



**International
Standard**

ISO 20948

**Vegetable fats and oils —
Determination of aflatoxins
B₁, B₂, G₁ and G₂ by
immunoaffinity column clean-
up and high-performance liquid
chromatography**

**First edition
2024-11**

Reference number
ISO 20948:2024(en)

Full document details: [ISO 20948-2024](#)

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Published in Switzerland

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Foreword

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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).

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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 B₁, B₂, G₁ and G₂ 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 B₁, B₂, G₁ and G₂ by immunoaffinity column clean-up and high-performance liquid chromatography

1 Scope

This document specifies a method for the determination of the aflatoxins B₁, B₂, G₁ and G₂ 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 B₁, B₂, G₁ and G₂, and for the sum of aflatoxins B₁, B₂, G₁ and G₂, 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 B₁ = 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, $c(\text{HCl}) = 12 \text{ mol/l}$.

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 B₁ (AFB₁, C₁₇H₁₂O₆, CAS Registry Number^① 1162-65-8), purity ≥ 98 %;
- aflatoxin B₂ (AFB₂, C₁₇H₁₄O₆, CAS RN[®] 7220-81-7), purity ≥ 98 %;
- aflatoxin G₁ (AFG₁, C₁₇H₁₂O₇, CAS RN[®] 1165-39-5), purity ≥ 98 %;
- aflatoxin G₂ (AFG₂, C₁₇H₁₄O₇, 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 AFB₁, AFB₂, AFG₁ and AFG₂ 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.