SLOVENSKI STANDARD
SIST EN 17641:2022

01-november-2022

Živila - Hkratna 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

Ta slovenski standard je istoveten z: **EN 17641:2022**

ICS:

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

SIST EN 17641:2022 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 17641

September 2022

ICS 67.050

English Version

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

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

This European Standard was approved by CEN on 24 July 2022.

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, Türkiye 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 17641:2022 E

Contents

Page

European foreword

This document (EN 17641:2022) 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 March 2023, and conflicting national standards shall be withdrawn at the latest by March 2023.

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.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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, Türkiye and the United Kingdom.

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 a method using isotopically labelled standards 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 food intended for infants and young children (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 measuring range of each mycotoxin in these naturally contaminated and/or spiked food samples were:

— AFB1: 0,085 7 µg/kg – 11,4 µg/kg;
— AFB2: 0,079 2 µg/kg – 12,5 µg/kg;
— AFG1: 0,062 8 µg/kg – 20,9 µg/kg;
— AFG2: 0,052 0 µg/kg – 15,0 µg/kg;
— AFM1: 0,034 2 µg/kg – 0,110 µg/kg;
— OTA: 0,448 µg/kg – 17,2 µg/kg;
— DON: 45,2 µg/kg – 743 µg/kg;
— ZEN: 9,57 µg/kg – 131 µg/kg;
— T-2: 10,3 µg/kg – 57,9 µg/kg;
— HT-2: 9,50 µg/kg – 81,8 µg/kg;
— FB1: 31,1 µg/kg – 4 260 µg/kg;
— FB2: 44,2 µg/kg – 1 300 µg/kg.

The measuring ranges of the method for each mycotoxin/matrix combination are given in Table 8.

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 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 food intended for infants and young children (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 resulting mixture 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 4 — Decontamination procedures for laboratory wastes of AFs 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, *m* + *m*).

Weigh 4,0 g ± 0,01 g of $MgSO_4$ (5.11) and 1,00 g ± 0,01 g of NaCl (5.12) into a 15 ml polypropylene tube.

Alternatively, a ready-to-use partitioning salt mixture may 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, *w*(HCl) = 37 %.

5.20 Hydrochloric acid diluted solution, (HCl) *c* = 0,1 mol/l.

Add 8,4 ml of hydrochloric acid solution (5.19) into a 1 l volumetric flask and fill-up to the mark 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 with approximately 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 (87 mmol/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. Fill-up to the mark 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 AFB1.

5.24.2 AFB2.

5.24.3 AFG1.

5.24.4 AFG2.

5.24.5 AFM1.

5.24.6 OTA.

5.24.7 DON.

5.24.8 ZEN.

5.24.9 T-2.

5.24.10 HT-2.

5.24.11 FB1.

5.24.12 FB2.

5.25 Mycotoxin stock standard solutions.

The individual stock standard solutions are either prepared by dissolving pure substance 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.

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 pipettes (6.1) and the mentioned solvents. These solutions are used to build the calibration curve (5.29) and for the preparation of positive control samples (7.6).

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

5.26.2 AFs working standard solution 2, AFB1, AFB2, AFG1 and AFG2, each at ρ = 0,01 µg/ml in acetonitrile.

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

5.26.4 AFM1 working standard solution 2, ρ = 0,01 µg/ml in acetonitrile.

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

Table 1 — [DON, T-2, HT-2, ZEN]- 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) 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 AFB1(^{13}C-AFB1), e.g. ($^{13}C_{17}$)-AFB1, ρ = 0,5 µg/ml in acetonitrile.

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

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

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

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

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

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

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

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

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

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

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

5.28 ISTD working standard solutions.

Prepare the ISTD solutions as described hereafter by combining the appropriate volumes of individual isotopically labelled mycotoxin solutions (5.27), using the appropriate pipettes (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-AFs 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 solution, ρ = 0,1 µg/ml in acetonitrile.

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

Table 2 — ^{13}C-[DON, T-2, HT-2, ZEN] ISTD 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-FBs 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 solution, ρ = 0,1 µg/ml in methanol-water solution (15 + 85, *V* + *V*).

5.29 Standard solutions for external calibration curve.

Prepare the standard solutions for calibration into nine separate 15 ml polypropylene tubes, as described in Table 3 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 4 for example.

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

Mass concentration of each mycotoxin in each calibrant solution is given in Table 5.

Table 3 — Example of 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 3 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 4 — Example of 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 3 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 5 — Example mass concentrations of mycotoxins and 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, pipettes, 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 cap.

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

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 min^{-1}.

6.7 Laboratory shaker.

6.8 Ultrasonic water bath.

6.9 Laboratory balance, accuracy of 0,01 g.

6.10 Analytical balance, accuracy of 0,1 mg.

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 1,5 ml volume, capable of generating a relative centrifugal force of 8 500 *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 IAC for AFB1, AFB2, AFG1, AFG2 and OTA.

The IAC contains antibodies raised against AFB1, AFB2, AFG1, AFG2 and OTA with a capacity greater than 100 ng [1].

6.19 IAC for AFM1.

The IAC contains antibodies raised against AFM1 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 also suitable [1].

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 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 Tandem 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.

[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.