BRITISH STANDARD

BS EN 14132:2009

Foodstuffs — Determination of ochratoxin A in barley and roasted coffee — HPLC method with immunoaffinity column clean-up

ICS 67.140.20

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British Standards

National foreword

This British Standard is the UK implementation of EN 14132:2009. It supersedes BS EN 14132:2003 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/-/3, Food analysis - Horizontal methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 June 2009.

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ISBN 978 0 580 64239 5

Amendments/corrigenda issued since publication

Date	Comments

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 14132

May 2009

ICS 67.140.20

Supersedes EN 14132:2003

English Version

Foodstuffs - Determination of ochratoxin A in barley and roasted coffee - HPLC method with immunoaffinity column clean-up

Produits alimentaires - Dosage de l'ochratoxine A dans l'orge et le café torréfié - Méthode par purification sur colonne d'immuno-affinité suivie d'une analyse par chromatographie liquide haute performance (CLHP)

Lebensmittel - Bestimmung von Ochratoxin A in Gerste und Röstkaffee - HPLC-Verfahren mit Reinigung an einer Immunoaffinitätssäule

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Ref. No. EN 14132:2009: E

Contents

Foreword

This document (EN 14132:2009) 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of an identical text or by endorsement, at the latest by November 2009, and conflicting national standards shall be withdrawn at the latest by November 2009.

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.

This document will supersede EN 14132:2003

Annex A is informative.

WARNING — Ochratoxin A is a potent nephrotoxin and liver toxin and has been reported to have immunosuppressant properties. It is classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (Group 2B). Acetonitrile is hazardous. Toluene is highly flammable and harmful. Observe appropriate safety precautions for handling such compounds.

Gloves and safety glasses shall be worn at all times and all standard and sample preparation stages shall be carried out in a fume cupboard. Operation outside the fume cupboard, such us measurement of standards by UV spectrophotometer, shall be performed with the standard in closed containers.

Decontamination procedures for laboratory wastes have been reported by the International Agency for Research on Cancer (IARC), see [1].

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

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1 Scope

This European Standard specifies a method for the determination of ochratoxin A content in barley and roasted coffee using immunoaffinity column clean up and high performance liquid chromatography (HPLC). This method has been validated for ochratoxin A contents in barley in the range from 0,1 µg/kg up to 4,5 µg/kg and for roasted coffee in the range from 0,2 µg/kg up to 5,5 µg/kg.

2 Normative reference

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, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

3 Principle

Ochratoxin A is extracted from barley by blending with aqueous acetonitrile. The extract is purified by passing it through an immunoaffinity column. Ochratoxin A is extracted from ground roasted coffee by blending with methanol and sodium hydrogen carbonate. The extract is cleaned up by passing it first through a phenyl silane column and then through an immunoaffinity column. Ochratoxin A is separated by reverse-phase HPLC and determined by fluorescence.

4 Reagents

4.1 General

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of grade 1 as defined in EN ISO 3696. Solvents shall be of quality for HPLC analysis.

Commercially available reagents with equivalent properties to the ones listed may be used.

4.2 Sodium chloride

4.3 Disodium hydrogen phosphate

4.4 Potassium dihydrogen phosphate

4.5 Potassium chloride

4.6 Sodium hydroxide solution, ρ(NaOH) = 8,0 g/l

Dissolve 8 g of sodium hydroxide in 900 ml of water, then dilute to 1 l with water.

4.7 Phosphate buffered saline (PBS)

Dissolve 8 g of sodium chloride (4.2), 1,2 g of disodium hydrogen phosphate (4.3), 0,2 g of potassium dihydrogen phosphate (4.4) and 0,2 g of potassium chloride (4.5) in 900 ml of water. Adjust the pH to 7,4 with sodium hydroxide solution (4.6) then dilute to 1 l with water.

Commercially available phosphate buffered saline tablets with equivalent properties may be used.

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4.8 Sodium hydrogen carbonate solution, $\rho(NaHCO_3)$ = 30 g/l

In a 1-l-volumetric flask dissolve 30 g sodium hydrogen carbonate in 900 ml of water. Dilute to volume with water.

4.9 Glacial acetic acid, $\varphi(CH_3COOH)$ = 98 %

4.10 Methanol

4.11 Acetonitrile

4.12 Toluene

4.13 Solvent mixture of toluene and glacial acetic acid

Mix 99 parts per volume of toluene (4.12) with 1 part per volume of glacial acetic acid (4.9).

4.14 Barley extraction solvent mixture

Mix 6 parts per volume acetonitrile (4.11) with 4 parts per volume of water.

4.15 Roasted coffee extraction solvent mixture

Mix 1 part per volume of methanol (4.10) with 1 part per volume of sodium hydrogen carbonate solution (4.8).

4.16 Injection solvent

Mix 30 parts per volume of methanol (4.10) with 70 parts per volume of water and 1 part per volume of glacial acetic acid (4.9).

4.17 Mobile phase

Mix 102 parts per volume of water with 96 parts per volume of acetonitrile (4.11) and 2 parts per volume of glacial acetic acid (4.9), filter through a 0,2 µm filter (5.12) and degas with for example helium before use.

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4.18 Phenyl silane column wash reagent 1

Mix 20 parts per volume of methanol (4.10) with 80 parts per volume of sodium hydrogen carbonate solution (4.8).

4.19 Phenyl silane column wash reagent 2, $\rho(NaHCO_3)$ = 1 g/100 ml

In a 100 ml volumetric flask dissolve 1 g of sodium hydrogen carbonate in 90 ml water. Dilute to volume with water.

4.20 Phenyl silane column elution reagent

Mix 7 parts per volume methanol (4.10) with 93 parts per volume of water.

4.21 Ochratoxin A stock solution

Dissolve 1 mg of the ochratoxin A (in crystal form) or the contents of 1 ampoule (if ochratoxin A has been obtained as a film) in solvent mixture (4.13) 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 5 nm steps in 1 cm quartz cells (5.14) and solvent mixture (4.13) as reference. Identify the wavelength for maximum absorption and 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 of ochratoxin A (M = 403,8 g/mol);

ε is the molar absorption coefficient of ochratoxin A in the solvent mixture (4.13), (here: 544 m^2/mol);

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

This solution is stable at –18°C for at least 4 years.

4.22 Ochratoxin A standard solution

Dilute the stock solution (4.21) with the solvent mixture (4.13) to obtain a standard solution with a mass concentration of ochratoxin A of 10 µg/ml. Store this solution in a refrigerator at approximately 4°C and check its stability.

4.23 Ochratoxin A calibration solution

Pipette 200 µl of the 10 µg/ml ochratoxin A standard solution (4.22) into a glass vial and dilute to 1 ml with 800 µl of solvent mixture (4.13). This gives 2 µg/ml ochratoxin A solution. Pipette 100 µl of the 2 µg/ml ochratoxin A solution into a glass vial (5.2). Evaporate the solvent under a stream of nitrogen. Redissolve in 10 ml injection solvent (4.16) which has been filtered through a 0,2 µm filter (5.12). This gives a calibration solution containing 20 ng/ml.

Prepare the calibration solutions at the beginning of every day of the analysis.

4.24 Spiking solution

Pipette 100 µl of the 10 µg/ml ochratoxin A standard solution (4.22) into a glass vial. Dilute to 2 ml with 1,9 ml of the mixture of toluene and acetic acid (4.13). This gives a mass concentration of 500 ng/ml ochratoxin A.

4.25 Immunoaffinity column

The immunoaffinity column contains antibodies raised against ochratoxin A. The column shall have a total capacity of not less than 100 ng of ochratoxin A. The performance of the column should be checked by applying a solution of 100 ng ochratoxin A in a solvent mixture of the same composition as the sample extract (6.1.3) to be applied. This shall give a recovery of not less than 85 %.

4.26 Phenyl silane solid phase extraction columns

500 mg sorbent weight and 3 ml reservoir volume (to ensure adequate column bed depth and prevent analyte breakthrough). The column should have a total capacity of not less than 100 ng ochratoxin A and should give a recovery of not less than 85 % when applied in a standard solution of ochratoxin A in the roasted coffee extraction solvent (4.15) containing 100 ng of ochratoxin A.

5 Apparatus

Usual laboratory equipment and, in particular, the following:

5.1 **Analytical balance,** accurate to 0,01 mg

5.2 **Glass vials,** of at least 10 ml

Certain types of vials might lead to losses of ochratoxin A during evaporation. To avoid this, silanization could be applied. Prepare vials by filling them with silanizing reagent and leave this reagent in the vial for 1 min. Rinse the vial twice with appropriate solvent (toluene, acetone or hexane) followed by water (twice) and dry the vial.

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5.3 Blender, explosion proof

With 1 l capacity jar and cover and with a high speed of approximately 20 000 $\text{min}^{-1.}$

5.4 Displacement pipettes of 5 ml, 1 ml and 200 µl capacity with appropriate pipettes tips

5.5 Vacuum manifold to accommodate phenyl silane and immunoaffinity columns

5.6 Reservoirs and attachments to fit to immunoaffinity columns

5.7 Vacuum pump, capable of pulling a vacuum of 10 mbar and pumping 18 l/min

5.8 Wrist action shaker or similar

5.9 Cooling centrifuge capable of 1300 *g* and operating at 4 °C

5.10 Centrifuge tubes, e.g. 50 ml capacity

5.11 Filter paper, pore size 20 µm to 25 µm or similar

5.12 Disposable syringe filters, of 0,2 µm pore size and 25 mm diameter polysulfone membrane

5.13 HPLC apparatus, consisting of:

5.13.1 Injection system, a syringe-loading injection valve with 100 µl injection loop or equivalent

5.13.2 HPLC pump, isocratic, capable of maintaining a volume flow rate of 1 ml/min

5.13.3 Analytical reverse phase separating column, for example C_{18} octyldecylsilane (ODS),which ensures resolution of ochratoxin A from all other peaks. The maximum overlapping of peaks shall be less than 10 % (it could be necessary to adjust the mobile phase for a sufficient baseline resolution). A suitable pre-column should be used.

5.13.4 Fluorescence detector, fitted with a flow cell and set at 333 nm (excitation) and 460 nm (emission)

5.13.5 Data system

5.14 UV spectrometer, with suitable quartz cells.

6 Procedure

6.1 Barley

6.1.1 Extraction

Weigh, to the nearest 0,1 g, a 25 g test portion of the ground (mesh size = 0,5 mm) barley sample into a blender jar (5.3). Add 100 ml of extraction solvent mixture (4.14). Seal the jar and blend for 3 min in a high speed blender at approximately 20 000 min^{-1}. Filter the extract through a filter paper (5.11).

6.1.2 Immunoaffinity column clean-up

Prepare the immunoaffinity column according to the suppliers instructions.

Pipette 4 ml of the sample filtrate (see 6.1.1) into a 100 ml glass beaker (or similar) and dilute with 44 ml of PBS

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(4.7). Connect the immunoaffinity column (4.25) to the vacuum manifold (5.5), and attach the reservoir (5.6) to the immunoaffinity column. Add all the diluted sample extract to the reservoir and pass through the immunoaffinity column. Flow rate should not exceed 5 ml/min. The immunoaffinity column shall not be allowed to run dry. Wash the immunoaffinity column with 10 ml of water. Dry the column by passing at least two column volumes of air through it. Remove the immunoaffinity column from the vacuum manifold and place it over a vial (5.2).

6.1.3 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 manufacturers instructions. Evaporate the immunoaffinity column eluate to dryness under a stream of nitrogen e.g. at approximately 50 °C. Redissolve in 1,0 ml injection solvent (4.16), which has been filtered through a 0,2 µm filter (5.12). Transfer to a HPLC vial (V_3). This is the sample test solution.

6.2 Roasted coffee

6.2.1 Extraction

Weigh, to the nearest 0,1 g, a 15 g test portion of ground (mesh size 1mm) roasted coffee sample into a 500 ml conical flask. Add 150 ml extraction solvent (4.15). Stopper the flask and shake gently for 30 min. Filter the extract through filter paper (5.11). Transfer an approximately 50 ml portion of the coffee extract into a centrifuge tube (5.10) and centrifuge for 15 min at 4 °C at 1300 *g*.

6.2.2 Phenyl silane column clean-up

Attach a phenyl silane column (4.26) to the vacuum manifold (5.5) but do not apply vacuum to column (if necessary, nitrogen gas can be used as positive pressure to assist the flow rate at the column preparation stage). Wash the column with 15 ml methanol (4.10) followed by 5 ml of sodium hydrogen carbonate solution (4.8). Discard the washings.

Pipette 10 ml of the centrifuged coffee extract (see 6.2.1) into a glass beaker and add 10 ml of sodium hydrogen carbonate solution (4.8). Pass this diluted extract through the phenyl silane column at a maximum speed of 5 ml/min. Wash the phenyl silane column with 10 ml wash reagent 1 (4.18), maximum flow rate of 5 ml/min, followed by 5 ml wash reagent 2 (4.19). Remove the phenyl silane column from the vacuum manifold and dry the column by pumping at least three 10 ml volumes of air through the packing material, using a syringe. Then place the column over a suitable collection vessel, and elute ochratoxin A with 10 ml phenyl silane column elution reagent (4.20), maximum flow rate of 5 ml/min.

6.2.3 Immunoaffinity column clean-up

Dilute the phenyl silane column eluate (6.2.2) with 30 ml PBS (4.7). Connect the immunoaffinity column (4.25) to the vacuum manifold (5.5), and attach the reservoir (5.6) to the immunoaffinity column. Add the diluted sample extract to the reservoir and pass through the immunoaffinity column. Flow rate should not exceed 5 ml/min. The immunoaffinity column shall not be allowed to run dry. Wash the immunoaffinity column with 10 ml water. Remove the immunoaffinity column from the vacuum manifold and place over a vial (5.2).

6.2.4 Preparation of the sample test solution

Elute the ochratoxin A into the vial (5.2) using four 1 ml portions of the column manufacturers recommended elution solvent. Evaporate the immunoaffinity column eluate to dryness under a stream of nitrogen e.g. at approximately 50 °C. Redissolve in 1 ml injection solvent (4.16) and filter through a 0,2 µm filter (5.12). Transfer to a HPLC vial (V_3). This is the sample test solution.

7 Spiking procedure

7.1 Barley spiking procedure

Weigh, to the nearest 0,1 g, 25 g blank barley sample into a blender jar. Pipette 200 µl of ochratoxin A spiking solution (4.24) onto the blank barley. Leave spiked solid material in a fume cupboard overnight, to allow the solvent to evaporate. Proceed as given in 6.1.

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7.2 Roasted coffee spiking procedure

Weigh, to the nearest 0,1 g, 15 g blank ground (mesh size 1 mm) roasted coffee sample into a 500 ml conical flask. Pipette 120 µl of ochratoxin A spiking solution (4.24) onto the blank coffee. Leave spiked solid material in a fume cupboard overnight, to allow the solvent to evaporate. Proceed as given in 6.2.

8 HPLC analysis

8.1 Calibration curve

Prepare a calibration curve at the beginning of every day of the analysis and whenever chromatographic conditions change.

Prepare five HPLC calibration solutions (4.23) in separate 5 ml volumetric flasks according to Table 1. Dilute each calibration solution to the mark with filtered injection solvent (4.16).

Table 1 — Preparation of HPLC calibration solutions

HPLC-calibration solution no	calibration solution (4.23) (µl)	injection solvent (4.16), filtered (µl)	mass concentration (ng/ml)
1	125	4875	0,5
2	250	4750	1,0
3	500	4500	2,0
4	1250	3750	5,0
5	2500	2500	10,0

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8.2 HPLC operating conditions

When a column meeting the specification in 5.13.3, (dimensions 4,6 mm x 250 mm with 5 µm particle size) and the mobile phase specified in 4.17 were used, the following settings were found to be appropriate.

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

— Fluorescence detection, emission wavelength: 460 nm;

— excitation wavelength: 333 nm;

— Injection volume: 100 µl (V_4).

8.3 Identification

Identify the analyte by comparing the retention time of the relevant peak in the sample with the peak of the standard substance in the chromatogram.

Sometimes it can be necessary to identify the peak by simultaneous injection of sample test solution and standard solution.

8.4 Determination

To carry out the determination by the external standard method, integrate the peak area or determine the peak height, and compare the results with the corresponding values for the standard substance with the nearest peak area or height, or use a calibration curve. In the case of a calibration curve, additional solutions with concentrations within the linear range may be prepared for the calibration curve.

Inject equal volumes of sample test solution and standard solution used for the calibration curve.

If the ochratoxin A response of the sample is outside the calibration range, adjust the amount of sample injected by diluting the sample test solution.

9 Calculation

Read off from the calibration curve the amount in nanogram of the ochratoxin A in the aliquot of test solution injected into the HPLC column.

Calculate the mass fraction of ochratoxin A, w_{OTA}, in micrograms per kilogram using Equation (2):

$$w_{\text{OTA}} = m_a \times \frac{V_3}{V_4} \times \frac{V_1}{V_2} \times \frac{1000}{m_s} \times \frac{1}{1000} \qquad (2)$$

where

m_a is the mass of ochratoxin A in the aliquot of test solution (9.1) in nanogram, injected into the column;

V_4 is the volume of the aliquot of test solution injected onto the column (8.2), in millilitres;

V_3 is the volume of the test solution (6.1.3, 6.2.3), in millilitres (V_3 = 1,0 ml);

V_2 is the volume of sample filtrate taken for clean-up, in millilitres (barley V_2 = 4,0 ml (6.1.2), roasted coffee V_2 = 10 ml (6.2.2));

V_1 is the volume of the extraction solvent, in millilitres (barley V_1 = 100 ml, roasted coffee V_1 = 150 ml);

m_s is the mass of the sample extracted, in gram (barley m_s = 25 g, roasted coffee m_s = 15 g);

Express the final result in micrograms per kilogram as this is equivalent to nanograms per gram.

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10 Precision

10.1 Interlaboratory test

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

10.2 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 barley are:	$\bar{x}$ = 0,1 µg/kg	r = 0,112 µg/kg	*(blank)*
	$\bar{x}$ = 1,3 µg/kg	r = 0,896 µg/kg	*(naturally contaminated)*
	$\bar{x}$ = 3,0 µg/kg	r = 1,036 µg/kg	*(naturally contaminated)*
	$\bar{x}$ = 3,7 µg/kg	r = 0,448 µg/kg	*(fortified)*
	$\bar{x}$ = 4,5 µg/kg	r = 1,792 µg/kg	*(naturally contaminated)*
The values for roasted coffee are:	$\bar{x}$ = 0,2 µg/kg	r = 0,112 µg/kg	*(blank)*

$\bar{x}$ = 1,2 µg/kg r = 0,756 µg/kg *(naturally contaminated)*

$\bar{x}$ = 2,7 µg/kg r = 0,868 µg/kg *(naturally contaminated)*

$\bar{x}$ = 3,5 µg/kg r = 0,588 µg/kg *(fortified)*

$\bar{x}$ = 5,5 µg/kg r = 0,308 µg/kg *(naturally contaminated)*

10.3 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 barley are: $\bar{x}$ = 0,1 µg/kg R = 0,280 µg/kg *(blank)*

$\bar{x}$ = 1,3 µg/kg R = 1,232 µg/kg *(naturally contaminated)*

$\bar{x}$ = 3,0 µg/kg R = 1,456 µg/kg *(naturally contaminated)*

$\bar{x}$ = 3,7 µg/kg R = 1,204 µg/kg *(fortified)*

$\bar{x}$ = 4,5 µg/kg R = 1,876 µg/kg *(naturally contaminated)*

The values for roasted coffee are: $\bar{x}$ = 0,2 µg/kg R = 0,308 µg/kg *(blank)*

$\bar{x}$ = 1,2 µg/kg R = 0,896 µg/kg *(naturally contaminated)*

$\bar{x}$ = 2,7 µg/kg R = 1,120 µg/kg *(naturally contaminated)*

$\bar{x}$ = 3,5 µg/kg R = 1,288 µg/kg *(fortified)*

$\bar{x}$ = 5,5 µg/kg R = 2,184 µg/kg *(naturally contaminated)*

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11 Test report

The test report shall contain the following data:

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

b) reference to this European Standard;

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

d) date of receipt;

e) date of test;

f) test results and 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)

Precision data

The following data were obtained in interlaboratory tests [2], [3] according to the harmonized protocol [4].

Table A.1 — Precision data for barley

Sample	Blank[a)]	Low level	Medium level	High level	Blind spike
Year of inter-laboratory test	1998/-99	1998/-99	1998/-99	1998/-99	1998/-99
Number of laboratories	15	15	15	15	15
Number of laboratories retained after eliminating outliers	14	15	14	12	12
Number of outliers (laboratories)	1	0	1	3	3
Number of accepted results	14	15	14	12	12
Mean value $\bar{x}$, µg/kg	0,1	1,3	3,0	4,5	3,7
Repeatability standard deviation s_r, µg/kg	0,04	0,32	0,37	0,64	0,16
Repeatability relative standard deviation RSD_r, %	26	24	12	14	4
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	0,112	0,896	1,036	1,792	0,448
Reproducibility standard deviation s_R, µg/kg	0,10	0,44	0,52	0,67	0,43
Reproducibility relative standard deviation RSD_R, %	72	33	17	15	12
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	0,280	1,232	1,456	1,876	1,204
Recovery, %	-	-	-	-	93 % ± 10 %

a) The set of data for the blank sample are at the level of the limit of detection.

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Table A.2 — Precision data for roasted coffee

Sample	**Blank**[a)]	**Low level**	**Medium level**	**High level**	**Blind spike**
Year of inter-laboratory test	1998/-99	1998/-99	1998/-99	1998/-99	1998/-99
Number of laboratories	15	15	15	15	15
Number of laboratories retained after eliminating outliers	14	15	14	12	13
Number of outliers (laboratories)	1	0	1	3	2
Number of accepted results	14	15	14	12	13
Mean value $\bar{x}$, µg/kg	0,2	1,2	2,7	5,5	3,5
Repeatability standard deviation s_r, µg/kg	0,04	0,27	0,31	0,11	0,21
Repeatability relative standard deviation RSD_r, %	28	22	11	2	6
Repeatability limit r [$r = 2,8 \times s_r$], µg/kg	0,112	0,756	0,868	0,308	0,588
Reproducibility standard deviation s_R, µg/kg	0,11	0,32	0,40	0,78	0,46
Reproducibility relative standard deviation RSD_R, %	71	26	15	14	13
Reproducibility limit R [$R = 2,8 \times s_R$], µg/kg	0,308	0,896	1,120	2,184	1,288
Recovery, %	-	-	-	-	88 % ± 15 %

a) The set of data for the blank sample are at the level of the limit of detection.

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