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