



**SLOVENSKI STANDARD**  
**oSIST prEN ISO 20122:2023**  
**01-december-2023**

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**Rastlinska olja - Določevanje nasičenih ogljikovodikov (MOSH) in aromatskih ogljikovodikov iz mineralnih olj (MOAH) z on-line sklopljeno analizo HPLC-GC-FID - Metoda za nizko mejo določljivosti (ISO/DIS 20122:2023)**

Vegetable oils - Determination of mineral oil saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH) with online coupled HPLC-GC-FID analysis - Method for low limit of quantification (ISO/DIS 20122:2023)

Pflanzliche Öle - Bestimmung von gesättigten Mineralölkohlenwasserstoffen (MOSH) und aromatischen Kohlenwasserstoffen (MOAH) mit online gekoppelter HPLC-GC-FID-Analyse - Verfahren für die niedrige Bestimmungsgrenze (ISO/DIS 20122:2023)

Huiles végétales - Dosage des hydrocarbures saturés d'huile minérale (MOSH) et des hydrocarbures aromatiques d'huile minérale (MOAH) par analyse par CLHP-CG-FID en ligne - Méthode pour une faible limite de quantification (ISO/DIS 20122:2023)

**Ta slovenski standard je istoveten z: prEN ISO 20122**

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**ICS:**

67.200.10	Rastlinske in živalske maščobe in olja	Animal and vegetable fats and oils
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### Vegetable oils — Determination of mineral oil saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH) with online coupled HPLC-GC-FID analysis — Method for low limit of quantification

*Huiles végétales — Détermination des hydrocarbures saturés d'huile minérale (MOSH) et des hydrocarbures aromatiques d'huile minérale (MOAH) par dosage HPLC-GC-FID en ligne — Méthode pour une faible limite de quantification*

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# Contents

Page

Foreword.....	iv
Introduction.....	v
<b>1 Scope.....</b>	<b>1</b>
<b>2 Normative references.....</b>	<b>1</b>
<b>3 Terms and definitions.....</b>	<b>1</b>
<b>4 Principle.....</b>	<b>2</b>
<b>5 Reagents.....</b>	<b>3</b>
<b>6 Apparatus.....</b>	<b>6</b>
<b>7 Sample.....</b>	<b>7</b>
7.1 Sampling.....	7
7.2 Preparation of the final sample for liquid and solid fats.....	7
<b>8 Procedures.....</b>	<b>7</b>
8.1 Hexane/ethanol distribution for removal of interfering substances.....	8
8.2 Saponification.....	8
8.3 Removal of biogenic n-alkanes with aluminium oxide for determination of the MOSH fraction.....	8
8.4 Clean-up before epoxidation to separate polar substances.....	8
8.5 Ethanolic epoxidation of the MOAH fraction to oxidize unsaturated non-aromatic compounds <sup>[8]</sup> .....	9
8.6 HPLC-GC separation.....	9
8.6.1 HPLC conditions.....	9
8.6.2 GC configuration.....	10
8.6.3 Solvent vapor exit configuration.....	10
8.6.4 Peak identification.....	11
8.6.5 System suitability test.....	12
8.7 Blank run.....	13
8.8 Quality control.....	13
<b>9 Result of the determination.....</b>	<b>13</b>
9.1 Testing the chromatograms for sufficient epoxidation and other relevant parameters.....	13
9.2 Calculation.....	14
<b>10 Precision of the method.....</b>	<b>15</b>
10.1 Repeatability limit ( <i>r</i> ).....	15
10.2 Reproducibility limit ( <i>R</i> ).....	15
<b>11 Test report.....</b>	<b>15</b>
<b>Annex A (informative) Graphics and chromatograms.....</b>	<b>16</b>
<b>Annex B (informative) Precision data.....</b>	<b>27</b>
<b>Annex C (Informative) Alternative method for the epoxidation of the MOAH fraction (performic acid epoxidation)<sup>[13]</sup>.....</b>	<b>40</b>
<b>Bibliography.....</b>	<b>41</b>

## ISO/DIS 20122:2023(E)

### 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)).

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

For an explanation of 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 [www.iso.org/iso/foreword.html](http://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*.

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](http://www.iso.org/members.html).

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## Introduction

In order to achieve a low limit of quantification (LOQ), the method contains additional and partially modified processing steps, specifications for the uniform processing of defined product groups and additional requirements for system suitability compared to EN 16995:2017.

The method has been tested in an interlaboratory study via the analysis of both naturally contaminated and spiked vegetable oil samples, ranging from 1 mg/kg to 75 mg/kg for MOSH, and from 1 mg/kg to 7 mg/kg for MOAH.

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# Vegetable oils — Determination of mineral oil saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH) with online coupled HPLC-GC-FID analysis — Method for low limit of quantification

## 1 Scope

This International standard method specifies a procedure for the determination of saturated and aromatic hydrocarbons (from C10 to C50) in vegetable fats and oils using the online-coupled HPLC-GC-FID ([1],[2] and[3]). This standard is not intended to be applied to other matrices.

The method is applicable for the analysis of mineral oil saturated hydrocarbons (MOSH) and/or mineral oil aromatic hydrocarbons (MOAH).

According to the results of the interlaboratory studies, the method has been proven suitable for MOSH mass concentrations above 3 mg/kg and MOAH mass concentrations above 2 mg/kg.

In case of suspected interferences, the fossil origin of the MOSH and MOAH fraction can be verified by examination by GC×GC-MS.

An alternative method for the epoxidation of the MOAH fraction (performic acid epoxidation) is proposed in [Annex C](#). This alternative method provides comparable results to the ethanolic epoxidation of the MOAH fraction described in [8.5](#). This alternative method for epoxidation has proven to be efficient for samples with a high amount of interferences in the MOAH fraction (e.g. tropical oils)<sup>[13]</sup>.

## 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 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/>

### 3.1

#### mineral oil saturated hydrocarbons

##### MOSH

Paraffinic (open-chain, usually branched) and naphthenic (cyclic, alkylated) hydrocarbons in the boiling range of *n*-alkanes with a chain length of 10 to 50 carbon atoms, which are obtained from mineral oil by this method by means of online-coupled HPLC-GC-FID.

## ISO/DIS 20122:2023(E)

### 3.2

#### mineral oil aromatic hydrocarbons

##### MOAH

Aromatic mainly alkylated hydrocarbons from mineral oil in the boiling range of *n*-alkanes with a chain length of 10 to 50 carbon atoms, determined by means of online HPLC-GC-FID.

### 3.3

#### unresolved complex mixture

##### UCM

Complex mixture of saturated or aromatic hydrocarbons not resolved by gas chromatography such as branched paraffins, alkylated naphthenes and alkylated aromatics, that produces a hump when analysed by GC-FID.

### 3.4

#### polyolefin oligomeric saturated hydrocarbons

##### POSH

Synthetic hydrocarbons from oligomers of polyolefins, such as polyethylene, polypropylene and polybutylenes. Food contact uses comprise plastic bags, containers or films, heat sealable layers and other lamination as well as adhesives and plasticizers. POSH can be distinguished from MOSH by their chromatographic pattern, but it is difficult to differentiate and chromatographically separate them from the MOSH if both are present<sup>[2]</sup>.

### 3.5

#### resin oligomeric saturated hydrocarbons

##### ROSH

Synthetic saturated hydrocarbons (oligomers from monoterpenes, cyclopentadienes and other C5- or C9-monomeres) that are ingredients of hot-melt adhesives and can migrate into the sample mostly via gas phase transfer or via direct contact.

### 3.6

#### resin oligomeric aromatic hydrocarbons

##### ROAH

Synthetic aromatic hydrocarbons that are ingredients of hot-melt adhesives and can migrate into the sample mostly via gas phase transfer or by direct contact.

### 3.7

#### poly-alpha-olefins

##### PAO

Synthetic iso-paraffins with short and long side chains, used as lubricants or in adhesives and hotmelts. When analysed by GC-FID, they are recognized by series of rather narrow humps of unresolved branched hydrocarbons with regular distance between them<sup>[2]</sup>.

## 4 Principle

The sample is saponified and from the unsaponifiable residue, purified fractions are obtained by further steps. These fractions are separated on a silica gel column of the HPLC-GC-FID system into MOSH and MOAH fractions and each is transferred separately to the GC by online coupling. Most of the solvent is removed via an early vapor exit between the uncoated precolumn and the GC separation column.

In order to meet the requirements of the various interfering accompanying substances occurring in the samples, specific sample preparation procedures are described for different product groups. However, epoxidation is a purification step that is necessary for the quantification of MOAH for all vegetable oil samples. This purification step allows the elimination of olefins like squalene, which elute within the MOAH fraction and interfere with quantification. Depending on the sample, this reaction may induce the epoxidation of a part of the MOAH or incomplete removal of the interfering olefins.

The signal area for mineral oil is calculated by subtracting riding peaks from the total area. The riding peaks can be caused by *n*-alkanes (naturally occurring hydrocarbons), terpenes, sterenes, squalene and their isomerization products as well as other substances. MOSH and MOAH are quantitated by

internal standard added before analysis. Verification standards are added for monitoring proper HPLC fractionation and GC transfer conditions.

NOTE 1 to entry Epoxidation step can induce degradation of MOAH with 3 or more aromatic rings.

## 5 Reagents

**WARNING — Reference is drawn to the regulations which specify the handling of hazardous substances. Technical, organisational and personal safety measures shall be followed.**

NOTE 1 to entry All materials shall be tested for their influence in a blank run. It is recommended to heat all glassware in an oven according to the instructions. All other materials that come into direct contact with the sample should also be heated and should not be made of polyethylene or polypropylene.

Unless otherwise stated

- analytical pure reagents shall be used;
- water shall be either distilled or of corresponding purity;
- a solution is understood to be an aqueous solution.

**5.1 Silica gel 60<sup>1)</sup>**, extra pure, for column chromatography, with a particle size between 60 µm and 200 µm (70 to 230 mesh) stored in a glass bottle (protection against contamination). The silica gel is heated in an oven at 400 °C for at least 16 h and cooled in a clean desiccator (without ground grease).

**5.2 Sodium sulfate**, anhydrous, analytical grade, purity ≥ 99 %.

NOTE 1 to entry In case of contamination, heat the sodium sulfate in an oven at 400 °C for at least 16 h and allow to cool down in a clean desiccator (without ground grease).

**5.3 *n*-Hexane**, free of hydrocarbons in the boiling range of the *n*-alkanes C10 - C50 and other impurities such as hexane oxidation products.

Check the purity of the *n*-hexane as follows: mix 30 ml *n*-hexane with 25 µl of the internal standard solution (5.17) and 2 drops of bis(2-ethylhexyl) maleate (5.26) and evaporate using an evaporator unit. Dissolve the residue in 0,2 ml *n*-hexane and inject 50 µl into the HPLC-GC-FID system for analysis. The hump signal (excluding any sharp single peaks of the solvent blank) should not exceed one-tenth of the LOQ.

NOTE 1 to entry Hydrocarbons in the boiling range under investigation interfere with the specific detection of mineral oil constituents in gas chromatography of the MOSH and MOAH fractions, while polar compounds such as hexane oxidation products interfere with the separation of long-chain *n*-alkanes in column chromatography on alumina.

**5.4 Dichloromethane (DCM)**, purity ≥ 99 %.

NOTE 1 to entry Test the purity as for *n*-hexane (5.3) with 30 ml DCM.

**5.5 Toluene**

**5.6 Perylene (PER)**, purity > 99 %.

**5.7 5- $\alpha$ -cholestane (CHO)**, purity ≥ 97 %.

1) Silica gel is available from Merck, reference 7754 or 7734 ([www.merck-chemicals.com](http://www.merck-chemicals.com)). It is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

## ISO/DIS 20122:2023(E)

5.8 *n*-Undecane (C11), purity  $\geq$  99 %.

5.9 *n*-Tridecane (C13), purity  $\geq$  99 %.

5.10 Tri-*tert*-butylbenzene (TBB), purity  $\geq$  97 %.

5.11 Bicyclohexyl (CYCY), purity  $\geq$  99 %.

5.12 1-Methyl naphthalene (1-MN), purity  $\geq$  95 %.

5.13 2-Methyl naphthalene (2-MN), purity  $\geq$  97 %.

5.14 Pentyl benzene (PB), purity  $\geq$  99 %.

5.15 **Stock solution, mass concentrations**  $\rho = 5, 10$  or  $20$  mg/ml: weigh e.g.  $50$  mg of C13 (5.9),  $100$  mg each of C11 (5.8), TBB (5.10), CYCY (5.11), 1-MN (5.12), 2-MN (5.13) and PB (5.14) as well as  $200$  mg CHO (5.7) and PER (5.6) to the nearest  $1$  mg and fill up to the mark in a  $10$  ml volumetric flask with toluene (5.5). Store at room temperature to keep the solutions stable. Dissolve any crystals formed during storage by gentle heating.

NOTE 1 to entry The verification of the start of the MOAH fraction based on TBB may result in losses of higher alkylated benzenes and naphthalenes, when present in the sample (i.e. cosmetics) and when the chromatographic performance of the column is limited. In such cases di(2-ethylhexyl) benzene (DEHB) may be used in addition as verification standard and the fractionation has to be adapted<sup>[7]</sup>.

5.16 **Internal standard solution 1 (ISTD1)<sup>2)</sup>**, mass concentrations  $\rho = 150$   $\mu$ g/ml (C13),  $300$   $\mu$ g/ml (C11, CYCY, PB, 1-MN, 2-MN and TBB) and  $600$   $\mu$ g/ml (CHO and PER): transfer  $300$   $\mu$ l stock solution (5.15) into a  $10$  ml volumetric flask and fill up to the mark with toluene (5.5).

5.17 **Internal standard solution 2 (ISTD2)**, mass concentrations  $\rho = 30$   $\mu$ g/ml (C13),  $60$   $\mu$ g/ml (C11, TBB, CYCY, 1-MN, 2-MN and PB) and  $120$   $\mu$ g/ml (PER and CHO): dilute ISTD1 solution (5.16) by a factor of  $5$ , e.g. fill up  $1\ 000$   $\mu$ l ISTD1 solution (5.16) to  $5$  ml with *n*-hexane.

5.18 **Aluminium oxide 90, alkaline**, for column chromatography,  $0,063$  mm to  $0,2$  mm, activated: heat the alumina before use for at least  $16$  h at  $500$  °C in an oven and cool down to room temperature in a desiccator.

5.19 ***meta*-chloroperbenzoic acid (mCPBA)**, stated quantities based on a purity of about  $\leq 77$  %;

NOTE 1 to entry Commercially available *m*CPBA contains varying amounts of *m*CPBA, *meta*-chlorobenzoic acid and water. For purification of the reagent remove contaminating hydrocarbons: e.g. finely suspend  $5$  g *m*CPBA with  $200$  ml *n*-hexane in a polyethylene terephthalate (PET) beaker in an ultrasonic bath and filter off by a vacuum frit. Let the purified *m*CPBA dry in a fume cupboard. Do not store in glass containers, as *m*CPBA decomposes on glass surfaces. Commercially available *m*CPBA still contains *meta*-chlorobenzoic acid and residual moisture to make handling in the laboratory safe. Pure *m*CPBA, on the other hand, is explosive, so isolation of *m*CPBA as a pure substance, which goes beyond the cleaning described here, is not advisable. Washing with a solvent mixture of  $200$  ml *n*-hexane and  $20$  ml DCM can remove further impurities, but also leads to significant higher losses (yields only  $75$  % of the initial *m*CPBA with a content of about  $74$  to  $84$  g *m*CPBA /  $100$  g starting material in the purified product).

2) This standard mixture is available from e.g. Restek Corp., Cat.# 31070. It is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.