



# SLOVENSKI STANDARD SIST EN ISO 15302:2007

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Animal and vegetable fats and oils - Determination of benzo[a]pyrene content - Reverse-phase high-performance liquid chromatography method (ISO 15302:1998)

Tierische und pflanzliche Fette und Öle - Bestimmung des Benzo[a]pyren-Gehalts - Umkehrphasen-HPLC-Verfahren (ISO 15302:1998)

Corps gras d'origines animale et végétale - Détermination de la teneur en benzo[a]pyrene - Méthode par chromatographie liquide à haute performance à polarité de phase inversée (ISO 15302:1998)

Ta slovenski standard je istoveten z: EN ISO 15302:2007

**ICS:**

67.200.10 Üæ dā • \ ^ Á Á ã ç ð \ ^      Animal and vegetable fats  
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**SIST EN ISO 15302:2007**                              en;fr;de

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ICS 67.200.10

English Version

Animal and vegetable fats and oils - Determination of  
benzo[a]pyrene content - Reverse-phase high-performance  
liquid chromatography method (ISO 15302:1998)

Corps gras d'origines animale et végétale - Détermination  
de la teneur en benzo[a]pyrène - Méthode par  
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Tierische und pflanzliche Fette und Öle - Bestimmung des  
Benzo[a]pyren-Gehalts - Umkehrphasen-HPLC-Verfahren  
(ISO 15302:1998)

This European Standard was approved by CEN on 23 December 2006.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

## Foreword

The text of ISO 15302:1998 has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 15302:2007 by Technical Committee CEN/TC 307 "Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis", the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 2007, and conflicting national standards shall be withdrawn at the latest by July 2007.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

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Endorsement notice

The text of ISO 15302:1998 has been approved by CEN as EN ISO 15302:2007 without any modifications.

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

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

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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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 15302 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Annexes A and B of this International Standard are for information only.

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

## 1 Scope

This International Standard 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 from 0,1 µg/kg to 10 µg/kg.

## 2 Normative reference

The following standard contains provisions, which through reference in this text, constitute provisions of this International Standard. At the time of publication the edition indicated was valid. All standards are subject to revision and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

[SIST EN ISO 15302:2007](https://standards.iteh.ai/catalog/standards/sist/1f695e97-0ac5-4327-8109-3e21e3111111/iso-661-1989)

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ISO 661:1989, *Animal and vegetable fats and oils*, 3.2 Preparation of test sample

## 3 Principle

Adsorption of a suitable amount of sample on an alumina column, followed by elution with light petroleum of any benzo[a]pyrene present, and subsequent analysis of the eluate by HPLC using a fluorimetric detector.

## 4 Reagents

All reagents shall be of recognized analytical grade. 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.

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

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

**4.3 Acetonitrile**, suitable for HPLC.

**4.4 Tetrahydrofuran**, suitable for HPLC.

**4.5 Toluene**, suitable for HPLC.

**4.6 Sodium sulfate**, granular, anhydrous.

**4.7 Alumina**, activity grade 4, prepared from neutral aluminium oxide, activity grade super 1, deactivated by the addition of 10 ml distilled water to 90 g of alumina.

**CAUTION — THE REACTION IS EXOTHERMIC AND PRESSURE MAY 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.

**4.8 Benzo[a]pyrene**, 99,0 % purity.

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

**4.9 Benzo[a]pyrene solutions**<sup>1)</sup>

**4.9.1 Stock solution**

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 (4.5) and fill to the mark.

This solution contains about 0,5 mg/ml benzo[a]pyrene and should be stored in the dark at 4 °C when it is stable for 6 months at least.

**4.9.2 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 (4.9.1) with acetonitrile (4.3).

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## 5 Apparatus

Usual laboratory apparatus and, in particular, the following.

**5.1 Glass column for chromatography**, 300 mm long, 15 mm internal diameter, fitted with sintered glass discs, and polytetrafluoroethylene (PTFE) tap.

**5.2 Water baths** (two), maintained at 35 °C ± 1°C and 65 °C ± 1°C.

**5.3 Flash evaporator**

A rotary evaporator with vacuum and a water bath set at 40 °C may be used. Care should be taken to prevent cross contamination. Clean the system thoroughly between determinations.

**5.4 High-performance liquid chromatograph**, consisting of an HPLC pump, injection valve with 10 µl sample loop, reverse-phase column, electronic integrator and chart recorder.

NOTE If an autosampler is used, the sample loop should be flushed with acetonitrile between subsequent injections.

1) A suitable reference material is available from the Commission of the European Community Bureau of Reference (BCR), rue de la Loi 200, B-1049, Brussels, Belgium.



## 5.5 Columns for HPLC analysis

**5.5.1 Reverse-phase guard column**, capable of resolving benzo[*a*]pyrene from co-extractives, together with appropriate precolumn [e.g. stainless-steel precolumn 75 mm long, 4,6 mm internal diameter, packed with Lichrosorb RP-18 (5 µm particle size).<sup>2)</sup>

**5.5.2 HPLC reverse-phase column**, 250 mm long, of 4,6 mm internal diameter (stainless steel), for polycyclic aromatic hydrocarbons (PAHs) (e.g. Chromspher 5 PAH or Vydac 201 TP5).<sup>3)</sup>

**5.6 Fluorescence detector**, with emission wavelength at 406 nm (slit 10 nm) and excitation wavelength at 384 nm (slit 10 nm). The detector shall be capable of the required performance to carry out the analysis.

**5.7 Crimp-top minivials**, of about 1 ml volume, with Teflon-layered septa and aluminium caps.

**5.8 Hand crimper**, for crimping the caps onto the vials.

**5.9 Disposable pipettes**

## 6 Sampling

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

It is important the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

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## 7 Preparation of test sample

Prepare the test sample in accordance with ISO 661.  
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## 8 Procedure

### 8.1 Clean up of sample

**8.1.1** Weigh, to the nearest 0,001 g, about 2 g of the oil sample into a 10 ml graduated flask. Dissolve in light petroleum (4.2) and dilute to the mark.

**8.1.2** Fill the chromatography column (5.1) to half its height with light petroleum (4.2). Rapidly weigh 22 g of alumina (4.7) into a small beaker and transfer the alumina immediately to the column, promoting settling of the alumina by gently tapping the column.

**8.1.3** Add anhydrous sodium sulfate (4.6) to the top of the column to form a layer about 30 mm deep.

**8.1.4** Open the tap and allow the light petroleum to fall to the level of the top of the sodium sulfate layer.

**8.1.5** Place a 20 ml graduated flask under the column.

**8.1.6** Pipette 2,00 ml of the oil solution (8.1.1) onto the column. Rinse the column with minimal amounts of light petroleum, allowing the solvent layer to run into the sodium sulfate layer between rinsings.

2) Lichrosorb RP-18 is an example of a product commercially available. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

3) Chromspher 5 PAH and Vydac 201 TP5 are examples of products commercially available. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.