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Foodstuffs - Determination of ochratoxin A in currants, raisins, sultanas, mixed dried fruit and dried figs - HPLC method with immunoaffinity column cleanup and fluorescence detection

Produits alimentaires - Dosage de l'ochratoxine A dans les raisins de Corinthe, les raisins secs, les raisins secs de Smyrne, les mélanges de fruits secs et les figues sèches - Méthode CLHP avec purification sur colonne d'immuno-affinité et détection par fluorescence

Lebensmittel - Bestimmung von Ochratoxin A in Korinthen, Rosinen, Sultaninen, gemischtem Trockenobst und getrockneten Feigen - HPLC-Verfahren mit Reinigung an einer Immunoaffinitätssäule und Fluoreszenzdetektion

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Foreword

This document (EN 15829:2010) 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of an identical text or by endorsement, at the latest by August 2010, and conflicting national standards shall be withdrawn at the latest by August 2010.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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1 Scope

This European Standard specifies a method for the determination of ochratoxin A in currants, raisins, sultanas, mixed dried fruit and dried figs by high performance liquid chromatography (HPLC) with immunoaffinity cleanup and fluorescence detection. This method has been validated in an interlaboratory study via the analysis of both naturally contaminated and spiked samples ranging from 1,1 µg/kg to 11 µg/kg.

For further information on the validation, see Clause 9 and Annex B.

WARNING — The use of this standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

2 Normative references

The following referenced documents are indispensable for the application 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 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

3 Principle

A test portion is extracted with a mixture of methanol and phosphoric acid. The extract is filtered, diluted with phosphate buffered saline, and applied to an immunoaffinity column containing antibodies specific for ochratoxin A. The ochratoxin A is isolated, purified and concentrated on the column then released with elution solvent. Ochratoxin A is quantified by reverse-phase high performance liquid chromatography (HPLC) with fluorescence detection.

4 Reagents

4.1 General

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696:1995, unless otherwise specified. Solvents shall be of quality for HPLC analysis. Commercially available solutions with equivalent properties to those listed may be used.

WARNING — Dispose of waste solvents according to applicable environmental rules and regulations. Decontamination procedures for laboratory wastes have been reported by the International Agency for Research on Cancer (IARC), see [1].

4.2 Helium purified compressed gas

4.3 Disodium hydrogen phosphate, anhydrous or $Na_2HPO_4 \cdot 12\ H_2O$

4.4 Potassium chloride

4.5 Potassium dihydrogen phosphate

4.6 Sodium chloride

4.7 Sodium hydroxide

4.8 Ammonium hydroxide solution, substance concentration $c(NH_4OH)$ = 1,1 mol/l, for post-column pH shift

Prepare fresh when required (optional, see 7.2).

4.9 Hydrochloric acid solution, mass fraction $w(HCl)$ = 37 % in water

4.10 Phosphoric acid solution, $c(H_3PO_4)$ = 0,1 mol/l

4.11 Hydrochloric acid solution, $c(HCl)$ = 0,1 mol/l

Dilute 8,28 ml of hydrochloric acid solution (4.9) to 1 l with water.

4.12 Sodium hydroxide solution, $c(NaOH)$ = 0,1 mol/l

Dissolve 4 g of sodium hydroxide (4.7) in 1 l of water.

4.13 Phosphate buffered saline (PBS) solution

Dissolve 8,0 g of sodium chloride (4.6), 1,2 g of anhydrous disodium hydrogen phosphate or 2,9 g of $Na_2HPO_4 \cdot 12\ H_2O$ (4.3), 0,2 g of potassium dihydrogen phosphate (4.5) and 0,2 g of potassium chloride (4.4) in 900 ml of water.

After dissolution, adjust the pH to 7,4 with hydrochloric acid solution (4.11) or sodium hydroxide solution (4.12) as appropriate, then dilute to 1 l with water. Alternatively, a PBS solution with equivalent properties can be prepared from commercially available PBS material.

4.14 Acetonitrile

WARNING — Acetonitrile is hazardous and samples shall be blended using an explosion proof blender which is housed within a fume cupboard. After blending, samples shall be filtered inside a fume cupboard.

4.15 Glacial acetic acid, $w(CH_3COOH) \geq 98\ \%$

4.16 Methanol

4.17 Toluene

4.18 Injection solvent

Mix 80 parts per volume of water with 20 parts per volume of acetonitrile (4.14) and two parts per volume of acetic acid (4.15).

4.19 HPLC mobile phase

Mix 99 parts per volume of water with 99 parts per volume of acetonitrile (4.14) and two parts per volume of glacial acetic acid (4.15). Degas the mobile phase solvent with for example helium (4.2).

4.20 Mixture of toluene and glacial acetic acid

Mix 99 parts per volume of toluene (4.17) with one part per volume of glacial acetic acid (4.15).

4.21 Immunoaffinity column

The immunoaffinity column contains antibodies raised against ochratoxin A. The column shall have a capacity of not less than 100 ng of ochratoxin A and shall give a recovery of not less than 70 % when 5 ng of ochratoxin A is applied in a solution of five parts per volume of acetonitrile (4.14) and 95 parts per volume phosphate buffered saline (4.13).

4.22 Surface silanising fluid (optional)

Mix one part per volume of the surface silanising fluid with 19 parts per volume of toluene (4.17).

4.23 Ochratoxin A, in crystal form or as a film in ampoules

4.24 Ochratoxin A stock solution

WARNING — Ochratoxin A is a potent nephrotoxin with immunotoxic, teratogenic and potential genotoxic properties. The International Agency for Research on Cancer (IARC) has classified ochratoxin A as a possible human carcinogen (group 2B). Protective clothing, gloves and safety glasses should be worn at all times, and all standard and sample preparation stages should be carried out in a fume cupboard.

Dissolve 1 mg of the ochratoxin A or the contents of 1 ampoule (if ochratoxin A has been obtained as a film) in solvent mixture (4.20) to give a solution containing approximately 20 µg/ml to 30 µg/ml of ochratoxin A.

To determine the exact concentration, record the absorption curve between a wavelength of 300 nm and 370 nm in a 1 cm quartz cell with solvent mixture (4.20) as reference using the spectrometer (5.12). Identify the wavelength for maximum absorption. Calculate the mass concentration of ochratoxin A, ρ_{ota}, in micrograms per millilitre using Equation (1):

$$\rho_{ota} = \frac{A_{max} \times M \times 100}{\varepsilon \times b} \qquad (1)$$

where

A_{max} is the absorption determined at the maximum of the absorption curve (here: at 333 nm);

M is the molar mass, in grams per mole, of ochratoxin A (M = 403,8 g/mol);

ε is the molar absorption coefficient, in square metres per mole, of ochratoxin A in the solvent mixture (4.20) (here: 544 m^2/mol, see [2]);

b is the optical path length, in centimetres, of the quartz cell.

Store this solution in a freezer at approximately - 18 °C. Allow to reach room temperature before opening. A solution stored in this way is usually stable for 12 months. Confirm the concentration of the solution if it is older than six months.

4.25 Ochratoxin A spiking solution

Transfer an aliquot of the stock solution (4.24) containing 12,5 µg of ochratoxin A to a 5 ml volumetric flask. Evaporate to dryness under nitrogen at no more than 50 °C. Redissolve immediately in methanol (4.16) and make up to volume. This solution contains 2,5 µg/ml ochratoxin A.

Store this solution in a freezer at approximately - 18 °C. Allow to reach room temperature before opening. A solution stored in this way is usually stable for 12 months. Confirm the concentration of the solution if it is older than six months.

4.26 Ochratoxin A standard solution

Transfer 500 µl of the ochratoxin A spiking solution (4.25) to a 5 ml volumetric flask, make up to volume with methanol (4.16). This solution contains 0,25 µg/ml ochratoxin A.

Store this solution in a freezer at approximately - 18 °C. Allow to reach room temperature before opening. A solution stored in this way is usually stable for 12 months. Confirm the concentration of the solution if it is older than six months.

5 Apparatus

5.1 General

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

5.2 Silanised glass vials (optional)

Prepare the vials by filling them with the silanising reagent (4.22) and leave this reagent in the vial for 1 min. Rinse the vial first with a solvent of low polarity, for example toluene (4.17) then with methanol (4.16) and dry before use.

WARNING — The use of silanised glassware may prevent ochratoxin A binding to glass during evaporation.

5.3 High speed blender or homogenizer

5.4 Analytical balance, capable of weighing to 0,000 1 g

5.5 Laboratory balance, capable of weighing to 0,1 g

5.6 Displacement pipettes, adjustable, of 10 ml, 5 ml, 1 ml and 200 µl capacity with appropriate pipette tips

5.7 Vacuum manifold, to accommodate immunoaffinity columns

5.8 Reservoirs and attachments, to fit to immunoaffinity columns

5.9 Vacuum pump, capable of pulling a vacuum of 1 kPa and pumping 18 l/min

5.10 Filter paper, with pore size of 20 µm to 25 µm

5.11 HPLC apparatus, comprising the following:

5.11.1 Injection system, capable of injecting e.g. 100 µl

5.11.2 Mobile phase pump, isocratic, pulse free, capable of maintaining a volume flow rate of 1 ml/min

5.11.3 Column oven (optional), capable of maintaining a constant temperature above any variability caused by fluctuations in the room temperature (e.g. (45 ± 1) °C, ± 0,5 °C temperature repeatability and stability).

5.11.4 Analytical reverse-phase HPLC separating column, for example C_{18} octadecylsilane (ODS), length of 25 cm, inner diameter of 4,6 mm and a particle size of 5 µm, which ensures resolution of ochratoxin A from

all other peaks. The maximum overlapping of peaks shall be less than 10 %. It can be necessary to adjust the mobile phase for a sufficient baseline resolution. A suitable corresponding reverse-phase guard column should be used.

5.11.5 Degasser (optional)

5.11.6 Fluorescence detector, fitted with a flow cell and set at 333 nm (excitation wavelength) and 477 nm (emission wavelength), or set at 390 nm (excitation wavelength) and 440 nm (emission wavelength), if an optional post-column system is used.

5.11.7 Recorder, integrator or computer based data processing system

5.11.8 Post-column system (optional), comprising pump, isocratic, pulse free, capable of maintaining a volume flow rate of 0,3 ml/min, zero dead volume t-piece, and reaction coil 1 500 mm × 0,25 mm internal diameter tubing (stainless steel or polyetheretherketone (PEEK))

5.12 UV spectrometer

6 Procedure

6.1 Sample slurry preparation

Weigh the laboratory sample received and record the weight. The sample may be minced to break it up. Add water in the proportion of five parts fruit to four parts water. Homogenize the sample and water for at least 30 min or until a slurry of a smooth consistency is achieved.

In all instances if the sample has been frozen allow it to thaw completely before sampling. Stir slurried samples thoroughly before removing an analytical test portion.

6.2 Extraction

Weigh, to the nearest 0,2 g, 45 g of fruit slurry into a beaker. Add 50 ml of methanol (4.16) and 5 ml of phosphoric acid solution (4.10). Blend using the homogenizer (5.3) for 3 min to 4 min. Filter the mixture through filter paper (5.10) by gravity.

NOTE 45 g of fruit slurry is equivalent to 25 g of dried fruit and 20 ml water when the slurry is prepared using the method described in 6.1. The extraction volume (V_1 = 75 ml) includes this water in addition to the methanol and phosphoric acid solution.

6.3 Immunoaffinity column cleanup

The cleanup may be carried out by using a vacuum, by positive pressure or by allowing the specified volumes to pass through the column under gravity. Do not exceed the maximum specified flow rates. Extra care is needed when a vacuum manifold is used.

Prepare the immunoaffinity column according to the manufacturers instructions. Accurately measure 12 ml of the filtrate into a 100 ml volumetric or conical flask and dilute to 100 ml with PBS (4.13) and shake well to mix.

Add 50 ml of diluted sample extract to the reservoir and pass it through the immunoaffinity column (V_3 = 6 ml). The flow rate should not exceed 5 ml/min. The immunoaffinity column should not be allowed to run dry. Wash the immunoaffinity column with 10 ml of water (or PBS depending on column manufacturer's instructions). Place a vial (5.2) under the immunoaffinity column.

NOTE Care should be taken not to exceed the capacity of the immunoaffinity column.

6.4 Preparation of the sample test solution

Elute the ochratoxin A into a vial (5.2) with a suitable solvent as recommended in the immunoaffinity column manufacturer's instructions. Extra care is needed when a vacuum manifold is used. Evaporate the immunoaffinity column eluate to dryness, under nitrogen. Redissolve in 1,0 ml of the injection solvent (4.18). Transfer to an HPLC vial (V_2 = 1 ml).

As an alternative, dilute an aliquot of the column eluate with water (or water with 2 % acetic acid depending on elution solvent) to prepare the test solution for analysis. To 1 ml of eluate add 2 ml of water (V_2 = 3 ml). Mix well.

The cleanup, sample test solution preparation and HPLC steps of this method may be carried out by an automated system such as automated solid phase extraction cleanup (ASPEC) system, provided that the conditions described in this method, e.g. volumes and flow rates are adhered to.

6.5 Spiking procedure

Weigh, to the nearest 0,2 g, 45 g of blank fruit slurry into a beaker or blender jar. Pipette 50 µl of the ochratoxin A spiking solution (4.25) onto the blank fruit slurry. Leave spiked fruit slurry in a fume cupboard for at least 30 min. Proceed as given in 6.2.

7 HPLC analysis

7.1 HPLC operating conditions

When a column meeting the specification in 5.11.4, and the mobile phase specified in 4.19 were used, the following settings were found to be appropriate. Ochratoxin A elutes at approximately 9 min to 10 min.

— Flow rate mobile phase (column): 1,0 ml/min;

— Fluorescence detection, emission wavelength: 477 nm;

— Fluorescence detection, excitation wavelength: 333 nm;

— Injection volume: 100 µl to 200 µl;

— Sample extracts diluted with water 300 µl to 600 µl.

7.2 Post-column reaction conditions (optional)

The use of a post-column reaction system can enhance sensitivity and improve the detection limits of ochratoxin A. It can reduce background interferences for some samples. It can be used to confirm ochratoxin A identity in contaminated samples. Using the post-column system described in 5.11.8, and a column as described in 5.11.4 the following HPLC conditions have been found to enhance the response of ochratoxin A by a factor of 3 to 4.

— Flow rate post-column reaction solution (4.8): 0,3 ml/min;

— Fluorescence detection, emission wavelength: 440 nm;

— Fluorescence detection, excitation wavelength: 390 nm;

— Post-column reaction loop: 1 500 mm × 0,25 mm internal diameter;

— Injection volume: 100 µl to 200 µl;

— Sample extracts diluted with water 300 µl to 600 µl.

NOTE 1 The eluent should be alkaline (typical pH ≈ 9) after the detector. Check it by using pH indicator paper.

NOTE 2 Spread of ammonia vapours should be minimised for example by putting a saturated citric acid solution into the waste reservoir.

7.3 Preparation of calibration solutions for HPLC

Prepare four HPLC calibration solutions in separate 5 ml volumetric flasks according to Table 1. Make up each calibration solution to 5 ml with injection solvent (4.18).

Table 1 — Preparation of HPLC calibration solutions

HPLC calibration solution	Injection solvent (4.18) µl	Standard solution (4.26) µl	Final ochratoxin A mass concentration in calibration solution ng/ml
1	4 960	40	2,0
2	4 900	100	5,0
3	4 840	160	8,0
4	4 800	200	10,0

NOTE In case that the content of ochratoxin A in the sample is outside of the calibration range, an appropriate calibration curve can be prepared. Alternatively the injection solution for HPLC analysis can be diluted to an ochratoxin A content appropriate for the established calibration curve.

7.4 Calibration curve

Prepare a calibration curve at the beginning of every day of the analysis using the calibration solutions from Table 1. Establish the calibration curve prior to analysis of test samples by plotting the concentration of ochratoxin A, in nanograms per millilitre on the x-axis against the peak signal as area or height on the y-axis and check the plot for linearity using linear regression ($r^2 \geq 0{,}998$).

7.5 Determination of ochratoxin A in sample test solutions

Inject aliquots of the sample test solutions (6.4) into the chromatograph using the same conditions used for the preparation of the calibration curve.

7.6 Peak identification

Identify the ochratoxin A peak in the sample test solution by comparing the retention time of the sample with that of the calibration solutions. The concentration of ochratoxin A in the sample test solution shall fall within the calibration range. In the case that the mass concentration of ochratoxin A in the sample test solution is outside the calibration range, an appropriate calibration curve can be prepared. Alternatively the sample test solution can be diluted to a mass concentration of ochratoxin A appropriate for the established calibration curve. The dilution factor shall be incorporated into all subsequent calculations.

8 Calculation

Determine the mass concentration of ochratoxin A in the sample test solution (6.4), in nanograms per millilitre, directly from the calibration curve (7.4). Calculate the mass fraction, w_{ota}, of ochratoxin A in micrograms per kilogram, using Equation (2):

$$w_{ota} = \frac{\rho_{ota} \times V_1 \times V_2}{V_3 \times m_s} \quad (2)$$

where

ρ_{ota} is the mass concentration of ochratoxin A, in nanograms per millilitre, in the aliquot of sample test solution injected and corresponding to the area of the ochratoxin A peak;

V_1 is the volume, in millilitres, of the solvent taken for extraction (here: 75 ml);

V_2 is the volume, in millilitres, achieved after elution from the immunoaffinity column (6.4) (here: 1 ml or 3 ml);

V_3 is the volume, in millilitres, of the extract aliquot used for immunoaffinity cleanup (here: 6 ml);

m_s is the mass, in grams, of sample material taken for analysis (here: 25 g).

9 Precision

9.1 General

Details of an interlaboratory test on the precision of the method are given in Table B.1. The values derived from this interlaboratory test may not be applicable to concentration ranges and/or matrices other than those given in Annex B.

9.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible interval will exceed the repeatability limit *r* in not more than 5 % of the cases.

The values for currants are:	$\bar{x}$ = 4,51 µg/kg	*r* = 0,73 µg/kg
The values for sultanas are:	$\bar{x}$ = 11,39 µg/kg	*r* = 1,79 µg/kg
The values for mixed dried fruit are:	$\bar{x}$ = 1,14 µg/kg	*r* = 0,27 µg/kg
The values for raisins are:	$\bar{x}$ = 7,55 µg/kg	*r* = 1,04 µg/kg
The values for dried figs are:	$\bar{x}$ = 2,55 µg/kg	*r* = 0,62 µg/kg

9.3 Reproducibility

The absolute difference between two single test results on identical test material reported by two laboratories will exceed the reproducibility limit *R* in not more than 5 % of the cases.

The values for currants are:	$\bar{x}$ = 4,51 µg/kg	*R* = 3,59 µg/kg
The values for sultanas are:	$\bar{x}$ = 11,39 µg/kg	*R* = 4,55 µg/kg
The values for mixed dried fruit are:	$\bar{x}$ = 1,14 µg/kg	*R* = 0,45 µg/kg
The values for raisins are:	$\bar{x}$ = 7,55 µg/kg	*R* = 2,95 µg/kg
The values for dried figs are:	$\bar{x}$ = 2,55 µg/kg	*R* = 1,28 µg/kg

10 Test report

The test report shall contain at least the following data:

a) all information necessary for the identification of the sample (kind of sample, origin of sample, designation);

b) a reference to this European Standard;

c) the date and type of sampling procedure (if known);

d) the date of receipt;

e) the date of test;

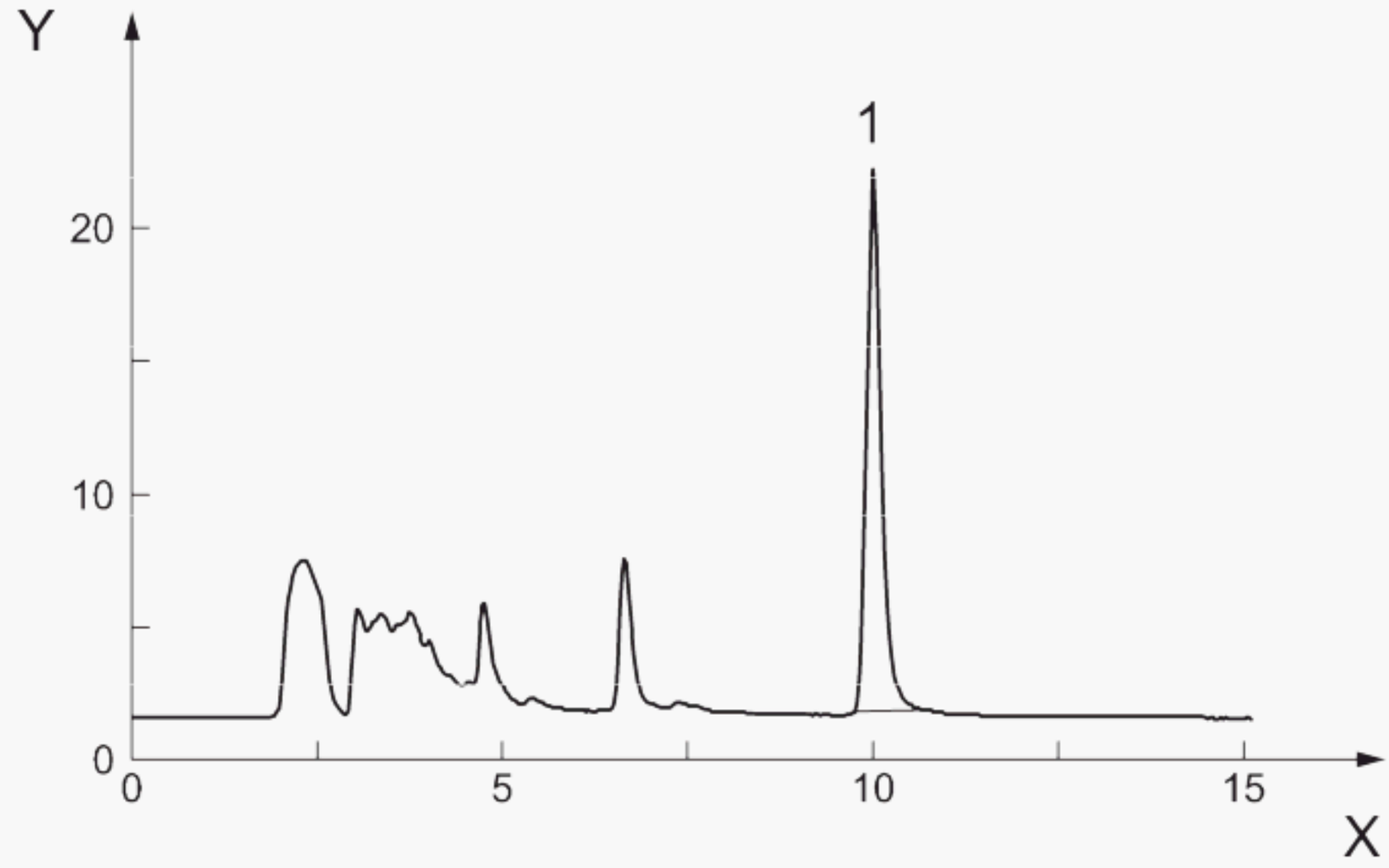
f) the test results and the units in which they have been expressed;

g) any particular points observed in the course of the test;

h) any operations not specified in the method or regarded as optional, which might have affected the results.

Annex A
(informative)

Typical chromatogram



Key
X time, in minutes
Y signal, in millivolts
1 ochratoxin A

Figure A.1 — Typical chromatogram of raisins with ochratoxin A at approximately 9 µg/kg

Annex B
(informative)

Precision data

The following data were obtained in an interlaboratory test [3] according to AOAC Guidelines for collaborative study procedures to validate characteristics of a method of analysis [4].

Table B.1 — Precision data

Sample	Currants	Sultanas	Mixed dried fruit	Raisins	Dried figs
Year of interlaboratory test	2002	2002	2002	2002	2002
Number of laboratories	20	24	24	24	24
Number of laboratories retained after eliminating outliers	20	22	20	21	22
Number of outliers (laboratories)	0	2	4	3	2
Number of accepted results	20	22	20	21	22
Mean value, $\bar{x}$, µg/kg	4,51	11,39	1,14	7,55	2,55
Repeatability standard deviation s_r, µg/kg	0,26	0,64	0,10	0,37	0,22
Repeatability relative standard deviation, RSD_r, %	5,7	5,6	8,6	4,9	8,7
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	0,73	1,79	0,27	1,04	0,62
Reproducibility standard deviation s_R, µg/kg	1,28	1,63	0,16	1,05	0,46
Reproducibility relative standard deviation, RSD_R, %	28,4	14,3	14,2	14,0	18,0
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	3,59	4,55	0,45	2,95	1,28
Recovery, % [a]		72	72	73	74
HorRat value, calculated using Predicted Standard Deviation ($PSRD_R$) from Thompson, see [5] and [6]	1,3	0,7	0,6	0,6	0,8

[a] Recovery values were derived independently from the analysis of single spiked samples of each matrix (5 µg/kg) by each laboratory that participated in the interlaboratory study.

Bibliography

[1] Castegnaro M., Barek J., Fremy J.M., Lafontaine M., Sansone E.B. and Telling G.M. Laboratory decontamination and destruction of carcinogens in laboratory wastes: some mycotoxins. IARC Scientific Publication No. 113, International Agency for Research on Cancer, Lyon (France), 1991, p. 63

[2] Wood, G. M., Patel, S., Entwisle, A.C. and Boenke, A., 1996, Ochratoxin A in wheat: a second intercomparison of procedures, *Food Additives and Contaminants*, **13**, 519-539

[3] MacDonald, S.J., Anderson, S., Brereton, P., and Wood, R. (2003). Determination of Ochratoxin A in currants, raisins, sultanas, mixed dried fruit, and dried frigs by immunoaffinity column cleanup with liquid chromatography: Interlaboratory Study, *Journal of AOAC International*., 86, 1164-1171

[4] AOAC International 1995, AOAC Official Methods Program, Associate Referee's Manual on Development, Study, Review, and Approval Process. Part IV AOAC Guidelines for Collaborative Studies p. 23-51

[5] Horwitz, W. and Albert, R., (2006), The Horwitz Ratio (HorRat): A Useful Index of Method Performance with Respect to Precision, *Journal of AOAC International*, 89, 1095-1109

[6] Thompson, M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, *Analyst*, **125**, 385-386