SLOVENSKI STANDARD
oSIST prEN 17203:2019

01-december-2019

Živila - Določevanje citrinina v živilih s HPLC-MS/MS

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

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

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

Ta slovenski standard je istoveten z: **prEN 17203**

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

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EUROPEAN STANDARD

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Will supersede EN 17203:2018

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 draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 275.

If this draft becomes a European Standard, 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.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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

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

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European foreword

This document (prEN 17203:2019) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

This document will supersede 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.

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.

5.20 Citrinin working solution, ρ = 100 µg/ml.

Pipette 1 ml of the stock solution (5.19) into 4 ml of the dilution solution (5.14) and homogenize the mixture.

A correction factor (see 5.19) shall be determined and used to correct the exact concentration of this working solution (mass concentration ρ = 100 µg/ml). This step may be omitted when using the certified standard solution (5.18).

The stock and working solutions can be used for 12 months if stored at < −18 °C and protected against light and moisture.

5.21 Citrinin intermediate solution, ρ = 1 µg/ml.

Pipette 50 µl of the citrinin working solution (5.20) into 4950 µl of the dilution solution (5.14) which results in 5 ml and homogenize the mixture.

Use this intermediate solution as spiking solution for recovery experiments. This solution can be used for 3 months if stored in amber flasks (6.13) at < −18 °C.

5.22 Citrinin-^{13}C stock solution, as standard solution at a given mass concentration, e.g. ρ = 100 µg/ml.

After opening, the stock solution can usually be used for 12 months if stored at < −18 °C in amber flasks (see its expiry date in the Certificate of Analysis).

If obtained as crystalline, dissolve the citrinin-^{13}C in a suitable amount of acetonitrile (5.2) to obtain a stock solution at a suitable mass concentration (e.g. ρ = 100 µg/ml). This stock solution can also usually be used for 12 months if stored at < −18°C

5.23 Citrinin-^{13}C intermediate solution, ρ = 100 ng/ml.

Dilute the citrinin-^{13}C stock solution (5.22) with dilution solution (5.14) and mix well to obtain the citrinin-^{13}C intermediate solution at a concentration of ρ = 100 ng/ml. Use this solution as internal standard (ISTD) and add it to each of the sample extracts, the calibration solutions (5.24) and citrinin control solution (5.25).

This solution can be used for 6 months if stored in amber flasks at < −18 °C. Check the stability of the solution when using after 6 months.

5.24 Calibration solutions.

Prepare e.g. the following calibration solutions (within a range from 0,25 ng/ml to 50 ng/ml) as outlined in Table 1 using the citrinin intermediate solution (5.21) and the dilution solution (5.14). Homogenize the mixtures.

Start with calibration solution 6 which is then used to prepare calibration solutions 1, 2 and 3 (see Table 1).

Table 1 — Examples of suitable calibration solutions

Calibration solution	Mass concentration ng/ml	Total volume µl	Citrinin intermediate solution (5.21) µl	Calibration solution 6 µl	Dilution solution (5.14) µl
1	0,25	1000		5	995
2	0,5	1000		10	990
3	1	1000		20	980
4	10	1000	10		990
5	20	1000	20		980
6	50	1000	50		950

Take 45 µl from every calibration solution (1 to 6), add 5 µl of the citrinin-^{13}C intermediate solution (5.23) and mix.

It is also possible to increase the volumes of the calibration and intermediate solutions in proportion to one another.

5.25 Citrinin control solution, ρ = 25 ng/ml.

Prepare a citrinin control solution from the certified standard solution (5.18). Alternatively, prepare a citrinin control solution from a suitable independent citrinin stock solution that has been checked photometrically or has been checked via a certified standard solution (5.18). Prepare the control solution (ρ = 25 ng/ml) by diluting the stock solution with dilution solution (5.14).

Add 5 µl of the citrinin-^{13}C intermediate solution (5.23) to 45 µl of this control solution before analysis.

This control solution can be used for 3 months if stored in amber flasks at < −18 °C.

6 Apparatus and equipment

Usual laboratory glassware and equipment, in particular, the following.

For glassware, use preferably amber coloured to reduce light effect and degradation of citrinin.

6.1 Laboratory balance, accuracy of 0,01 g.

6.2 Analytical balance, accuracy of 0,1 mg.

6.3 Laboratory shaker.

6.4 Centrifuge, suitable for 50 ml centrifuge tubes.

6.5 Centrifuge, suitable for 1,5 ml centrifuge tubes.

6.6 Pipettes, adjustable e.g. 1 µl to 1000 µl, suitable for organic solvents, with appropriate tips.

6.7 Centrifuge tubes, 50 ml.

6.8 Centrifuge tubes, polypropylene, (e.g. Eppendorf®[1] tube), 1,5 ml.

6.9 Syringe, 2 ml.

6.10 Filters for syringe, polytetrafluoroethylene (PTFE), with 0,2 µm pore diameter.

6.11 Amber vials for injection (1,5 ml), with screw cap or equivalent.

6.12 Glass inserts.

6.13 Amber flasks.

6.14 LC-MS/MS system, comprising the following**:**

6.14.1 LC pump, suitable for gradient elution.

6.14.2 Injection system, capable of injecting an appropriate volume of injection solution with sufficient accuracy.

6.14.3 LC column, e.g. HSST3 C18 column, 1,8 µm, 2,1 mm ×100 mm and corresponding pre-filter or pre-column.

Columns of different dimensions may also be used that ensure base line separation to distinguish peaks of the citrinin from all other signals.

6.14.4 Column thermostat.

6.14.5 Tandem mass spectrometer (MS/MS), capable of performing ionization of citrinin and multiple reaction monitoring (MRM) with a sufficiently wide dynamic range.

6.14.6 Data evaluation system.

7 Procedure

7.1 Preparation of the test sample

Finely grind the laboratory sample, homogenize and store in the dark before taking a test portion for analysis.

7.2 Extraction of citrinin

Weigh a test portion of 4,00 g of the homogenized laboratory sample to the nearest 0,02 g into a 50-ml centrifugation tube (6.7).

Add 10 ml of the extraction solution 1 (5.12) followed immediately by 20 ml (V_{ex}) of extraction solution 2 (5.13) and cap the tube.

Shake the mixture for approximately 1 h using a laboratory shaker (6.3) at room temperature.

Thereafter, add 6,0 g of magnesium sulfate (5.5) and 1,5 g of sodium chloride (5.6) to the mixture and immediately shake vigorously for 30 s to avoid aggregation of the salts.

NOTE This step is exothermic and results in moderate but noticeable warming of the tube and its contents.

[1] Eppendorf is a trade name of a centrifugation tube supplied by Eppendorf International. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent tubes may be used if they can be shown to lead to the same results.