

Foodstuffs - Determination of citrinin in food by
HPLC-MS/MS

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN 17203:2021 sisaldab Euroopa standardi EN 17203:2021 ingliskeelset teksti.	This Estonian standard EVS-EN 17203:2021 consists of the English text of the European standard EN 17203:2021.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation and Accreditation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 05.05.2021.	Date of Availability of the European standard is 05.05.2021.
Standard on kättesaadav Eesti Standardimis- ja Akrediteerimiskeskusest.	The standard is available from the Estonian Centre for Standardisation and Accreditation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

ICS 67.050

Standardite reprodutseerimise ja levitamise õigus kuulub Eesti Standardimis- ja Akrediteerimiskeskusele. Andmete paljundamine, taastekitamine, kopeerimine, salvestamine elektroonsesse süsteemi või edastamine ükskõik millises vormis või millisel teel ilma Eesti Standardimis- ja Akrediteerimiskeskuse kirjaliku loata on keelatud.

Kui Teil on küsimusi standardite autorikaitse kohta, võtke palun ühendust Eesti Standardimis- ja Akrediteerimiskeskusega: Koduleht www.evs.ee; telefon 605 5050; e-post info@evs.ee

The right to reproduce and distribute standards belongs to the Estonian Centre for Standardisation and Accreditation. No part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, without a written permission from the Estonian Centre for Standardisation and Accreditation.

If you have any questions about copyright, please contact Estonian Centre for Standardisation and Accreditation: Homepage www.evs.ee; phone +372 605 5050; e-mail info@evs.ee

English Version

Foodstuffs - Determination of citrinin in food by HPLC-MS/MS

Produits alimentaires - Dosage de la citrinine dans les produits alimentaires par CLHP-SM/SM

Lebensmittel - Bestimmung von Citrinin in Lebensmitteln mit HPLC-MS/MS

This European Standard was approved by CEN on 19 March 2021.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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.....	8
7 Procedure.....	9
7.1 Preparation of the test sample.....	9
7.2 Extraction of citrinin.....	9
7.3 Spiking procedure.....	10
7.4 Preparation of sample test solutions.....	10
7.5 LC-MS/MS analysis.....	10
7.5.1 General.....	10
7.5.2 LC-MS/MS operating conditions	11
7.6 Identification	12
8 Calculation.....	12
8.1 General.....	12
8.2 Calculation with the internal standard	12
9 Precision.....	14
9.1 General.....	14
9.2 Repeatability.....	14
9.3 Reproducibility	14
10 Test report.....	14
Annex A (informative) Example conditions for suitable LC-MS/MS systems	15
Annex B (informative) Typical chromatograms	17
Annex C (informative) Precision data.....	20
Bibliography.....	23

European foreword

This document (EN 17203:2021) 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 November 2021, and conflicting national standards shall be withdrawn at the latest by November 2021.

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.

This document supersedes EN 17203:2018.

This document has been prepared under a standardization request given to CEN by the European Commission and the European Free Trade Association.

The alterations to the version of 2018 are as follows:

- 5.24 The necessity to prepare calibration solutions freshly every day was deleted.
- 6.14.2 The requirement for cross contamination below 1 % was deleted.
- 7.5.1 First sentence was re-worded in better language.
- 7.5.2 The last part “when negative ion mode is used” of second para before Table 2, starting with “When an adduct ion is used as precursor ion” was deleted as not applicable for this method.
- 7.5.2 Table 2, the last column for the 2nd qualifier was re-introduced again.
- 7.6 Para 2 line 1 was re-worded in a clearer way.
- 8.1 Para 3 was aligned with other standards of CEN/TC 275/WG 5.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

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 3 000 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 <https://www.electropedia.org/>
- ISO Online browsing platform: available at <https://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, internal standard (ISTD) solution is added and analysed by reversed phase LC-MS/MS. Quantification is based on matching citrinin/citrinin-¹³C 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 (CH₃COOH), analytical grade or higher.

5.4 Glacial acetic acid (CH₃COOH), LC-MS grade.

5.5 Magnesium sulfate; anhydrous (MgSO₄), analytical grade or higher.