# INTERNATIONAL STANDARD



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# Animal and vegetable fats and oils — Determination of polycyclic aromatic hydrocarbons by on-line donor-acceptor complex chromatography and HPLC with fluorescence detection

Corps gras d'origines animale et végétale — Détermination de la teneur en hydrocarbures aromatiques polycycliques par chromatographie de complexe donneur-accepteur et CLHP avec détection par fluorescence



Reference number ISO 22959:2009(E)

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## Foreword

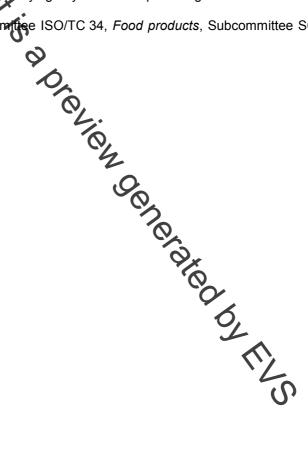
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ISO 22959 was prepared by Technical Committee ISO/TC 34, Food products, Subcommittee SC 11, Animal and vegetable fats and oils.



### Introduction

Polycyclic aromatic hydrocarbons (PAHs) are formed during pyrolytic processes such as the incomplete combustion of organic substances or have a petrogenic origin (mineral oils). Edible fats and oils may be contaminated by environmental pollution and/or processing steps prior to refining. The presence of PAHs in fats and oils is a health concern due to their carcinogenicity. Different levels of PAHs have been observed in crude edible oils. Befining of the oils (deodorization, bleaching, charcoal treatment) under the appropriate conditions reduces the content of the individual PAHs to the microgram per kilogram level. The known methods of analysis of PAHs in edible fats and oils include complex and laborious extraction and clean-up procedures to isolate the low levels of PAHs present.

With the donor-acceptor complex-chromatography (DACC) technique, PAHs can be extracted from different matrices. PAHs are electron donors ( $\pi$ -electrons) and the strong interaction of the PAHs with an electron acceptor stationary phase results in retention of the PAHs and elution of (the bulk of) the other components of the oil. This International Standard specifies an automated on-line method for the determination of PAHs in edible oils and fats, which can easily be applied as a routine analysis. The method consists of an LC-LC coupling of a clean-up DACC column to an analytical column for the separation. PAHs are quantified by fluorescence detection.

Compared to older techniques, this automated on-line method significantly reduces the amount of solvent used and saves considerable time. The DACC column clean-up is fast and is carried out during the HPLC run of the previous sample. The total analysis time for one sample is approximately 90 min, compared with the traditional methods which require 8 h to 10 h Moreover, the system can run 24 h/day. Finally, losses of volatile PAHs during solvent evaporation, for example, are eliminated. The quantification limits of 0,1 µg/kg of the individual PAHs have been retained with the DACC method, which automatically corrects for possibly incomplete recoveries because the calibration samples are subjected to the same treatment as the samples to be analysed. The system uses conventional HPLC instrumentation.

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# Animal and vegetable fats and oils — Determination of polycyclic aromatic hydrocarbons by on-line donor-acceptor complex chromatography and HPLC with fluorescence detection

#### 1 Scope

This International Standard specifies a high performance liquid chromatographic (HPLC) procedure for the determination of polycyclic aromatic hydrocarbons (PAHs) in edible fats and oils.

The method has been validated for coconut (CN), olive (OV), sunflower (SF), and soybean (BO) oil, and is possibly applicable to other oils, dependent on the determination of appropriate parameters.

The lowest level of quantification for the PAHs is  $0,1 \mu g/kg$ . The lowest possible amount of each PAH which can be distinguished from the baseline hoise has not been determined. The validated concentration range of the method is 0,1 µg/kg to 3,5 µg/kg for each individual PAH. For samples containing (light) PAH contents > 3,5 µg/kg, dilution to bring the contents into the validated range is possible. It is also possible to adjust the range of the calibration curves. However, ranges exceeding 3,5 µg/kg have not been validated.

 $\mathbf{O}$ PAHs which can be determined by this method are: anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[e]pyrene, benzo[a]pyrene, perylene, benzo[*ghi*]perylene, anthanthrene, dibenzo[a,h]anthracene, coronene, indeno[1,2,3-colovrene, benzo[a]fluoranthene, benzo[b]fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene.

#### 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced The following ... references, only the edition once ... document (including any amendments) applies. ISO 661, Animal and vegetable fats and oils — Preparation of test sample of the second sec

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 whose content can be determined according to the method specified in this International Standard