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Animal and vegetable fats and oils — Determination of benzo[*a*]pyrene — Reverse-phase high performance liquid chromatography method

Corps gras d'origines animale et végétale — Détermination du benzo[a]pyrène — Méthode par chromatographie liquide à haute performance à polarité de phase inversée

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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 documents 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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

For an explanation on 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 the following URL: 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*.

This third edition cancels and replaces the second edition (ISO 15302:2007), of which it constitutes a minor revision. The scope of this document has been revised to exclude its application to milk and milk products and their derivatives.

Animal and vegetable fats and oils — Determination of benzo[*a*]pyrene — Reverse-phase high performance liquid chromatography method

1 Scope

This document specifies a method for the determination of benzo[*a*]pyrene in crude or refined edible oils and fats by reverse-phase high performance liquid chromatography (HPLC) using fluorimetric detection in the range 0,1 µg/kg to 50 µg/kg.

Milk and milk products (or fat coming from milk and milk products) are excluded from the scope of this document.

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 661, *Animal and vegetable fats and oils — Preparation of test sample*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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

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

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

3.1
benzo[*a*]pyrene content
mass fraction of benzo[*a*]pyrene in the test portion, as determined using the method specified in this document

Note 1 to entry: The content is expressed in micrograms per kilogram.

4 Principle

A test portion is dissolved in light petroleum and benzo[*b*]chrysene is added as internal standard. A suitable amount of sample is adsorbed on an alumina column and eluted with light petroleum to remove any benzo[*a*]pyrene present.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified. Where analytical grade solvents other than the recommended ones are used, a full blank analysis shall be carried out and the results of this blank analysis reported.

SAFETY PRECAUTIONS — Attention is drawn to regulations which specify handling procedures for dangerous substances. Users should be aware of and comply with technical, organizational, and personal safety measures.

5.1 Water, double distilled, filtered through a membrane filter of pore size 0,45 µm; deionized water obtained by purifying demineralized water systems may also be used.

5.2 Light petroleum, (boiling point range 40 °C to 60 °C), or **hexane**, redistilled over potassium hydroxide pellets (4 g/l).

5.3 Acetonitrile, suitable for HPLC.

5.4 Tetrahydrofuran, suitable for HPLC.

5.5 Acetonitrile-tetrahydrofuran mixture, prepared by mixing 90 ml acetonitrile (5.3) and 10 ml tetrahydrofuran (5.4).

5.6 Toluene, suitable for HPLC.

5.7 Sodium sulfate, granular, anhydrous.

5.8 Alumina activity grade 4, prepared from neutral aluminium oxide, activity grade super 1[1)], deactivated by the addition of 10 ml water (5.1) to 90 g of alumina.

Due to the differences in activity of alumina of various brands, a check is recommended to confirm that the deactivation procedure is appropriate for total benzo[*a*]pyrene recovery from a reference sample.

CAUTION — THE DEACTIVATION REACTION IS EXOTHERMIC AND PRESSURE CAN BUILD UP.

Shake the container for about 15 min and allow the contents to equilibrate for 24 h. Store the alumina in a closed vessel at ambient temperature.

5.9 Benzo[*a*]pyrene[2)], of purity 99,0 % by mass.

CAUTION — BENZO[*a*]PYRENE IS A KNOWN CARCINOGEN. CARRY OUT ALL WORK WITH IT IN A FUME HOOD, WEARING GLOVES TO MINIMIZE EXPOSURE.

5.9.1 Benzo[*a*]pyrene stock solution in toluene, 0,5 mg/ml.

Weigh, to the nearest 0,1 mg, about 12,5 mg of benzo[*a*]pyrene in a 25 ml graduated flask. Dissolve it in toluene (5.6) and make up to the mark with that solvent.

Store the solution in the dark at 4 °C where it is stable for at least 6 months.

5.9.2 Benzo[*a*]pyrene standard solutions.

Prepare two standard solutions containing approximately 0,2 µg/ml and 0,01 µg/ml of benzo[*a*]pyrene, respectively, by diluting aliquots of the stock solution (5.9.1) with acetonitrile.

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1) "Aluminium oxide 90 active neutral" is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

2) A suitable reference material is available from the Joint Research Centre of the European Commission, Institute for Reference Materials and Measurements (IRMM). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.