is based on matching citrinin/citrinin-^{13}C ratios and citrinin concentrations.

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 also be used.

5.1 Ethyl acetate, analytical grade or higher.

5.2 Acetonitrile, LC-MS grade. .

5.3 Glacial acetic acid (CH_3COOH), analytical grade or higher.

5.4 Glacial acetic acid (CH_3COOH), LC-MS grade.

5.5 Magnesium sulfate; anhydrous ($MgSO_4$), analytical grade or higher.

5.6 Sodium chloride (NaCl), analytical grade or higher.

5.7 Hydrochloric acid solution (HCl), analytical grade or higher, volume fraction φ(HCl) = 37 % (acidimetric).

5.8 Water (H_2O), deionised (Ultrapure).

5.9 Water (H_2O), LC-MS grade.

5.10 Methanol (MeOH), LC-MS grade.

5.11 Ammonium acetate (CH_3COONH_4), LC-MS grade.

5.12 Extraction solution 1.

Add 10 ml of glacial acetic acid (5.3) to 990 ml of water (5.8) and mix (water + glacial acetic acid, 99+1, v+v). Dissolve 100 g of sodium chloride (5.6) in 1 l of this mixture and add 16 ml of hydrochloric acid solution (5.7). This solution can be used for 1 month if stored at room temperature.

5.13 Extraction solution 2.

Mix 240 ml of acetonitrile (5.2) with 750 ml of ethyl acetate (5.1) and 10 ml of glacial acetic acid (5.3). This solution (ethyl acetate + acetonitrile + glacial acetic acid, 75+24+1, v+v+v) can be used for 1 month if stored at room temperature.

5.14 Dilution solution.

Mix 80 ml of methanol (5.10), 18 ml of water (5.9) and 2 ml of glacial acetic acid (5.4). This solution (methanol + water + glacial acetic acid, 80+18+2, v+v+v) can be used for 1 month if stored at room temperature.

5.15 Ammonium acetate/glacial acetic acid in water.

Dissolve 9,5 g of ammonium acetate (5.11) in 12,5 ml of water (5.9), then add 12,5 ml of glacial acetic acid (5.4) and mix thoroughly. This solution can be used for 12 months if stored at < −18 °C.

5.16 Mobile phase A: ammonium acetate/glacial acetic acid in water, c = 5 mmol/l.

Add 1 ml of ammonium acetate/glacial acetic acid in water (5.15) to 999 ml of water (5.9) and mix thoroughly.

5.17 Mobile phase B: ammonium acetate/glacial acetic acid in methanol, c = 5 mmol/l.

Add 1 ml of ammonium acetate/glacial acetic acid in water (5.15) to 999 ml of methanol (5.10) and mix thoroughly.

5.18 Citrinin, analytical standard > 99 %, e.g. crystalline or as certified standard solution.

5.19 Citrinin stock solution, mass concentration ρ = 500 µg/ml.

Weigh 5 mg of crystalline citrinin to the nearest 0,1 mg into a 10 ml volumetric flask and dissolve with acetonitrile (5.2) by filling up to the mark. The mass concentration of this stock solution shall be checked. This can be achieved via LC-MS/MS analysis against the certified standard solution (5.18) or by a photometric determination of concentration using the molar extinction coefficient [8].

The certified standard solution (5.18) can alternatively be used as stock solution.