SLOVENSKI STANDARD
SIST EN 17279:2019

01-december-2019

Živila - Multirezidualna presejalna metoda za ugotavljanje prisotnosti aflatoksina B1, deoksinivalenola, fumonizinov B1 in B2, ohratoksina A, toksina T-2 in HT-2 ter zearalenona v živilih, razen v hrani za dojenčke in majhne otroke, s HPLC-MS/MS

Foodstuffs - Multimethod for the screening of aflatoxin B1, deoxynivalenol, fumonisin B1 and B2, ochratoxin A, T-2 toxin, HT-2 toxin and zearalenone in foodstuffs, excluding foods for infants and young children, by LC-MS/MS

Lebensmittel - Multiverfahren mit HPLC-MS/MS zum Screening auf Aflatoxin B1, Deoxynivalenol, Fumonisin B1 und B2, Ochratoxin A, T2-HT-2-Toxin und Zearalenon in Lebensmitteln außer Lebensmittel für Säuglinge und Kleinkinder

Produits alimentaires - Multiméthode de dépistage de l'aflatoxine B1, du déoxynivalénol, de la fumonisine B1 et B2, de l'ochratoxine A, de la toxine T-2, de la toxine HT-2 et de la zéaralénone dans les produits alimentaires, à l'exception des aliments pour nourrissons et jeunes enfants, par CLHP-SM/SM

Ta slovenski standard je istoveten z: **EN 17279:2019**

ICS:

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

SIST EN 17279:2019 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 17279

October 2019

ICS 67.050

English Version

Foodstuffs - Multimethod for the screening of aflatoxin B1, deoxynivalenol, fumonisin B1 and B2, ochratoxin A, T-2 toxin, HT-2 toxin and zearalenone in foodstuffs, excluding foods for infants and young children, by LC-MS/MS

Produits alimentaires - Multiméthode de dépistage de l'aflatoxine B1, du déoxynivalénol, de la fumonisine B1 et B2, de l'ochratoxine A, de la toxine T-2, de la toxine HT-2 et de la zéaralénone dans les produits alimentaires, à l'exception des aliments pour nourrissons et jeunes enfants, par CLHP-SM/SM

Lebensmittel - Multiverfahren mit HPLC-MS/MS zum Screening auf Aflatoxin B1, Deoxynivalenol, Fumonisin B1 und B2, Ochratoxin A, T2-HT-2-Toxin und Zearalenon in Lebensmitteln außer Lebensmittel für Säuglinge und Kleinkinder

This European Standard was approved by CEN on 5 August 2019.

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

Ref. No. EN 17279:2019 E

Contents

Page

European foreword

This document (EN 17279:2019) 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 April 2020, and conflicting national standards shall be withdrawn at the latest by April 2020.

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 has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

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

Mycotoxins are fungal metabolites that may occur in various foodstuffs. Cereals and cereal products, peanuts, dried fruits and relevant derived products are most likely to be affected by the mycotoxins covered by this document (aflatoxin B_1, deoxynivalenol, fumonisin B_1 and B_2, ochratoxin A, HT-2 and T-2 toxins, and zearalenone).

WARNING 1 — Suitable precaution and protection measures need to be taken when carrying out working steps with harmful chemicals. The hazardous substances ordinance Regulation (EU) 1907/2006 [2] 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.

1 Scope

This document specifies a screening method for the determination of aflatoxin B_1, deoxynivalenol, fumonisin B_1 and B_2, ochratoxin A, T-2 toxin, HT-2 toxin, and zearalenone in foodstuffs by high performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS).

The aim of the screening method is to determine whether a certain pre-defined concentration (the screening target concentration, *STC*) is exceeded or not. The result of the screening is either "negative" or "suspect". "Negative" (screen negative) means that the targeted mycotoxins are not detected or potentially present but below the *STC*. "Suspect" (screen positive) means that the established cut-off value is exceeded and the sample can contain one or more mycotoxins at a concentration higher than the *STC*.

For full identification and accurate quantification a confirmatory quantitative analysis method is required which is outside the scope of this document.

The method is suitable for various types of foodstuff and has been validated for representative matrices from four commodity groups (see detailed data in Annex C):

— high starch and/or protein content and low water and fat content: wheat, cereal mixture, wheat flour and cornflakes;

— high oil content: peanuts;

— high sugar and low water content: dried figs;

— high water content: grape juice.

During validation, cut-off values were established for the following screening target concentrations:

— aflatoxin B_1: 2 µg/kg to 5 µg/kg;

— deoxynivalenol: 250 µg/kg to 865 µg/kg;

— fumonisin B_1: 200 µg/kg to 790 µg/kg;

— fumonisin B_2: 110 µg/kg to 230 µg/kg;

— ochratoxin A: 4 µg/kg to 9 µg/kg;

— T-2 toxin: 25 µg/kg;

— HT-2 toxin: 25 µg/kg to 50 µg/kg;

— zearalenone: 30 µg/kg to 100 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 https://www.iso.org/obp

4 Principle

The mycotoxins are extracted from the homogenized sample material, after addition of water, by shaking with acidified acetonitrile. After a salt-induced phase partitioning and centrifugation, the acetonitrile extract is diluted with water, optionally filtered, and analysed by HPLC coupled with MS/MS. The relative response of each mycotoxin to its isotopic labelled analogue added to the final extract at the screening target concentration (*STC*), is tested against an established cut-off value.

5 Reagents

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696, unless otherwise specified. Solvents shall be of quality for LC analysis, unless otherwise specified.

5.1 Water, deionised.

5.2 Water, LC-MS grade, double distilled or water of grade 1 as defined in EN ISO 3696.

5.3 Acetonitrile, pro analysis (p.a.).

5.4 Acetic acid, purity greater than 98 % (m/m).

5.5 Magnesium sulfate ($MgSO_4$) - anhydrous, p.a.

5.6 Aflatoxin B_1 (AB1) e.g. crystalline, as a film or as certified standard solution.

5.7 Deoxynivalenol (DON) e.g. crystalline, as a film or as certified standard solution.

5.8 Fumonisin B_1 (FB1) e.g. crystalline, as a film or as certified standard solution.

5.9 Fumonisin B_2 (FB2) e.g. crystalline, as a film or as certified standard solution.

5.10 HT-2 toxin (HT-2) e.g. crystalline, as a film or as certified standard solution.

5.11 Ochratoxin A (OTA) e.g. crystalline, as a film or as certified standard solution.

5.12 T-2 toxin (T-2) e.g. crystalline, as a film or as certified standard solution.

5.13 Zearalenone (ZEA) e.g. crystalline, as a film or as certified standard solution.

5.14 ^{13}C Aflatoxin B_1 (^{13}C-AB1) e.g. solution ρ = 0,5 mg/l, in acetonitrile.

5.15 ^{13}C Deoxynivalenol (^{13}C-DON) e.g. solution ρ = 25 mg/l, in acetonitrile.

5.16 ^{13}C Fumonisin B_1 (^{13}C -FB1) e.g. solution ρ = 25 mg/l, in acetonitrile/water.

5.17 **^{13}C Fumonisin B$_2$ (^{13}C-FB2)** e.g. solution ρ = 25 mg/l, in acetonitrile/water.

5.18 **^{13}C HT-2 toxin (^{13}C-HT2)** e.g. solution ρ = 25 mg/l, in acetonitrile.

5.19 **^{13}C Ochratoxin A (^{13}C-OTA)** e.g. solution ρ = 10 mg/l, in acetonitrile.

5.20 **^{13}C T-2 toxin (^{13}C-T2)** e.g. solution ρ = 25 mg/l, in acetonitrile.

5.21 **^{13}C Zearalenone (^{13}C-ZEA)** e.g. solution ρ = 25 mg/l, in acetonitrile.

5.22 **Extraction solution,** acetonitrile containing 1 % of acetic acid.

Add 1 part per volume of acetic acid (5.4) to 99 parts per volume of acetonitrile (5.3) and mix. This solution can be used for six months if stored at room temperature.

5.23 Individual stock solutions

The individual solutions are either prepared by dissolving neat (solid) standards in an appropriate solvent, or from individual stock solutions purchased as such. The mycotoxins covered in this European Standard dissolve well in acetonitrile, with the exception of fumonisins for which a mixture of acetonitrile and water (50+50, v+v) is recommended for preparing individual stock solutions.

Calculate for each of the individual mycotoxins the mass concentration, ρ, in ng/ml according to Formula (1):

$$\rho = 20 \times D \times STC \quad (1)$$

where

D is the dilution factor (g sample per ml final extract) (D = 0,25 by default), in g/ml;

STC is the screening target concentration (mass fraction) in the sample, in µg/kg.

EXAMPLE For a mycotoxin with an *STC* of 1 000 µg/kg in the sample, the mass concentration of this mycotoxin in the mixed mycotoxin stock solution is 5 000 ng/ml.

5.24 Mixed stock solution

Prepare a mixed stock solution containing all individual mycotoxins at a mass concentration calculated according to Formula (1), using the appropriate pipets (6.6) and a mixture of acetonitrile and water (80+20, v+v). This solution can be used for six months if stored in the dark at 4°C.

This mixed stock solution may be used for the preparation of positive control samples (7.4).

5.25 Mixed internal standard (ISTD) solution (isotopically labelled mycotoxins).

Isotopically labelled mycotoxins are generally available as certified standard solutions. Prepare a mixed ISTD solution in a mixture of acetonitrile and water (80+20, v+v), containing all isotopically labelled mycotoxins at a mass concentration calculated according to Formula (1).

EXAMPLE For a mycotoxin with an *STC* of 10 µg/kg in the sample, the mass concentration of the corresponding isotopic label in the mixed ISTD solution is 50 ng/ml.

This solution can be used for six months if stored in the dark at 4 °C.

This solution is used as internal calibrant and is to be added to the mixed standard solution (5.26) and each of the sample extracts (7.3).

5.26 Mixed standard solution.

Prepare a mixed standard solution by combining 1 part per volume of the mixed mycotoxin stock solution (5.24), 1 part per volume of the mixed ISTD solution (5.25), 8 parts per volume of extraction solution (5.22), and 10 parts per volume of water (5.2). A volume of typically 400 µl is freshly prepared for each batch of analysis.

EXAMPLE Preparation in vial:

20 µl	mixed stock solution (5.24);
20 µl	mixed ISTD solution (5.25);
160 µl	extraction solution (5.22);
200 µl	water (5.2).

The mixed standard solution is used to check correct measurement of the mycotoxins and their isotopic labelled analogues (7.5.4).

6 Apparatus and equipment

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

6.1 Conical polypropylene screw cap centrifuge tubes, 50 ml with caps.

6.2 Analytical balance, accuracy: 0,01 mg.

6.3 Laboratory balance, accuracy: 0,01 g.

6.4 Adjustable mechanical vertical or **horizontal shaker** or **rotary tumbling machine.**

6.5 Laboratory shaker.

6.6 Pipets, adjustable, e.g. 10 µl to 100 µl and 100 µl to 1000 µl, suited for organic solvents (e.g. positive displacement pipets), with appropriate tips.

6.7 Centrifuge, capable of generating a relative centrifugal force of 3 000 *g*.

6.8 Vials, 1,5 ml to 2 ml, made of glass or polypropylene with screw cap.

6.9 Syringe filter or **centrifuge filter,** 0,20 µm to 0,45 µm, made of nylon or polytetrafluoroethylene (PTFE).

6.10 Auto sampler vials, of appropriate size for the auto sampler in use, e.g. glass with insert vials, filter vials (PTFE, 0,45 µm), with crimp cap or equivalent.

6.11 LC-MS/MS system, with the following components:

6.11.1 LC pump, capable of delivering a binary gradient at flow rates appropriate for the analytical column in use with sufficient accuracy.

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

6.11.3 LC column, capable of retaining the target mycotoxins, preferably with a retention factor of at least two.

6.11.4 Column oven, capable of maintaining a constant temperature.

6.11.5 Tandem mass spectrometer (MS/MS), capable of ionization of the mycotoxins (either resulting in positive or negative ions), performing Multiple Reaction Monitoring (MRM), and with a sufficiently wide dynamic range.

NOTE Instruments capable of alternating measurement of positive and negative ions (pos/neg switching) are beneficial because these can cover all target analytes within one run.

6.11.6 Data evaluation system.

7 Procedure

7.1 Preparation of the test sample

Finely grind the laboratory sample and homogenize it.

7.2 Extraction

The amount of homogenized test sample examined is 5 g. For samples homogenized by slurry mixing, the amount of slurry corresponding to 5 g of the original sample is used.

Weigh the test portion indicated in Table 1 to the nearest 0,01 g into a 50 ml centrifuge tube (6.1).

Add water (5.1) and acidified acetonitrile (5.22) to the sample as indicated in Table 1. Close the tube, and shake thoroughly by hand. Make sure dry samples are suspended into the liquid.

Table 1 — Sample set-up

Sample	Test portion	Added water	Extraction solution (5.22)[d]	$MgSO_4$ (5.5)[e]
	g	ml	ml	g
Sample with moisture content < 15 %[a]	5	10	10	5
Slurried sample 1+1[b]	10	5	10	5
Slurried sample 1+1,5[b]	12,5	2,5	10	5
Slurried sample 1+2[b]	15	0	10	5
Slurried sample 1+3[b]	20	0	10	7,5
Samples with moisture content > 85 %[c]	5	5	10	5

[a] e.g. dry milled cereals.

[b] x + y means: x g sample with y ml of water.

[c] most fresh fruits/vegetables, liquids .

[d] ratio of water and extraction solvent is 1+1, v+v, except for 'Slurried sample 1+3' (1+0,67, v+v).

[e] the amount of magnesium sulfate is 0,5 g per ml of total water (from sample and added) in the extraction tube.

Place the tubes in a mechanical shaker (6.4) and shake for 30 min.

Open the tube, add the amount of magnesium sulfate (5.5) indicated in Table 1 to the tube, close the tube, immediately shake for approximately 5 s to avoid formation of lumps of magnesium sulfate. Shake the tube vigorously for approximately 30 s by hand or in a mechanical shaker (6.5).

Centrifuge the tube at approximately 3 000 *g* for at least 5 min to aid settlement of particulate matter and phase separation.

NOTE After phase partitioning, the volume of the acetonitrile phase (upper layer) is approximately 10,7 ml and contains approximately 17 % of water [4].

7.3 Preparation of the sample test solution

Using the appropriate pipets, add 10 parts per volume of extract into a vial (6.8 or 6.10), furthermore add 1 part per volume of mixed ISTD solution (5.25) and 9 parts per volume of water (5.1). Mix using a shaker (6.5) for at least 5 s.

NOTE 1 Volumes of 200 µl of extract, 20 µl of mixed ISTD solution and 180 µl of water have shown to work well.

The final extract can be turbid. Turbid extracts can be injected without adverse effects on the analysis. In case precipitates occur, these shall be removed by centrifugation or by filtration, either using a filter vial (6.10), centrifuge filter, or a syringe filter (6.9). In the latter case, the total volume of 400 µl indicated above might be too small.

NOTE 2 The final extract composition is acidic acetonitrile and water (approximately 1+1, v+v). A lower content of organic solvent can result in solubility limitations of higher concentrations of less water soluble mycotoxins such as zearalenone.

7.4 Preparation of control samples

With each batch of samples, one negative control and one positive control is used.

The negative control is either a sample free of the target mycotoxins (non-detectable or < 10 % of the *STC*), or, if not available, a reagent blank.

To create a reagent blank, perform extraction (7.2) and subsequent steps without adding the sample test portion.

The positive control is a sample free of the target mycotoxins (non-detectable or < 10 % of the *STC*) which is spiked with the mycotoxins at the *STC*. Alternatively, a reference material known to contain the target mycotoxins at a concentration close to the *STC* is used.

For preparation of a positive control sample: spike a sample free of the target mycotoxins by adding 1,0 ml of mixed stock solution (5.24) to a test sample free of the target mycotoxins.

7.5 LC-MS/MS analysis

7.5.1 General

The LC-MS/MS system, injection volume, mobile phase composition and gradient, acquisition conditions, and data processing parameters shall be such that the targeted mycotoxins are detected at ≤ 25 % of the [cut-off value × *STC*], with sufficient selectivity to obtain an acceptable low false suspect rate.

Examples of LC-MS/MS settings are given in Annex A.