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**Animal and vegetable fats and oils —  
Determination of polycyclic aromatic  
hydrocarbons**

*Corps gras d'origines animale et végétale — Détermination des  
hydrocarbures aromatiques polycycliques*

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## 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 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](http://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](http://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 meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](http://www.iso.org/foreword)

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This second edition cancels and replaces the first edition (ISO 15753:2006), of which it constitutes a minor revision. It also incorporates Amendment ISO 15753:2006/Amd.1:2011. A non-applicability statement for milk and milk products has been added to the Scope.

<https://standards.iteh.ai/catalog/standards/iso/e7068979-a51b-4eba-bac6-7084b5d14e6b/iso-15753-2016>

# Animal and vegetable fats and oils — Determination of polycyclic aromatic hydrocarbons

## 1 Scope

This International Standard describes two methods for the determination of 15 polycyclic aromatic hydrocarbons (PAHs) in animal and vegetable fats and oils:

- a general method;
- a specific method for coconut oil and vegetable oils with short-chain fatty acids.

These methods are not quantitative for the very volatile compounds such as naphthalene, acenaphthene and fluorene. Due to interferences provided by the matrix itself, palm oil and olive pomace oil cannot be analysed using this method.

The quantification limit is 0,2 µg/kg for almost all compounds analysed, except for fluoranthene and benzo(*g,h,i*)perylene, where the quantification limit is 0,3 µg/kg, and indeno(1,2,3-*c,d*)pyrene, where the quantification limit is 1,0 µg/kg.

NOTE The results for olive pomace oil in [Annex B](#) show that this method is not applicable to this type of oil. The precision data determined are very poor.

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

## 2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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.

### 3.1

#### polycyclic aromatic hydrocarbon

#### PAH

compound that contains two or more condensed (fused) aromatic hydrocarbon rings and the content of which can be determined according to the method specified in this International Standard

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

Note 2 to entry: In general, PAHs are divided into light PAHs with two to four aromatic rings, and heavy PAHs with five or more aromatic rings.

EXAMPLE Light PAHs include:

naphthalene (CAS RN [91-20-3]), acenaphthene (CAS RN [83-32-9]), acenaphthylene (CAS RN [208-96-8]), fluorene (CAS RN [86-73-7]), anthracene (CAS RN [120-12-7]), phenanthrene (CAS RN [85-01-8]), fluoranthene (CAS RN [206-44-0]), chrysene (CAS RN [218-01-9]), benz(*a*)anthracene (CAS RN [56-55-3]), pyrene (CAS RN [129-00-0]).

Heavy PAHs include:

benzo(*a*)pyrene (CAS RN [50-32-8]), benzo(*b*)fluoranthene (CAS RN [205-99-2]), benzo(*k*)fluoranthene (CAS RN [207-08-9]), benzo(*g,h,i*)perylene (CAS RN [191-24-2]), dibenz(*a,h*)anthracene (CAS RN [53-70-3]), indeno(1,2,3-*c,d*)pyrene (CAS RN [193-39-5]).

## 4 Principle

The polycyclic aromatic hydrocarbons are extracted with an acetonitrile/acetone mixture followed by purification on C18 reversed-phase and then Florisil bonded-phase cartridges. Determination of the content of the individual polycyclic aromatic hydrocarbons after separation is achieved by means of high-pressure liquid chromatography (HPLC) and by measuring the fluorescence at various excitation and emission wavelengths.

## 5 Reagents and materials

**WARNING — Attention is drawn to the regulations governing the handling of dangerous matter. Technical, organizational and personal safety measures should be followed.**

Use only reagents of recognized analytical grade unless otherwise stated.

Check the quality of solvents before use by concentrating the solvent about 1 000 times by evaporation and analysing the concentrate by HPLC (300 ml to 300 µl). The chromatogram shall be free from peaks in the elution area of PAHs.

**5.1 Methanol**, “ultra resi-analysed” grade.<sup>1)</sup>

**5.2 Hexane**, HPLC grade.<sup>1)</sup>

**5.3 Acetonitrile**, HPLC grade.<sup>1)</sup>

**5.4 Acetone**, HPLC grade.<sup>1)</sup>

**5.5 Dichloromethane**, HPLC grade.<sup>1)</sup>

**5.6 Toluene**, HPLC grade.<sup>1)</sup>

**5.7 Water**, HPLC grade.<sup>1)</sup>

**5.8 Tetrahydrofuran**, HPLC grade.<sup>1)</sup>

**5.9 Solvent mixture 1**: acetonitrile/acetone (volume fraction 60 %/40 %).

Quantity used per sample: 41 ml for general method, 36 ml for specific method for coconut oil.

**5.10 Solvent mixture 2**: acetonitrile/acetone (volume fraction 80 %/20 %).

Quantity used per sample: 2 × 11 ml for method specific for coconut oil.

**5.11 Solvent mixture 3**: hexane/dichloromethane (volume fraction 75 %/25 %).

Quantity used per sample: 7 ml for general method, 2 × 7 ml for method specific for coconut oil.

<sup>1)</sup> These can be obtained from, for example, Baker. The information given in the footnotes is for the convenience of users of this document and does not constitute an endorsement by ISO of these products. Equivalent products may be used if they can be shown to lead to the same results.

**5.12 Mixture of tetrahydrofuran/methanol** (volume fraction 50 %/50 %).

**5.13 Standard solution with 16 certified EPA Priority PAHs in toluene,**<sup>2)</sup> at a concentration of 100 µg/ml (100 mg/l): naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenz(*a,h*)anthracene, benzo(*g,h,i*)perylene, indeno(1,2,3-*c,d*)pyrene.

NOTE 1 This is stored at -20 °C.

Before use, allow the solution to warm up to ambient temperature for at least 1 h.

NOTE 2 Acenaphthylene is not fluorescent and, thus, it cannot be determined by these methods.

**5.14 Stock standard solution**, 200 ng/ml (200 µg/l).

Add 100 µl of standard solution (5.13) with a 250 µl syringe (6.11) to a 50 ml volumetric flask (6.20) and dilute to the mark with acetonitrile.

**5.15 Working standard solution**, 50 ng/ml (50 µg/l).

Add 250 µl of stock standard solution (5.14) with a 250 µl syringe (6.11) to 750 µl of THF/methanol mixture (5.12) or acetonitrile (5.3).

**5.16 C18 bonded-phase cartridges,**<sup>3)</sup> 2 g phase, 12 ml capacity.

**5.17 Florisil bonded-phase cartridges,**<sup>3)</sup> 500 mg phase, 3 ml capacity.

**5.18 Stream of nitrogen**, pressure regulated at 34,5 kPa (5 psi, about 1,5 l/min).

## 6 Apparatus

Usual laboratory apparatus and, in particular, the following.

The use of disposable glass tubes is acceptable. The general use of glass is necessary as plastics can contain PAHs.

**6.1 Centrifuge**, capable of attaining at least 4 000 min<sup>-1</sup>, suitable for 100 ml and 10 ml tubes.

**6.2 HPLC system with binary gradient elution**, with solvent reservoir of 1 l capacity, a mobile phase liner filter, pump, autosampler, column temperature regulation set at 25 °C, fluorescence detector programmable over time for various excitation and emission wavelengths, and computer-assisted acquisition and data treatment.

**6.3 C18 reversed-phase column,**<sup>4)</sup> 250 mm in length, 4,6 mm internal diameter, 5 µm particles, suitable for PAH analysis.

**6.4 Vortex mixer.**

2) This can be obtained from, for example, Promochem.

3) This can be obtained from, for example, Varian.

4) This can be obtained from, for example, Vydac, ref. 201TP54.

**6.5 Automatic evaporator**,<sup>5)</sup> for 10 ml tube (optional), or water bath (6.6).

Recommended operating conditions:

- temperature of water bath            35 °C;
- nitrogen pressure                    34,5 kPa.

**6.6 Water bath**, regulated at 35 °C.

**6.7 Balance**, with readability of 0,1 mg.

**6.8 Centrifuge tubes**, of 100 ml capacity (one per sample).

**6.9 Conical centrifuge tubes**, of 11 ml capacity (three per sample), with PTFE septa and closed top screw caps (one per sample).

**6.10 Graduated measuring cylinders**, ISO 4788[6], class A.

**6.11 Microsyringe**, 250 µl.

**6.12 Syringe**, 1 000 µl.

**6.13 Graduated pipette**, capacity 5 ml, ISO 835[4], class A.

**6.14 Syringe**, 5 ml, equipped with an adapter cap for SPE cartridges.

**6.15 Vials for autosampler**.

**6.16 Microvials**, of 250 µl capacity, adapted for HPLC system.

**6.17 Ultrasonic bath**, with water temperature not higher than 40 °C.

**6.18 Pasteur pipettes**, with cotton wool in the top part to prevent contamination, ISO 7712[7].

**6.19 Device composed of stand and pincers**,<sup>6)</sup> to hold SPE cartridges or, if available, an automatic SPE work station.

NOTE Depending on the SPE sample processing station used, the proposed extraction methods may require slight adaptations (times, pressure, volumes).

**6.20 One-mark volumetric flask**, capacity 50 ml, ISO 1042[5], class A.

## 7 Sampling

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

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555.

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5) This can be obtained from, for example, Zymark, Zymark TurboVap LV evaporator.

6) This can be obtained from, for example, Zymark, Zymark Rapid Trace.



## 8 Sample preparation

Prepare the test sample in accordance with the method given in ISO 661. Before sampling, the liquid samples shall be at room temperature and homogenized by magnetic agitation.

Sample the solid matrix by melting the entire sample or by melting and homogenizing several core samples.

## 9 Procedure for determination of PAHs from fats and oils: General method

### 9.1 Preliminary remarks

In order to obtain repeatable results, the ambient temperature of the laboratory shall be regulated ( $\leq 20$  °C). This is a very important condition for the extraction of PAHs from coconut oil (or vegetable oils containing short-chain fatty acids). These oils contain fatty acids with short and long chains; when the ambient temperature is higher than 20 °C, the solubility of short-chain fatty acids increases.

Before use, rinse the whole vessel three times with hexane (5.2).

Each sequence of samples shall include a blank (9.2), and a standard solution extracted under the same conditions as the sample in order to calculate the recovery values of the extraction (9.3). The recovery values shall be within the range 70 % to 110 %. The mean recovery values are given in Table A.1.

For a quantitative analysis, two test portions shall be extracted and analysed separately, the final result being the mean value of the results of these two subsamples.

It is not possible to complete the entire analysis within a single day. Sample extracts shall be stored overnight under deep-freeze conditions of at least -18 °C:

- 1st day: step 1, step 2 and step 3, up to purification on C18 cartridge (see Figure A.1);
- 2nd day: step 3, purification on Florisil cartridge and preparation of HPLC system for sample analysis (see Figure A.1);
- following night and day(s): analysis of the samples (see Table A.2).

### 9.2 Blank

To ensure the absence of contamination of solvents and cartridges, the purification procedure (according to 9.5, 9.6 and Clause 11) shall first be carried out on a blank sample (sample with solvent mixture but with the oil omitted). The chromatogram obtained shall be free from the compounds of interest. If the chromatogram contains interferences, the source of interferences shall be determined and eliminated. Blank values cannot be used to correct sample values as blank values are generally not homogenous (repeatability).

### 9.3 Determination of recovery values (without matrix)

In order to verify the extraction efficiency of cartridges, carry out a test with a standard solution. Spike 1 750 µl of solvent mixture 1 (5.9) with 250 µl of working standard solution (5.15) with a 250 µl syringe (6.11). Transfer to a C18 cartridge and treat as described in 9.5, 9.6 and Clause 11.

**WARNING — When removing solvents under a stream of nitrogen (see 9.5.6), do not evaporate to dryness but leave about 50 µl in the vial, otherwise, volatile PAHs will be lost.**

### 9.4 Extraction (liquid/liquid extraction)

**9.4.1** The flow chart of the isolation procedure is given in Figure A.1.

**9.4.2** Weigh, to the nearest 1 mg, about 2,5 g of the sample into a 100 ml centrifuge tube (6.8).

Add 10 ml of solvent mixture 1 (5.9).

**9.4.3** Agitate the centrifuge tube for 30 s with the vortex mixer (half speed), and then put the tube in an ultrasonic bath (6.17) for 5 min.

**9.4.4** Centrifuge for 5 min at 4 000 min<sup>-1</sup>.

**9.4.5** Carefully remove the top layer with a Pasteur pipette (6.18) and transfer it to a weighed conical tube (6.9).

**9.4.6** Evaporate the solvent from the conical tube for 30 min to 40 min, under a stream of nitrogen (5.18), using either a water bath at 35 °C (6.6) or an automatic evaporator (6.5).

**9.4.7** Repeat the extraction twice with a further 10 ml of solvent mixture 1 (5.9).

Concentrate the extracts in the same conical tube under a stream of nitrogen (5.18) using water bath set at 35 °C (6.6) or using an automatic evaporator (6.5). The fat residue should be about 200 mg to 800 mg.

If the fat residue mass is higher than 800 mg, then the general method (see Clause 9) is not suitable and the method specific for coconut oil should be used (see Clause 10).

## **9.5 Purification on C18-bonded phase cartridge (solid/liquid extraction)**

**9.5.1** Cartridge conditioning: Put the cartridge (5.16) on a stand (6.19).

Rinse the cartridge with 2 volumes of 12 ml of methanol (5.1) then 2 volumes of 12 ml of acetonitrile (5.3). Allow the solvent to flow through under atmospheric pressure.

**9.5.2** Put a weighed conical tube (6.9) under the cartridge (5.16).

**9.5.3** With a syringe (6.12) or a graduated pipette (6.13), introduce 2 ml of solvent mixture 1 (5.9) into the conical tube containing residual fat material (9.4.6).

Agitate the tube with the vortex mixer (6.4) for 15 s. Centrifuge for 30 s. Transfer the top layer to the cartridge (5.16) with a Pasteur pipette (6.18). Repeat the operation twice (2 ml of solvent mixture 1, mixing, centrifuging and transferring onto the cartridge). Collect the solvent eluting from the cartridge together with the elution solvent.

**9.5.4** Add 5 ml of solvent mixture 1 (5.9) to the top of the cartridge (5.16) and allow the elution to proceed under atmospheric pressure.

**9.5.5** Using a syringe (6.14), inject air into the cartridge in order to elute the remaining solvent and any PAHs which could be retained in the phase.

**9.5.6** Remove solvents under a stream of nitrogen (5.18) using a water bath set at 35 °C (6.6) or an automatic evaporator (6.5).

The fat residue should be not more than 50 mg.

**9.5.7** Dilute the residue in 1 ml of hexane (5.2), measured with a syringe (6.12).

Close the conical tube hermetically and store at -18 °C until further use.