SLOVENSKI STANDARD
oSIST prEN 17641:2021

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Živila - Večelementna metoda za določevanje aflatoksina, deoksinivalenola, fumonizinov, ohratoksina A, toksinov T-2 in HT-2 ter zearalenona z LC-MS/MS

Foodstuffs - Multimethod for the determination of aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, T-2 toxin, HT-2 toxin and zearalenone by LC-MS/MS

Lebensmittel — Multimethode für die Bestimmung von Aflatoxinen, Deoxynivalenol, Fumonisinen, Ochratoxin A, T 2 Toxin, HT 2 Toxin und Zearalenon mittels LC MS/MS

Produits alimentaires — Multiméthode de détermination de la teneur en aflatoxines, déoxynivalénol, fumonisines, ochratoxine A, toxine T-2, toxine HT-2 et zéaralénone par CL-SM/SM

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Foodstuffs - Multimethod for the determination of aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, T-2 toxin, HT-2 toxin and zearalenone by LC-MS/MS

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

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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 17641:2021) 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.

Introduction

Mycotoxins are fungal metabolites that may occur in various foodstuffs such as cereals, nuts, spices, fruits, oil seeds, or coffee. Mycotoxins can be produced before harvest in the crop and even after harvest if climate conditions are favourable for further fungal growth. Milk can be contaminated as well by Aflatoxin M_1, the major metabolite of Aflatoxin B_1, when cows are fed with Aflatoxin B_1 contaminated feed. To protect consumer health, maximum levels for mycotoxins in foodstuffs have been established in a broad range of food commodities including those intended for infants and young children consumption.

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 [1], 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 — Aflatoxins are known to have carcinogenic effects and to be both acutely and chronically toxic. Aflatoxins B_1, B_2, G_1, G_2 and M_1 are classified as carcinogenic to humans (Group 1) by the International Agency for Cancer Research (IARC). Fumonisin B_1, fumonisin B_2 and ochratoxin A have been classified as possibly carcinogenic to humans (Group 2B) and zearalenone, deoxynivalenol and T-2 as not classifiable as to their carcinogenicity to humans (Group 3) [2].

1 Scope

This document describes an isotope dilution method for the quantitative determination of aflatoxins B_1, B_2, G_1, G_2 and M_1 (AFB1, AFB2, AFG1, AFG2 and AFM1), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEN), T-2 and HT-2 toxins (T-2 and HT-2) and fumonisins B_1 and B_2 (FB1 and FB2) in foods by liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS).

A specific immunoaffinity column (IAC) clean-up is needed for aflatoxins (AFs) and OTA in infant foods (e.g. infant cereals, milk-based powders), in spices, in dried fruits and in nuts.

The method has been validated through an intercollaborative study on different commodity groups: cereals and cereal-based products including food for infant and young children, nuts, spices, dried fruits and milk powder. The ranges of concentrations of each mycotoxin in these naturally contaminated and/or spiked food samples were:

— aflatoxin B_1: 0,0857 µg/kg – 11,4 µg/kg;
— aflatoxin B_2: 0,0792 µg/kg – 12,5 µg/kg;
— aflatoxin G_1: 0,0628 µg/kg – 20,9 µg/kg;
— aflatoxin G_2: 0,0520 µg/kg – 15,0 µg/kg;
— aflatoxin M_1: 0,0342 µg/kg – 0,110 µg/kg;
— ochratoxin A: 0,448 µg/kg – 17,2 µg/kg;
— deoxynivalenol: 45,2 µg/kg – 743 µg/kg;
— zearalenone: 9,57 µg/kg – 131 µg/kg;
— T-2 toxin: 10,3 µg/kg – 57,9 µg/kg;
— HT-2 toxin: 9,50 µg/kg – 81,8 µg/kg;
— fumonisin B_1: 31,1 µg/kg – 4 262 µg/kg;
— fumonisin B_2: 44,2 µg/kg – 1 299 µg/kg.

The measuring ranges of the method for each mycotoxin/matrix combination are given in Table 7.

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 Principles

The mycotoxins are extracted from the test portion with water and acidified acetonitrile and a liquid-liquid partition is initiated after addition of magnesium sulphate and sodium chloride salts. The resulting acetonitrile supernatant is then defatted with hexane. Depending on the mycotoxin/matrix combination, the sample extract is then submitted to two different protocols (a general scheme is given in Annex B):

— *without IAC clean-up*: generic procedure for the determination of all mycotoxins in cereals and cereal-based products. An aliquot of the acetonitrile supernatant is evaporated to dryness, then reconstituted in a methanol-water solution and subsequently analysed by LC-MS/MS;

— *with IAC clean-up*: specific procedure for AFs and OTA determination in infant foods (e.g. infant cereals, milk powder) for sensitivity purpose and in spices, nuts and dried fruits to prevent matrix effects in the mass spectrometer instrument. An aliquot of the acetonitrile supernatant is first diluted in a phosphate buffered saline (PBS) solution and the whole extract is then applied onto an IAC containing antibodies specific to AFs and OTA. The IAC is washed with water and the mycotoxins are eluted with methanol. The eluate is evaporated to dryness, reconstituted in a methanol-water solution, and subsequently analysed by LC-MS/MS.

Quantification is performed by the isotopic dilution approach using ^{13}C isotopically labelled mycotoxins as internal standards (ISTDs).

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 LC-MS grade for LC-MS analysis, unless otherwise specified. Commercially available solutions with equivalent properties to those listed may be used.

WARNING 1 — Decontamination procedures for laboratory wastes of aflatoxins were developed by the IARC [3, 4].

5.1 Nitrogen compressed gas, purity equivalent to 99,99 % or better.

5.2 Water, HPLC grade.

5.3 Water, LC-MS grade.

5.4 Methanol (MeOH), analytical grade.

5.5 Methanol (MeOH), LC-MS grade.

5.6 Acetonitrile (MeCN), analytical grade.

5.7 Formic acid, analytical grade.

5.8 Ammonium formate, LC-MS grade.

5.9 Acetic acid glacial, analytical grade.

5.10 n-Hexane, analytical grade.

5.11 Magnesium sulphate ($MgSO_4$) anhydrous, analytical grade.

5.12 Sodium chloride (NaCl), analytical grade.

5.13 Partitioning salts mixture, $MgSO_4$-NaCl (4 + 1, *w* + *w*).

Weigh 4,0 g ± 0,2 g of $MgSO_4$ (5.11) and 1,00 g ± 0,05 g of NaCl (5.12) into a 15 ml polypropylene tube.

Alternatively, a ready-to-use partitioning salt mixture can be supplied by commercial sources.

5.14 Potassium chloride, (KCl) analytical grade.

5.15 Potassium dihydrogen phosphate, (KH_2PO_4) analytical grade.

5.16 Disodium hydrogen orthophosphate, (Na_2HPO_4) analytical grade.

5.17 Sodium hydroxide, (NaOH) analytical grade.

5.18 Sodium hydroxide solution, (NaOH) molar concentration c = 0,1 mol/l.

Dissolve 0,4 g of sodium hydroxide (5.17) in 100 ml water (5.2).

5.19 Hydrochloric acid solution, (HCl) analytical grade, φ(HCl) = 37 %.

5.20 Hydrochloric acid diluted solution, c = 0,1 mol/l.

Add 8,4 ml of hydrochloric acid solution (5.19) into a 1-l volumetric flask and complete to volume with water (5.2).

5.21 Phosphate buffered saline (PBS) solution, pH 7,3 ± 0,2

Weigh 0,20 g of KCl (5.14), 0,20 g of KH_2PO_4 (5.15), 1,15 g of Na_2HPO_4 (5.16) and 8,00 g of NaCl (5.12) to the nearest 0,01 g and transfer into a 1-l volumetric flask. Dissolve in water (5.2) and add 900 ml of water (5.2).

After dissolution adjust the pH to 7,3 ± 0,2 with either HCl solution (5.20) or NaOH solution (5.18), then fill up to the mark with water (5.2).

Alternatively, a PBS solution with equivalent properties may be prepared from commercially available PBS material.

5.22 Extraction solution, acetic acid 5 ml/l in acetonitrile.

Mix 500 ml of acetonitrile (5.6) and 5 ml of acetic acid glacial (5.9) into a 1-l volumetric flask. Complete to volume with acetonitrile (5.6) and mix well. This solution can be used for 3 months if stored at room temperature.

5.23 Diluting solution, methanol-water (15 + 85, *V* + *V*).

Mix 15 ml of methanol (5.4) with 85 ml of water (5.2) into a 100-ml volumetric flask. This solution can be used for 3 months if stored at room temperature.

5.24 Mycotoxins analytical standard, e.g. crystalline, as a film or as a certified standard solution.

5.24.1 Aflatoxin B_1 (AFB1).

5.24.2 Aflatoxin B_2 (AFB2).

5.24.3 Aflatoxin G_1 (AFG1).

5.24.4 Aflatoxin G_2 (AFG2).

5.24.5 Aflatoxin M_1 (AFM1).

5.24.6 Ochratoxin A (OTA).

5.24.7 Deoxynivalenol (DON).

5.24.8 Zearalenone (ZEN).

5.24.9 T-2 Toxin (T-2).

5.24.10 HT-2 Toxin (HT-2).

5.24.11 Fumonisin B_1 (FB1).

5.24.12 Fumonisin B_2 (FB2).

5.25 Mycotoxin stock standard solutions.

The individual stock standard solutions are either prepared by dissolving neat (solid) standards in an appropriate solvent or prepared from dried-down films and subsequently reconstituted according to the certificate of each individual standard or purchased as ready-to-use solutions (5.24).

The mycotoxins covered in this document dissolve well in acetonitrile, with the exception of fumonisins for which acetonitrile/water solution (50 + 50, *V* + *V*) is recommended for the preparation of individual stock solutions.

5.26 Mycotoxin working standard solutions.

Prepare the working standard solutions as described hereafter by combining the appropriate volumes of each individual stock standard solutions (5.25), using the appropriate pipets (6.1) and the mentioned solvent. These solutions are used to build the calibration curve (5.29) and for the preparation of positive control samples (7.6).

5.26.1 Aflatoxins (AFs) composite working standard solution, AFB1, AFB2, AFG1 and AFG2, each at mass concentration ρ = 0,1 µg/ml in acetonitrile.

5.26.2 Aflatoxins (AFs) composite working standard solution, AFB1, AFB2, AFG1 and AFG2, each at ρ = 0,01 µg/ml in acetonitrile.

5.26.3 AFM1 working standard solution, ρ = 0,1 µg/ml in acetonitrile.

5.26.4 AFM1 working standard solution, ρ = 0,01 µg/ml in acetonitrile.

5.26.5 [DON, T-2, HT-2, ZEN]-composite working standard solution, in acetonitrile at mass concentrations given in Table 1.

Table 1 — [DON, T-2, HT-2, ZEN]-composite working standard solution

Compound	Mass concentration µg/ml
DON	5,0
T-2	2,5
HT-2	2,5
ZEN	2,0

5.26.6 Fumonisins (FBs) composite working standard solution, FB1 and FB2, each at ρ = 5,0 µg/ml in acetonitrile-water solution (50 + 50, $V + V$).

5.26.7 OTA working standard solution, ρ = 0,1 µg/ml in methanol-water solution (15 + 85, $V + V$).

5.27 ^{13}C-isotopically labelled mycotoxin analytical standards, as a certified standard solution.

5.27.1 ^{13}C-isotopically labelled aflatoxin B_1 (^{13}C-AFB1), e.g. ($^{13}C_{17}$)-aflatoxin B_1, ρ = 0,5 µg/ml in acetonitrile.

5.27.2 ^{13}C-isotopically labelled aflatoxin B_2 (^{13}C-AFB2), e.g. ($^{13}C_{17}$)-aflatoxin B_2, ρ = 0,5 µg/ml in acetonitrile.

5.27.3 ^{13}C-isotopically labelled aflatoxin G_1 (^{13}C-AFG1), e.g. ($^{13}C_{17}$)-aflatoxin G_1, ρ = 0,5 µg/ml in acetonitrile.

5.27.4 ^{13}C-isotopically labelled aflatoxin G_2 (^{13}C-AFG2), e.g. ($^{13}C_{17}$)-aflatoxin G_2, ρ = 0,5 µg/ml in acetonitrile.

5.27.5 ^{13}C-isotopically labelled aflatoxin M_1 (^{13}C-AFM1), e.g. ($^{13}C_{17}$)-aflatoxin M_1, ρ = 0,5 µg/ml in acetonitrile.

5.27.6 ^{13}C-isotopically labelled ochratoxin A (^{13}C-OTA), e.g. ($^{13}C_{20}$)-ochratoxin A, ρ = 10 µg/ml in acetonitrile.

5.27.7 ^{13}C-isotopically labelled deoxynivalenol (^{13}C-DON), e.g. ($^{13}C_{15}$)-deoxynivalenol, ρ = 25 µg/ml in acetonitrile.

5.27.8 ^{13}C-isotopically labelled zearalenone (^{13}C-ZEN), e.g. ($^{13}C_{18}$)-zearalenone, ρ = 25 µg/ml in acetonitrile.

5.27.9 ^{13}C-isotopically labelled T-2 toxin (^{13}C-T2), e.g. ($^{13}C_{24}$)-T-2 toxin, ρ = 25 µg/ml in acetonitrile.

5.27.10 ^{13}C-isotopically labelled HT-2 toxin (^{13}C-HT2), e.g. ($^{13}C_{22}$)-HT-2 toxin, ρ = 25 µg/ml in acetonitrile.

5.27.11 ^{13}C-isotopically labelled fumonisin B_1 (^{13}C-FB1), e.g. ($^{13}C_{34}$)-fumonisin B_1, ρ = 25 µg/ml in acetonitrile-water solution (50 + 50, $V + V$).

5.27.12 ^{13}C-isotopically labelled fumonisin B_2 (^{13}C-FB2), e.g. ($^{13}C_{34}$)-fumonisin B_2, ρ = 10 µg/ml in acetonitrile-water solution (50 + 50, $V + V$).

5.28 Internal standard (ISTD) solutions.

Prepare the ISTD solutions as described hereafter by combining the appropriate volumes of individual isotopically labelled mycotoxin solutions, using the appropriate pipets (6.1) and the mentioned solvent. These solutions are used to build the calibration curve (5.29) and to spike each test portion before extraction (7.3).

5.28.1 ^{13}C-Aflatoxins (^{13}C-AFs) internal standard solution, **^{13}C-AFB1, ^{13}C-AFB2, ^{13}C-AFG1, ^{13}C-AFG2**, each at ρ = 0,1 µg/ml in acetonitrile.

5.28.2 ^{13}C-AFM1 internal standard solution, ρ = 0,1 µg/ml in acetonitrile.

5.28.3 [13]C-[DON, T-2, HT-2, ZEN] internal standard solution, in acetonitrile at concentrations given in Table 2.

Table 2 — [13]C-[DON, T-2, HT-2, ZEN] internal standard solution

Compound	Mass concentration µg/ml
^{13}C-DON	5,0
^{13}C-T-2	2,5
^{13}C-HT-2	2,5
^{13}C-ZEN	2,0

5.28.4 [13]C-Fumonisins ([13]C-FBs) internal standard solution, **[13]C-FB1 and [13]C-FB2**, each at ρ = 10 µg/ml in acetonitrile-water solution (50 + 50, *V* + *V*).

5.28.5 [13]C-OTA internal standard solution, ρ = 0,1 µg/ml in methanol-water solution (15 + 85, *V* + *V*).

5.29 Standard solutions for external calibration curve.

Into nine separate 15 ml polypropylene tubes, prepare the standard solutions for calibration as described in Table 3a for example.

Evaporate to dryness under a stream of nitrogen at 40 °C then continue to prepare the standard solutions for calibration as described in Table 3b for example.

Sonicate the calibrants CAL 0 to CAL 8 for about 1 min. Transfer these solutions into glass vials and store them at −20 °C protected from light for up to 3 months.

Concentration of each mycotoxin in each calibrant solution is given in Table 4.

Table 3a — Example pipetting scheme for the preparation of the calibration solutions before the evaporation step

Mycotoxin	CAL 0 µl	CAL 1 µl	CAL 2 µl	CAL 3 µl	CAL 4 µl	CAL 5 µl	CAL 6 µl	CAL 7 [a] µl	CAL 8 [a] µl
AFs (5.26.1)	0	-	-	-	20	40	160	320	640
AFs (5.26.2)	-	5	20	40	-	-	-	-	-
AFM1 (5.26.3)	0	-	-	-	20	40	160	320	640
AFM1 (5.26.4)	-	5	20	40	-	-	-	-	-
[DON, T-2, HT-2, ZEN] (5.26.5)	0	5	10	20	40	80	160	320	640
FBs (5.26.6)	0	5	10	20	40	80	160	320	640
^{13}C-AFs (5.28.1)	20	20	20	20	20	20	20	20	20
^{13}C-AFM1(5.28.2)	20	20	20	20	20	20	20	20	20
^{13}C-[DON, T-2, HT-2, ZEN] (5.28.3)	20	20	20	20	20	20	20	20	20
^{13}C-FBs (5.28.4)	20	20	20	20	20	20	20	20	20

[a] The calibration range can be extended for quantification of highly contaminated samples (9.2). Typically, CAL 7 and CAL 8 can be prepared as described in Table 3a to extend the range by a factor of 2 (CAL 7) and a factor of 4 (CAL 8).

NOTE Robustness of the method is not affected as long as the same ISTD solutions are used for both preparing calibration standard solutions and spiking test portions (7.3).

Table 3b — Example pipetting scheme for the preparation of the calibration solutions following the evaporation step

Compound	CAL 0 µl	CAL 1 µl	CAL 2 µl	CAL 3 µl	CAL 4 µl	CAL 5 µl	CAL 6 µl	CAL 7 [a] µl	CAL 8 [a] µl
OTA (5.26.7)	0	2,5	5	10	20	40	160	320	640
^{13}C-OTA (5.28.5)	20	20	20	20	20	20	20	20	20
MeOH-H_2O (15 + 85, *V* + *V*) (5.23)	1 980	1978	1 975	1 970	1 960	1 940	1 820	1 660	1 340

[a] The calibration range can be extended for quantification of highly contaminated samples (9.2). Typically, CAL 7 and CAL 8 can be prepared as described in Table 3a to extend the range by a factor of 2 (CAL 7) and a factor of 4 (CAL 8).

NOTE 1 Robustness of the method is not affected as long as the same ISTD solutions are used for both preparing calibration standard solutions and spiking test portions (7.3).

NOTE 2 OTA solutions are added after the evaporation step to avoid unpredictable OTA losses upon evaporation.

Table 4 — Example mass concentrations of mycotoxins and related ISTD in calibration solutions

Mycotoxin	CAL 0 ng/ml	CAL 1 ng/ml	CAL 2 ng/ml	CAL 3 ng/ml	CAL 4 ng/ml	CAL 5 ng/ml	CAL 6 ng/ml	CAL 7 ng/ml	CAL 8 ng/ml
AFs	0	0,025	0,1	0,2	1	2	8	16	32
AFM1	0	0,025	0,1	0,2	1	2	8	16	32
DON	0	12,5	25	50	100	200	400	800	1600
T-2 and HT-2	0	6,25	12,5	25	50	100	200	400	800
ZEN	0	5	10	20	40	80	160	320	640
FBs	0	12,5	25	50	100	200	400	800	1600
OTA	0	0,125	0,25	0,5	1	2	8	16	32
^{13}C-AFs	1	1	1	1	1	1	1	1	1
^{13}C-AFM1	1	1	1	1	1	1	1	1	1
^{13}C-DON	50	50	50	50	50	50	50	50	50
^{13}C-T-2 & ^{13}C-HT-2	25	25	25	25	25	25	25	25	25
^{13}C-ZEN	20	20	20	20	20	20	20	20	20
^{13}C-FBs	100	100	100	100	100	100	100	100	100
^{13}C-OTA	1	1	1	1	1	1	1	1	1

6 Apparatus and equipment

Glassware and equipment (graduated cylinders, glass funnels, beakers, pipette, etc.) and, in particular, the following.

6.1 Pipettes, suited for organic solvent in the range 1 µl to 1 ml.

6.2 Conical polypropylene screw cap centrifuge tube, 50 ml with caps.

6.3 Conical polypropylene screw cap centrifuge tube, 15 ml with caps.

6.4 Polypropylene microcentrifuge tube, 1,5 ml.

6.5 HPLC glass vial, 1,5 ml with screw cap.

6.6 Adjustable mechanical vertical or horizontal shaker, capable to shake at 300 r/min.

6.7 Laboratory shaker.

6.8 Ultrasonic water bath.

6.9 Laboratory balance, accuracy: 0,01 g.

6.10 Analytical balance, accuracy: 0,000 1 g.

6.11 Centrifuge, with rotors adapted for polypropylene tubes of 15 ml and 50 ml volume, capable of generating a relative centrifugal force of 4 000 *g*.

6.12 Centrifuge, with rotors adapted for polypropylene tubes of 15 ml volume, capable of generating a relative centrifugal force of 8 400 *g*.

6.13 Sample concentrator, with temperature control and nitrogen gas supply.

6.14 Vacuum manifold for SPE clean-up, with taps.

6.15 Polypropylene reservoirs (approx. 25 ml), adapted for SPE columns.

6.16 Disposable syringe, 5 ml.

6.17 Plastic Pasteur pipette, non-sterile, 7 ml.

6.18 Immunoaffinity columns (IAC) for AFB1, AFB2, AFG1, AFG2 and OTA.

The IAC contains antibodies raised against AFB_1, AFB_2, AFG_1, AFG_2 and OTA with a capacity greater than 100 ng[1)].

6.19 Immunoaffinity columns for AFM1.

The IAC contains antibodies raised against aflatoxin M_1 with a capacity greater than 100 ng.

Alternatively, an IAC containing antibodies raised against AFB1, AFB2, AFG1, AFG2 with a cross-reactivity to AFM1 might be also suitable[1)].

[1] AFLAOCHRA PREP® column from R-biopharm, is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

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

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

6.20.2 Degasser, optional, for degassing LC mobile phases.

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

6.20.4 LC column, capable to retain the first eluting analyte at at least twice the retention time corresponding to the void volume of the column. Examples of suitable columns and gradients are given in Annex C.

6.20.5 LC pre-column, optional, with the same stationary phase material as the LC column (6.20.4).

6.20.6 Column oven, capable to maintain a constant temperature.

6.20.7 Triple stage mass spectrometer (e.g. triple quadrupole or quadrupole linear ion trap), equipped with an electrospray ionization (ESI) interface and operated in multiple reaction monitoring (MRM) mode. Any ionization mode (typically negative or positive) giving sufficient yield may be employed.

6.20.8 Computer-based instrument control and data evaluation system.

7 Procedure

7.1 Preparation of the laboratory sample

Samples shall be stored in air-tight containers, protected from light and thoroughly mixed before analysis. Finely grind and/or extensively homogenize the laboratory samples.

7.2 Test portions weighing

7.2.1 Cereals, cereals-based products

Weigh a test portion of 5,00 g of the homogeneous laboratory sample to the nearest 0,05 g into a 50 ml polypropylene tube (6.2).

7.2.2 Milk powders, nuts, spices, dried fruits

Weigh a test portion of 2,00 g of the homogeneous laboratory sample to the nearest 0,02 g into a 50 ml polypropylene tube (6.2).

7.3 Spiking with internal standard (ISTD) solutions

Spike each test portion with 50 µl of ISTD solutions (5.28) as shown in Table 5. The choice of the ISTD to be spiked depends on the final purpose of the analysis, i.e. the mycotoxin(s) to be monitored.

NOTE Robustness of the method is not affected as long as the same ISTD solutions are used for both preparing calibration standards (5.29) and spiking test portions.