International Standard

ISO 20948

First edition
2024-11

Vegetable fats and oils — Determination of aflatoxins B_1, B_2, G_1 and G_2 by immunoaffinity column clean-up and high-performance liquid chromatography

Reference number
ISO 20948:2024(en)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*, in collaboration with AOAC INTERNATIONAL.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Aflatoxins (AFs) are carcinogenic toxins that can naturally contaminate oleaginous seeds and fruits, leading to the potential risk of the consumption of edible oils contaminated by aflatoxins. Regulatory limits for AFs in edible oils have been established in several countries. This document specifies a method for the determination of the aflatoxins B_1, B_2, G_1 and G_2 in vegetable fats and oils. The method is based on AOAC Official Method 2013.05[1] and the validation has been extended to include corn oil, sunflower oil, rapeseed oil and coconut oil.

Vegetable fats and oils — Determination of aflatoxins B_1, B_2, G_1 and G_2 by immunoaffinity column clean-up and high-performance liquid chromatography

1 Scope

This document specifies a method for the determination of the aflatoxins B_1, B_2, G_1 and G_2 in vegetable fats and oils, including peanut oil, sesame oil, olive oil, corn oil, sunflower oil, rapeseed oil and coconut oil, using immunoaffinity column clean-up and high-performance liquid chromatography with post-column derivatization.

The limits of quantification for the aflatoxins B_1, B_2, G_1 and G_2, and for the sum of aflatoxins B_1, B_2, G_1 and G_2, are 1 µg/kg, 0,25 µg/kg, 0,5 µg/kg, 0,25 µg/kg and 1 µg/kg, respectively.

The validation was carried out over the following concentration ranges:

— aflatoxin B_1 = 1 µg/kg to 20 µg/kg;

— total aflatoxins = 2 µg/kg to 52 µg/kg.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle

Test samples are extracted with methanol–water (a volume fraction of 55 + 45). After shaking and centrifuging, the lower layer is filtered, diluted with water, and filtered through glass microfibre filter paper. The filtrate is passed through an immunoaffinity column, and the toxins are eluted with methanol. The toxins are subjected to high-performance liquid chromatography with fluorescence detector (HPLC-FLD) analysis after post column derivatization.

WARNING — Aflatoxins are generally considered to be carcinogenic, neurotoxic and immunosuppressive. Observe appropriate safety precautions[2] for handling such compounds and in particular avoid handling in dry form as their electrostatic nature can result in dispersion and inhalation. Glassware can be decontaminated with 4 % sodium hypochlorite solution. Attention is drawn to the statement made by the International Agency for Research on Cancer (WHO)[3][4].

5 Reagents

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

5.1 Methanol, LC grade or equivalent.

5.2 Acetonitrile, LC grade or equivalent.

5.3 Sodium chloride (NaCl).

5.4 Potassium chloride (KCl).

5.5 Hydrochloric acid, *c*(HCl) = 12 mol/l.

5.6 Disodium hydrogen phosphate (Na_2HPO_4).

5.7 Potassium dihydrogen phosphate (KH_2PO_4).

5.8 Phosphate-buffered saline (PBS) buffer, pH 7,40.

Dissolve 8 g NaCl (5.3), 1,2 g Na_2HPO_4 (5.6), 0,2 g KH_2PO_4 (5.7) and 0,2 g KCl (5.4) in about 990 ml of water. Adjust the pH to 7,4 with HCl (5.5) and make up to 1 l with water. Alternatively, a PBS solution of equivalent properties may be prepared from commercially available PBS material.

5.9 Potassium bromide (KBr).

5.10 Nitric acid, 65 %

5.11 Extraction solvent, mix 55 volume parts of methanol (5.1) and 45 volume parts of water.

5.12 Washing solution, mix 10 volume parts of methanol (5.1) and 90 volume parts of water.

5.13 Aflatoxin (AF) standards:

— aflatoxin B_1 (AFB_1, $C_{17}H_{12}O_6$, CAS **Registry Number**©[1] 1162-65-8), purity ≥ 98 %;

— aflatoxin B_2 (AFB_2, $C_{17}H_{14}O_6$, CAS RN© 7220-81-7), purity ≥ 98 %;

— aflatoxin G_1 (AFG_1, $C_{17}H_{12}O_7$, CAS RN© 1165-39-5), purity ≥ 98 %;

— aflatoxin G_2 (AFG_2, $C_{17}H_{14}O_7$, CAS RN© 7241-98-7), purity ≥ 98 %.

All standards shall be either certified standard solutions or in a crystalline form. Store all materials at –18 °C.

5.14 AF stock standard solutions

Prepare each of the four AFs at a concentration of 10 µg/ml in acetonitrile. Weigh 1 mg of AFB_1, AFB_2, AFG_1 and AFG_2 to the nearest 0,01 mg. Dissolve them with acetonitrile in 100 ml volumetric flasks (6.12). Store AF stock standard solutions at –18 °C. If crystalline AFs are used to prepare the stock standard solutions, the exact concentrations of the stock standard solutions shall be determined as described in Annex B. The concentrations of certified standard solutions can be checked according to the method in Annex B.

1) CAS Registry Number® is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

5.15 Intermediate AF standard solution:

Prepare a 260 ng/ml aflatoxin mixture solution (combination of AFB_1, AFB_2, AFG_1 and AFG_2 at 100 ng/ml, 30 ng/ml, 100 ng/ml and 30 ng/ml, respectively) by adding the appropriate amount of each aflatoxin stock standard solution to the same volumetric flask (6.12) and adjust to the volume with acetonitrile. Use the intermediate AF standard solution as the spiking solution for recovery studies. Store the intermediate AF standard solution at −18 °C. Equilibrate to room temperature for at least 30 min before use.

5.16 Working AF standard solution:

Prepare working standard solutions daily in separate 10 ml volumetric flasks (6.12) according to Table A.1. Adjust to volume with methanol–water (a volume fraction of 1 + 1).

5.17 4 M nitric acid:

Dilute 13,9 ml of 65 % nitric acid (5.10) with water to a volume of 50 ml.

5.18 HPLC mobile phase solvent A:

Mix methanol (5.1), acetonitrile (5.2) and water (v: v: v = 25:17:60). Degas the solution before use if an online system is not available on the HPLC (6.15) instrument.

5.19 HPLC mobile phase solvent B:

Mix methanol (5.1), acetonitrile (5.2) and water (v: v: v = 25:17:60). Add 120 mg of KBr (5.9) and 350 µl of nitric acid (4 M, 5.17) in 1 l mobile phase. Degas the solution before use if an online system is not available on the HPLC (6.15) instrument.

5.20 Sodium hypochlorite solution, concentration (NaOCl) = 4 g/100 ml.

6 Apparatus and equipment

The usual laboratory equipment and, in particular, the following shall be used.

6.1 Balance, sensitivity 0,01 g and 0,000 01 g.

6.2 Pipettes, suitable for handling volumes of 10 µl to 100 µl, 200 µl to 1 000 µl and 1 ml to 10 ml.

Automatic pipettes or 10 ml graduated glass pipettes may be used.

6.3 Vibration device, e.g. Vortex.

6.4 Rotary shaker, shaker capable of 400 r/min.

6.5 Column manifold, Vicam G1104 12-position pump stand[2)], or equivalent.

6.6 Centrifuge, suitable for relative centrifugal force of 6 000*g*.

6.7 Injection vials, 2 ml, suitable for LC autosampler.

6.8 Centrifuge tubes with screw caps, 50 ml.

2) These are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the products named. Equivalent products may be used if they can be shown to lead to the same results.

6.9 Glass syringe, 10 ml.

6.10 Glass cylinder, 25 ml and 50 ml.

6.11 Erlenmeyer flask, 125 ml.

6.12 Volumetric flasks, 2 ml, 10 ml and 100 ml.

6.13 Filter paper.

6.13.1 Folded filter paper.

6.13.2 Glass microfibre filter paper.

6.14 Immunoaffinity column (IAC): The AF IAC contains antibodies, which are specific for aflatoxins B_1, B_2, G_1 and G_2. The columns should have a capacity of not less than 200 ng AF and should give a recovery of not less than 80 % for AFB_1, AFB_2, AFG_1 and AFG_2 when 5 ng of each AF is applied in 10 ml methanol–PBS; a volume fraction of 10 + 90.

6.15 HPLC-FLD system, including an eluent reservoir, a pump, an injection system, column oven, a fluorescence detector with variable wavelength setting and a data processor, e.g. an integrator with plotter.

6.16 Post-column derivatization systems for AFs, equipped with post-column derivatization with photochemical reactor cell or electrochemical cell.

6.16.1 System for derivatization by photochemical reaction, e.g. photochemical reactor for enhanced detection (PriboFast®KRC or PHRED™)[2], only to be used with mobile phase A (5.18). The photochemical reactor is inserted between the HPLC column and the detector inlet.

6.16.2 System for derivatization with electrochemically generated bromine, e.g. Kobra® Cell[2], which shall only be used with mobile phase B (5.19). The system is inserted between the HPLC column and the detector inlet, with a current of 100 µA.

WARNING — Never flush 100 % organic solvent through the system as this can damage the membrane. Always switch the system current source off first before switching off the HPLC pump.

6.17 Analytical reverse-phase HPLC separating column, C18, which ensures a baseline resolved resolution of AFB_1, AFB_2, AFG_1 and AFG_2 peaks from all other peaks.

6.18 UV-spectrometer with quartz cuvettes.

7 Procedure

7.1 Sampling

A representative sample should be sent to the laboratory. It should not have been damaged or changed during transport and storage.

7.2 Sample pre-treatment

7.2.1 Extraction

Weigh 5 g, weighed to the nearest 0,01 g, of test portion in a 50 ml centrifuge tube (6.8). Add 1 g NaCl (5.3) and 25 ml extraction solvent (5.11). Vortex until sample particles and extract solvent are well mixed. Shake

at 400 r/min for 10 min. For coconut oil that can be in solid state, after the addition of 1 g NaCl (5.3) and 25 ml extraction solvent (5.11), heat the centrifuge tube in a water bath for 10 min at 40 °C to make sure the coconut oil is in liquid state, and then vortex and shake. Centrifuge at 6 000*g* for 10 min. Aspirate and discard the upper oil layer. Pass the lower aqueous methanol layer through folded filter paper (6.13.1). Measure 15 ml extract after filtration with a 25 ml graduate cylinder and place in a 125 ml Erlenmeyer flask. Add 30 ml water, mix, and filter through glass microfibre paper (6.13.2). Collect 30 ml filtrate (equivalent to 10 ml extract) into a 50 ml graduate cylinder and proceed immediately with IAC clean-up.

7.2.2 IAC clean-up

IACs are equilibrated at room temperature for at least 15 min before use. Remove the top cap from the column and connect to the reservoir of the column manifold. Remove the bottom cap from the column and let the liquid in the column pass through until it reaches 2 mm above the column packing. Add 30 ml of filtrate into the column reservoir. Let the filtrate flow through the IAC column by gravity force until the liquid level reaches 2 mm above the column packing. Add 10 ml of washing solution (5.12) to the column reservoir. Let the column run dry, then force 10 ml of air through the column with a syringe. Place a 2 ml volumetric flask under the column. Elute with 0,6 ml of LC grade methanol, collect the AF in a 2 ml volumetric flask, and let drip freely. Let the column run dry. Let stand for 1 min. Elute with an additional 0,6 ml of methanol and collect into the same volumetric flask. Let the column run dry and force 10 ml of air through the column and collect the eluate into the same volumetric flask. Dilute the eluate to volume with water and perform LC analysis.

7.3 Analysis

7.3.1 HPLC conditions

Column temperature: 35 °C.

Injection volume: 50 µl.

Flow rate: 1,0 ml/min.

FLD: excitation wavelength 362 nm, emission wavelength 440 nm.

7.3.2 Post-column derivatization

The options described in 6.16 have all proven to be suitable for post-column derivatization.

7.3.3 Calibration graph

Prepare a calibration graph by injecting working AF standard solutions (5.16). These solutions shall be injected at the beginning of the analysis and whenever the chromatographic conditions have changed. Plot peak areas (y-axis) of the individual analytes against the corresponding mass concentrations (ng/ml). Perform a linearity check.

7.3.4 Identification

Identify each aflatoxin peak in the sample chromatogram by comparing the retention times with those of corresponding reference standards[5]. Sometimes, it can be necessary to identify the AF peak by simultaneous injection of sample test solution and standard solution.

7.3.5 Determination

To carry out the determination by external standard method, the peak area of each aflatoxin in the sample solution is integrated, which is then related to the corresponding value for the standard substance in the calibration solutions (5.16).

Inject a reagent blank, AF working standards or test solution into the LC column. AFs elute in the order of AFG_2, AFG_1, AFB_2 and AFB_1. After passing through the post-column derivatization device, the AFG_1 and AFB_1 are derivatized to form AFG_{2a} (derivative of AFG_1) and AFB_{2a} (derivative of AFB_1). The peaks should be baseline resolved. If necessary, adjust the mobile phase by adding water, methanol or acetonitrile for maximum peak resolution and improved chromatographic performance.

8 Calculations

Calculate the mass fraction X of each AF in micrograms per kilogram in test sample using Formula (1) (external standard method):

$$X = \frac{\rho \times V_1 \times V_3 \times 1\,000}{V_2 \times m \times 1\,000} \quad (1)$$

where

ρ is the concentration of AFs in the final solution used in the LC determination, in ng/ml;

V_1 is the volume of the solvent used for extraction, in millilitres (V_1 = 25 ml);

V_2 is the volume of the extract used for clean-up, in millilitres (V_2 = 10 ml);

V_3 is the final volume (ml) of the test solution (V_3 = 2 ml);

m is the mass for test portion in grams, (m = 5 g);

1 000 is the conversion factor.

The total AFs is the sum of AFB_1, AFB_2, AFG_1, and AFG_2.

Report the result in the relevant format after rounding to two decimal places.

Indicate whether or not a correction for recovery has been applied.

NOTE The results of collaborative studies were not corrected for recovery.

9 Precision

9.1 Results of interlaboratory test

An interlaboratory test carried out at different levels (see Annex C) gave the statistical results (evaluated in accordance with ISO 5725-1[6] and ISO 5725-2[7]) shown in Tables C.1 to C.7.

9.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method with identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, is the repeatability limit r given in Tables C.1 to C.7.

9.3 Reproducibility

The absolute difference between two single test results, obtained using the same method with identical test material in different laboratories with different operators using different equipment, is the reproducibility limit R given in Tables C.1 to C.7.

10 Test report

The test report shall contain at least the following data:

— all information necessary for the identification of the sample;

— the test method used, with reference to this document, i.e. ISO 20948:2024;

— the results and the units in which the results have been expressed;

— the date and type of sampling (if known);

— the date of receipt of the laboratory sample;

— the date of the test;

— any particular points observed in the course of the test;

— any operations not specified in the method or regarded as optional which can have affected the results.

Annex A
(normative)

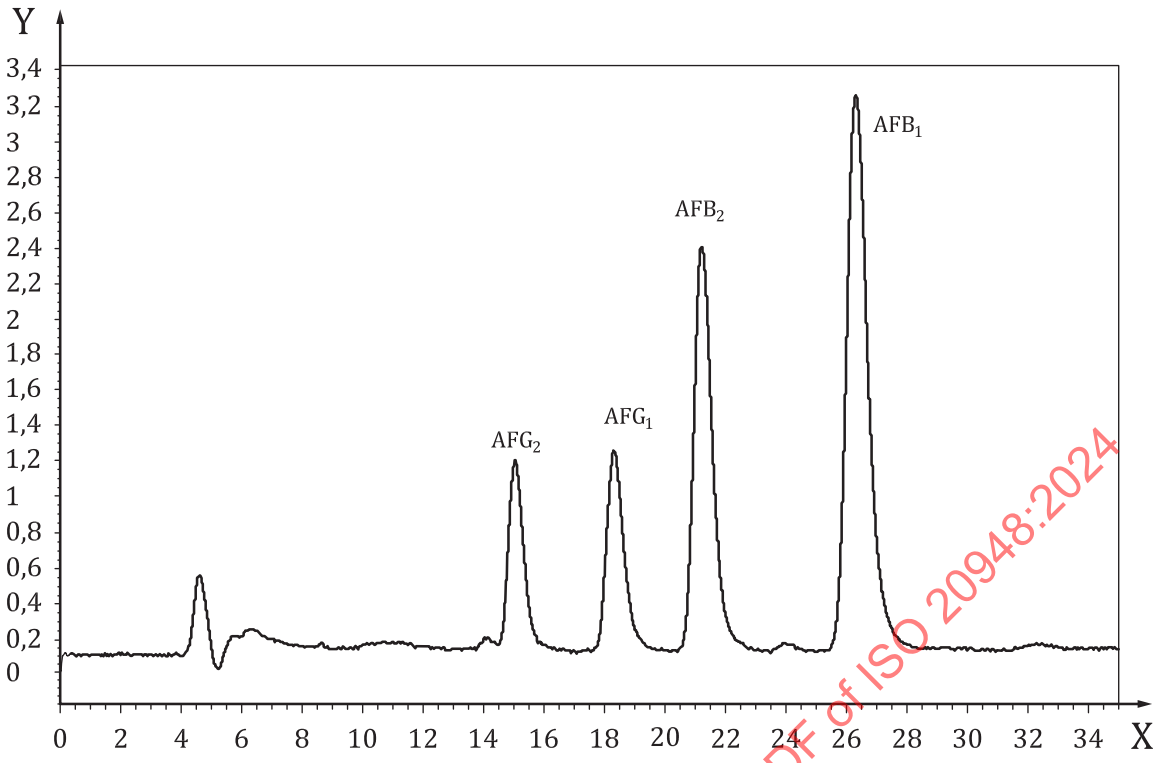
Reference standard solutions and typical chromatogram of AFs

Table A.1 — Concentration of standard solutions

Working standard solution	Intermediate AF standard solution µl	Final AF concentration of working standard solution ng/ml				
		AFB_1	AFB_2	AFG_1	AFG_2	AF total
1	0	0	0	0	0	0
2	50	0,5	0,15	0,5	0,15	1,3
3	200	2	0,6	2	0,6	5,2
4	500	5	1,5	5	1,5	13
5	1 000	10	3	10	3	26
6	2 000	20	6	20	6	52
7	4 000	40	12	40	12	104

The concentrations of the standard solutions and the calibration curve are given as references. Inject at least four calibration solutions of different suitable concentrations.

An example of a chromatogram containing the four main aflatoxins is given in Figure A.1



Key

X	retention time (min)
Y	intensity (uV)
AFG_2	Aflatoxin G_2
AFG_1	Aflatoxin G_1
AFB_2	Aflatoxin B_2
AFB_1	Aflatoxin B_1

Figure A.1 — Typical LC chromatogram of aflatoxins in corn oil

Annex B
(normative)

Determination of the exact concentration of AF stock standard solutions

Record the ultraviolet (UV) spectrum of aflatoxin solution from 330 nm to 370 nm against the acetonitrile in the reference cell (see ISO 16050[9], EN 17424:2020[10], and References [1] and [8]). Determine the concentration of the aflatoxin solution by measuring the absorbance (A) at a wavelength of maximum absorption close to 360 nm using Formula (B.1):

$$\rho = \frac{A_{max} \times M \times 1\,000}{\varepsilon \times d} \quad \text{(B.1)}$$

where

A_{max} is the absorbance determined at the maximum of the absorption curve;

M is the molecular weight of each aflatoxin, in g/mol;

ε is the molar absorption coefficient of each aflatoxin in acetonitrile, in m^2/mol;

d is the path length of the quartz cell, in cm.

Molecular weight (M) and molar absorption coefficient (ε) values are provided in Table B.1

Table B.1 — Molecular mass molar absorption coefficient of AFB_1, AFB_2, AFG_1 and AFG_2

Aflatoxins	M/(g/ mol)	ε (m^2/mol)
AFB_1	312	20 700
AFB_2	314	22 500
AFG_1	328	17 600
AFG_2	330	18 900

Annex C
(informative)

Results of collaborative trial

C.1 General

The values of the repeatability limit and reproducibility limit for this method were derived from the results of an international interlaboratory test programme carried out in accordance with ISO 5725-2[7] For olive oil, peanut oil and sesame oil, the collaborative trial was performed as part of the AOAC 2013.05[1] method programme and was organized in 2011 by the Technical Center of Qingdao Customs (named Technical Center of Shandong Entry & Exit Inspection and Quarantine Bureau before April, 2018), China. Sixteen laboratories from seven countries registered to take part but only 15 sets of results were received. The data are listed in Tables C.1 to C.3.

For corn oil, coconut oil, rapeseed oil and sunflower seed oil, six samples with three different spiked levels were tested for each oil type, respectively. Nine laboratories from three countries took part. The test programme was organized in 2022 to 2023 by the Technical Center of Qingdao Customs, China. The results obtained were subjected to statistical analysis in accordance with ISO 5725-1[6], ISO 5725-2[7] and ISO 5725-6[11] to give the precision data shown in Tables C.4 to C.7. HorRat values[12] are listed in each table.

C.2 Precision data

Table C.1 — Interlaboratory study results for aflatoxins in olive oil samples

Parameter	**AF total:** 2 µg/kg				**AF total:** 4 µg/kg				**AF total:** 20 µg/kg			
	AFB_1 (1 µg/kg)	**AFB_2** (0,25 µg/kg)	**AFG_1** (0,5 µg/kg)	**AFG_2** (0,25 µg/kg)	**AFB_1** (2 µg/kg)	**AFB_2** (0,5 µg/kg)	**AFG_1** (1 µg/kg)	**AFG_2** (0,5 µg/kg)	**AFB_1** (10 µg/kg)	**AFB_2** (2,5 µg/kg)	**AFG_1** (5 µg/kg)	**AFG_2** (2,5 µg/kg)
Number of participating laboratories (N)	15	15	15	15	15	15	15	15	15	15	15	15
Number of laboratories retained after elimination outliers (n)	14	13	14	13	15	15	15	14	15	15	15	14
Number of individual test results of all laboratories on each sample (z)	28	26	28	26	30	30	30	28	30	30	30	28
Mean value (µg/kg)	0,86	0,23	0,45	0,21	1,77	0,47	0,93	0,42	8,70	2,28	4,40	2,00
Repeatability standard deviation (s_r)	0,070	0,010	0,050	0,010	0,190	0,040	0,090	0,030	0,300	0,070	0,290	0,100
Repeatability coefficient of variation ($C_{V,r}$) %	8,09	5,40	11,75	5,08	10,48	8,30	9,72	7,64	3,50	3,24	6,51	4,82
Repeatability limit (r) (µg/kg)	0,196	0,028	0,140	0,028	0,532	0,112	0,252	0,084	0,840	0,196	0,812	0,280
Reproducibility standard deviation (s_R)	0,080	0,020	0,060	0,030	0,220	0,050	0,120	0,050	0,650	0,170	0,480	0,190
Reproducibility coefficient of variation ($C_{V,R}$) %	8,72	10,51	11,65	12,22	12,34	10,50	13,22	10,97	7,45	7,36	10,80	9,61
Reproducibility limit (R) (µg/kg)	0,224	0,056	0,168	0,084	0,616	0,140	0,336	0,140	1,820	0,476	1,344	0,532
HorRat value	0,40	0,48	0,53	0,56	0,56	0,48	0,60	0,50	0,34	0,33	0,49	0,44

Table C.2 — Interlaboratory study results for aflatoxins in peanut oil samples

Parameter	**AF total:** 2 µg/kg				**AF total:** 4 µg/kg				**AF total:** 20 µg/kg			
	AFB_1	**AFB_2**	**AFG_1**	**AFG_2**	**AFB_1**	**AFB_2**	**AFG_1**	**AFG_2**	**AFB_1**	**AFB_2**	**AFG_1**	**AFG_2**
	(1 µg/kg)	(0,25 µg/kg)	(0,5 µg/kg)	(0,25 µg/kg)	(2 µg/kg)	(0,5 µg/kg)	(1 µg/kg)	(0,5 µg/kg)	(10 µg/kg)	(2,5 µg/kg)	(5 µg/kg)	(2,5 µg/kg)
Number of participating laboratories (N)	15	15	15	15	15	15	15	15	15	15	15	15
Number of laboratories retained after elimination outliers (n)	13	15	15	14	15	14	15	14	14	14	14	13
Number of individual test results of all laboratories on each sample (z)	26	30	30	28	30	28	30	28	28	28	28	26
Mean value (µg/kg)	0,86	0,24	0,47	0,21	1,77	0,46	0,88	0,4	8,61	2,23	4,25	1,9
Repeatability standard deviation (s_r)	0,07	0,01	0,05	0,01	0,07	0,02	0,07	0,03	0,57	0,14	0,28	0,13
Repeatability coefficient of variation ($C_{V,r}$) %	8,39	7,94	11,40	6,08	3,98	4,18	8,12	7,43	6,67	6,20	6,60	6,68
Repeatability limit (r) (µg/kg)	0,196	0,028	0,14	0,028	0,196	0,056	0,196	0,084	1,596	0,392	0,784	0,364
Reproducibility standard deviation (s_R)	0,08	0,02	0,05	0,03	0,27	0,06	0.16	0.07	1	0,25	0,63	0,22
Reproducibility coefficient of variation ($C_{V,R}$) %	11,18	7,52	13,18	7,57	15,35	13,62	18,12	17,56	11,62	11,15	14,72	11,45
Reproducibility limit (R) (µg/kg)	0,224	0,056	0,14	0,084	0,756	0,168	0,448	0,196	2,8	0,7	1,764	0,616
HorRat value	0,51	0,34	0,60	0,34	0,70	0,62	0,82	0,80	0,53	0,51	0,67	0,52

Table C.3 — Interlaboratory study results for aflatoxins in sesame oil samples

Parameter	**AF total:** 2 µg/kg				**AF total:** 4 µg/kg			
	AFB_1 (1 µg/kg)	**AFB_2** (0,25 µg/kg)	**AFG_1** (0,5 µg/kg)	**AFG_2** (0,25 µg/kg)	**AFB_1** (2 µg/kg)	**AFB_2** (0,5 µg/kg)	**AFG_1** (1 µg/kg)	**AFG_2** (0,5 µg/kg)
Number of participating laboratories (N)	15	15	15	15	15	15	15	15
Number of laboratories retained after elimination outliers (n)	13	13	15	13	14	14	15	15
Number of individual test results of all laboratories on each sample (z)	26	26	30	26	28	28	30	30
Mean value (µg/kg)	0,91	0,24	0,49	0,20	1,85	0,48	0,96	0,39
Repeatability standard deviation (s_r)	0,040	0,010	0,070	0,010	0,130	0,030	0,140	0,030
Repeatability coefficient of variation ($C_{V,r}$) %	4,00	3,91	14,16	7,63	7,03	6,16	14,89	6,77
Repeatability limit (r) (µg/kg)	0,112	0,028	0,196	0,028	0,364	0,084	0,392	0,084
Reproducibility standard deviation (s_R)	0,07	0,02	0,08	0,04	0,2	0,04	0,15	0,07
Reproducibility coefficient of variation ($C_{V,R}$) %	8,05	7,13	15,92	18,12	10,82	8,32	15,09	17,03
Reproducibility limit (R) (µg/kg)	0,196	0,056	0,224	0,112	0,56	0,112	0,42	0,196
HorRat value	0,37	0,32	0,72	0,82	0,49	0,38	0,69	0,77