International Standard

ISO 20122

First edition
2024-04

Vegetable oils — Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with online-coupled high performance liquid chromatography-gas chromatography-flame ionization detection (HPLC-GC-FID) analysis — Method for low limit of quantification

Reference number
ISO 20122:2024(en)

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Published in Switzerland

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Foreword

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This document was prepared by Technical Committee ISO/TC 34 *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 307, *Oilseeds, vegetable and animal fats and oils and their by-products — Methods of sampling and analysis*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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.

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.

Vegetable oils — Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with online-coupled high performance liquid chromatography-gas chromatography-flame ionization detection (HPLC-GC-FID) analysis — Method for low limit of quantification

1 Scope

This document specifies a procedure for the determination of saturated and aromatic hydrocarbons (from C10 to C50) in vegetable fats and oils using the online-coupled high performance liquid chromatography-gas chromatography-flame ionization detection (HPLC-GC-FID).[4][5][6] This document does not apply 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 8.6. 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).[14]

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 high performance liquid chromatography-gas chromatography-flame ionization detection (HPLC-GC-FID)

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-coupled high performance liquid chromatography-gas chromatography-flame ionization detection (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 gas chromatography-flame ionization detection (GC-FID)

3.4
polyolefin oligomeric saturated hydrocarbons
POSH
synthetic hydrocarbons from oligomers of polyolefins, such as polyethylene, polypropylene and polybutylenes

Note 1 to entry: Food contact uses comprise plastic bags, containers or films, heat sealable layers and other lamination as well as adhesives and plasticizers.

Note 2 to entry: POSH can be distinguished from mineral oil saturated hydrocarbons (MOSH) by their chromatographic pattern, but it is difficult to differentiate and chromatographically separate them from the MOSH if both are present.[5]

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

Note 1 to entry: When analysed by gas chromatography-flame ionization detection (GC-FID), they are recognized by series of rather narrow humps of unresolved branched hydrocarbons with regular distance between them.[5]

4 Principle

The sample is saponified and from the unsaponifiable residue, purified fractions are obtained following additional steps. These fractions are separated on a silica gel column of the HPLC-GC-FID system into MOSH

and MOAH fractions; each is transferred separately to the GC by online coupling. Most of the solvent is removed via a solvent vapour exit between the uncoated pre-column 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 such as squalene, which elute within the MOAH fraction and interfere with quantification. Depending on the sample, this reaction can 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 Epoxidation step can induce degradation of MOAH with three or more aromatic rings.

5 Reagents

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

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[1)], 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 %.

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 to 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 two drops of bis(2-ethylhexyl) maleate (5.26);

— evaporate using an evaporator unit;

— dissolve the residue in 0,2 ml *n*-hexane;

— inject 50 µl into the HPLC-GC-FID system for analysis.

1) Silica gel is available from Merck, reference 7754 or 7734. It 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.

The hump signal (excluding any sharp single peaks of the solvent blank) should not exceed one-tenth of the LOQ.

NOTE 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 %.

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 %.

5.8 *n*-Undecane (C11), purity ≥ 99 %.

5.9 *n*-Tridecane (C13), purity ≥ 99 %.

5.10 Tri-tert-butylbenzene (TBB), purity ≥ 97 %.

5.11 Bicyclohexyl (CYCY), purity ≥ 99 %.

5.12 1-Methyl naphthalene (1-MN), purity ≥ 95 %.

5.13 2-Methyl naphthalene (2-MN), purity ≥ 97 %.

5.14 Pentyl benzene (PB), purity ≥ 99 %.

5.15 Stock solution, mass concentrations, ρ = 5 mg/ml, 10 mg/ml or 20 mg/ml. Weigh, for example, 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.

The verification of the start of the MOAH fraction, based on TBB, can result in losses of higher alkylated benzenes and naphthalenes, if present in some samples (i.e. cosmetics) and when the chromatographic performance of the column is limited. In such cases, di(2-ethylhexyl) benzene (DEHB) can be used in addition as verification standard and the fractionation shall be adapted.[10]

5.16 Internal standard solution 1 (ISTD1)[2], mass concentrations ρ = 150 µg/ml (C13), 300 µg/ml (C11, CYCY, PB, 1-MN, 2-MN and TBB) and 600 µg/ml (CHO and PER). Transfer 300 µl 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 ρ = 30 µg/ml (C13), 60 µg/ml (C11, TBB, CYCY, 1-MN, 2-MN and PB) and 120 µg/ml (PER and CHO) Dilute ISTD1 solution (5.16) by a factor of 5, e.g. fill up 1 000 µl 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 cleaned desiccator (without ground grease).

2) This standard mixture is available from, for example, Restek Corp., Cat.# 31070. It is an example of a suitable product commercially available. 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.

5.19 *meta*-chloroperbenzoic acid (*m*CPBA), stated quantities based on a purity of about ≤ 77 %;

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 by using 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 recommended. 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 g to 84 g *m*CPBA per 100 g starting material in the purified product).

To determine the *m*CPBA content of the reagent, weigh about 0,2 g *m*CPBA into a PET beaker, add 50 ml distilled water and mix thoroughly. Add 5 ml concentrated acetic acid and 10 ml sodium iodide solution (10 g sodium iodide in 100 ml water). Then pre-titrate with 0,1 N sodium thiosulfate solution from dark red to light yellow. Add a few drops of starch indicator solution and titrate from dark blue to colourless at the end point (consumption usually below 20 ml).

Calculate the content w (*m*CPBA) in per cent by mass as shown by Formula (1):

$$w = \frac{N \times V \times 86{,}29 \times 100}{E} \tag{1}$$

where

N is the normality of the sodium thiosulfate solution;

V is the total volume of consumed sodium thiosulfate solution in l;

E is the mass of the reagent in g.

5.20 *m*CPBA solution in ethanol, ρ = 100 mg/ml, e.g. dissolve 1 g *m*CPBA (5.19) in 10 ml ethanol (5.28). Prepare the solution fresh every working day.

5.21 Sodium thiosulfate, anhydrous, purity > 90,0 %.

5.22 Sodium hydrogen carbonate (or sodium carbonate), anhydrous, purity > 90,0 %.

5.23 Solution for deactivation of the excess of *m*CPBA: Sodium thiosulfate and sodium carbonate solution, ρ = 50 g/l, e.g. dissolve 5 g of sodium thiosulfate and 5 g of sodium hydrogen carbonate (or sodium carbonate) in 100 ml distilled water and mix thoroughly.

5.24 Alumina column with silica gel cover. Place a filter (6.4) in a glass column (6.3). Add and compress 10 g of alumina (5.18), 3 g of silica gel (5.1) and 1 g of sodium sulfate (5.2).

5.25 Clean-up column. Place a filter (6.4) in an empty SPE glass cartridge (volume 6 ml), add 3 g of silica gel (5.1), compress and cover with 1 g of sodium sulfate (5.2).

5.26 bis (2-ethylhexyl) maleate, purity 90 %. Check the purity in a blank run.

Bis (2-ethylhexyl) maleate may be replaced by bis(2-ethylhexyl) sebacate in order to limit the risk of epoxidation process disturbance.

5.27 Standard solution of the *n*-alkanes with chain lengths of 10 to 50 in the same mass concentration for checking for discrimination against low- or high-boiling substances, ρ = 1 µg/ml. Store this solution at room temperature, otherwise C50 can crystallize.

5.28 Ethanol, absolute.

Test the purity as for *n*-hexane (5.3) with 30 ml ethanol.

5.29 Mixture of ethanol and *n*-hexane, volume fraction φ = 50 %, e.g. mix 50 ml of ethanol (5.28) with 50 ml of *n*-hexane (5.3).

5.30 Elution mixture of *n*-hexane and DCM, e.g. mix 30 ml DCM (5.4) with 70 ml *n*-hexane (5.3). Due to the volatility of DCM, the solution shall be freshly prepared.

5.31 Potassium hydroxide solution, e.g. 50 g potassium hydroxide in 100 ml distilled water, w = 33 g/100 g.

6 Apparatus

In order to achieve a sufficiently low blank level, the following process has proven to be effective: glassware (except volumetric flasks) should be heated in an oven at 430 °C for 4 h or overnight at 400 °C and kept in desiccators or other containers for use. In addition, it is recommended to:

— perform multiple determinations in different series and not directly one after another;

— not use grease for ground joints;

— not use hand cream;

— handle samples with gloves, only;

— use glassware where possible;

— check nitrogen for purity, when drying in a stream of nitrogen;

— rinse volumetric flasks, glass pipettes and other required glassware with *n*-hexane before use.

6.1 Analytical balance, readability 0,000 1 g, weighing accuracy 0,001 g.

6.2 Centrifuge and centrifuge tubes.

6.3 Glass column, without stopcock, 15 cm to 20 cm long and 15 mm to 20 mm inner diameter.

6.4 Filter for glass column, extracted or heated, filter made of quartz wool/glass fibre.

6.5 Glass vial, 40 ml, with polytetrafluoroethylene (PTFE) sealed screw cap.

6.6 Rotary evaporator, with vacuum and water bath at 35 °C.

Comparable devices can also be used. Take care to prevent contamination. If necessary, clean the system thoroughly between determinations.

6.7 HPLC column, e.g. LiChrospher Si 60 or Allure Silica[3], 5 µm material, 2 × 250 mm or comparable.

6.8 Uncoated GC guard column, fused silica or metal capillary, e.g. HydroGuard® MXT®[4], 10 m × 0,53 mm or comparable.

3) LiChrospher Si 60 and Allure Silica are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

4) HydroGuard® MXT® 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.

NOTE The capillaries in 6.8 to 6.12 have proven to be suitable, but can be adapted to the system to meet the requirements and yield comparable results.

6.9 GC separation column, fused silica or metal capillary, programmed temperature stable up to at least 370 °C: 100 % dimethylpolysiloxane or 95 % dimethyl and 5 % phenyl methylpolysiloxane as stationary phase, length 15 m, internal diameter (ID) 0,32 mm or 0,25 mm and film thickness 0,10 µm to 0,25 µm.

6.10 Fused silica or metal capillary, deactivated, for transfer the HPLC fractions from the valve to the T-connector of the GC, 1 m long, 0,1 mm ID.

6.11 Capillary, deactivated, from the T-connector between pre- and separation column to the vapour exit.

6.12 Restriction capillary at the vapour exit, deactivated, 1 m long, ID 0,05 mm.

6.13 Syringe, 100 µl, suitable for injection of 5 to 100 µl in liquid chromatography.

6.14 Pasteur pipette made of glass.

NOTE The use of plastic pipette tips and polyethylene foil leads to increased blank levels.

6.15 Online-coupled HPLC-GC-FID system, consisting of an HPLC instrument capable of running a binary gradient, injection valve, HPLC column (6.7), ultraviolet light (UV) detector (detection wavelength: 230 nm), switching valves for column backflush and fraction transfer into GC, GC with solvent vapour exit (SVE), pneumatic control and evaluation system. In addition, an automatic control system is recommended.

6.16 Test tube shaker with temperature control and agitation (e.g. 500 r/min or comparable).

7 Sample

7.1 Sampling

Sampling is not part of this method. The sample may only be stored in glass bottles, aluminium or other materials that do not release hydrocarbons. Packaging made of paper, polyethylene or polypropylene is unsuitable. Containers made of PET or foil bags made of a high-performance polyamide such as RILSAN[5)] may be used in some cases. Attention shall also be paid to the closure and sealing materials of the containers. The use of hand cream should be avoided when handling samples. The sampling shall be checked by blank runs using *n*-hexane instead of a sample.

A recommended sampling method is given in ISO 5555.

7.2 Preparation of the final sample for liquid and solid fats

Prepare the test sample in accordance with ISO 661.

Special treatments of the test sample (e.g. filtering, melting) shall be mentioned.

5) RILSAN 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.

8 Procedures

8.1 General

Depending on the type of fats and oils, the samples shall be prepared differently. The specific cases A to C are as follows:

— A: Prepare oils and fats with unknown or high content of biogenic, long-chain alkanes and unsaturated compounds[7] such as olive oil, rapeseed oil, sunflower oil and comparable samples according to 8.2 (ethanol-hexane distribution). Using two separate 10 ml fractions of the extract, run 8.3 (saponification) on the first fraction for the determination of the MOSH content according to 8.4 (Alox column), and run 8.3 (saponification) on the second fraction for the determination of the MOAH content according to 8.5 (clean-up) and 8.6 (epoxidation).

— B: Prepare oils and fats with low biogenic, long-chain alkanes and disturbing unsaturated compounds such as coconut fat, linseed oil, palm oil and comparable samples without aluminium oxide column according to 8.2 (ethanol-hexane distribution), 8.3 (saponification), 8.5 (clean-up) and 8.6 (epoxidation). Determine the MOSH and MOAH fractions from the solution obtained.

— C: For automated application of the alumina column, prepare oils and fats according to 8.2 (ethanol-hexane distribution), 8.3 (saponification), 8.5 (clean-up) and 8.6 (epoxidation). Inject into the HPLC-GC-FID system. After separation of the MOSH, which are passed to an integrated online Alox column, the MOAH fraction can be determined in the same run using a two-channel system.

Every laboratory using automated procedures shall carry out tests to ensure that the results obtained with the automated procedures do not deviate from results obtained with the manual procedure.

Unknown samples or mix oils samples may be analysed first without Alox cleanup. If the presence of interfering long-chain *n*-alkanes significantly impact the hump and do not allow a proper integration, the extract shall be re-injected using the alumina column to reduce interferences (e.g. for online Alox clean up, the same sample extract used to determine MOAH is used for the subsequent separation of long-chain *n*-alkanes with the aluminium oxide column for the determination of MOSH).

NOTE Only the manual purification method for MOSH fraction was validated during the collaborative study.

8.2 Hexane/ethanol distribution for removal of interfering substances

Weigh 3 g of the sample for oils and fats into a 40 ml centrifuge tube with screw cap. Add 30 ml of the mixture of *n*-hexane (5.3) and ethanol (5.29) and 20 µl ISTD1 (5.16) or 100 µl ISTD2 (5.17), and homogenize. Use 10 ml of this solution for the further procedure (see 8.3).

NOTE In case of oils and fats, no phase separation will be observed. Nevertheless, this step ensures a complete saponification of oils and fats. If necessary, other quantities of internal standard can be added.

8.3 Saponification

Transfer an aliquot of 10 ml (see 8.2) into another sample tube and add 3 ml potassium hydroxide solution (5.31). Saponify the solution for 30 min at 60 °C in a water bath while shaking until the solution becomes clear. Cool down, add 5 ml *n*-hexane (5.3) and 5 ml mixture of ethanol and water (a volume fraction of 1:1), shake the mixture again, transfer the lower phase after phase separation into a new vial and extract again with an additional 5 ml of *n*-hexane. Combine both *n*-hexane extracts.

Depending on the sample preparation method, continue using the solution to separate biogenic, long-chain *n*-alkanes with aluminium oxide (according to 8.4) or for epoxidation (according to 8.5 and 8.6).

NOTE The addition of ethanol after saponification enables a better phase separation and avoids foaming.

8.4 Removal of biogenic *n*-alkanes with aluminium oxide for determination of the MOSH fraction

Pre-clean the alumina column (5.24) with 20 ml of *n*-hexane in order to remove interfering substances (see text on interfering substances below). Transfer the solution from 8.3 to the alumina column and elute the hydrocarbons with 25 ml *n*-hexane (5.3). Collect the hydrocarbons beginning with the sample transfer. Evaporate the solvent under vacuum (≥260 mbar) at 35 °C after adding two drops of bis (2-ethylhexyl) maleate (5.26).

Dissolve the residue in about 1 ml *n*-hexane, centrifuge if necessary and fill into a vial. Inject 60 µl to 90 µl of the solution for analysis with the HPLC-GC-FID system to determine the MOSH fraction.

The MOAH remain on the alumina column and shall not be determined from this eluate. It is recommended to remove interfering substances from the alumina by pre-rinsing the column with 20 ml *n*-hexane. However, clean alumina does not need to be pre-rinsed.

Removal of biogenic *n*-alkanes can also remove paraffinic waxes at the same time if present in the sample. In these cases, analysis shall be carried out according to procedure B.

NOTE Paraffinic waxes are characterized by *n*-alkanes with no odd carbon predominance of their chain lengths, while naturally occurring *n*-alkanes in edible oils present an odd carbon predominance. As an example, main *n*-alkanes in sunflower oils are C27, C29 and C31 alkanes.

8.5 Clean-up before epoxidation to separate polar substances

Transfer the combined upper phases from the saponification step (see 8.3) to a clean-up column (5.25) and collect the eluting hydrocarbons: start collecting the eluent immediately after transferring the upper phase from the saponification step to the clean-up column. Complete the elution of hydrocarbons from the column with additional 15 ml solvent mixture of *n*-hexane and DCM (volume fraction of 7 + 3) (5.30). Use the eluate for epoxidation (according to 8.6).

It is recommended to remove interfering substances from the clean-up column by pre-rinsing the column with *n*-hexane. However, clean glassware and reagents do not need to be pre-rinsed.

8.6 Ethanolic epoxidation of the MOAH fraction to oxidize unsaturated non-aromatic compounds

NOTE For further information, see References [11] and [13].

After adding two drops of bis (2-ethylhexyl) maleate (5.26) as a keeper, concentrate the solution of 8.5 at 35 °C under vacuum, retaining all internal standards, and adjust to a volume of 1 ml with *n*-hexane.

In this step, any DCM should be completely removed to ensure reliable fractionation in the HPLC-GC-FID system.

Add 1 ml ethanolic *m*CPBA solution (5.20) to the obtained extract and place the sample on a shaker (6.16) for 20 min at 40 °C at, for example, 500 r/min. Then add 500 µl ethanol (5.28) and 2 ml solution for deactivation of the excess of *m*CPBA (5.23) and shake the sample vial for about 1 min at about 750 r/min to deactivate any excess of *m*CPBA. Transfer the upper hexane phase to a fresh sample vial and dry the hexane solution with a spatula tip of sodium sulfate (5.2). Inject 90 µl of the dried solution into the HPLC-GC-FID system for the determination of MOAH.

It is optional to use a more concentrated final volume of, for example, 300 µl and a corresponding injection volume as long as the enrichment is improved and the internal standards are retained during evaporation.

For sample preparation according to procedures A and B, this solution is used directly for the determination of MOAH. In automated sample preparation according to procedure C, this solution is used for the subsequent separation of long-chain *n*-alkanes with the aluminium oxide column (5.24) for the additional determination of MOSH.

Continuous intense shaking of the reaction solution for epoxidation, at the specified reaction temperature, is necessary to ensure sufficient and reproducible removal of interfering substances.

8.7 HPLC-GC separation

8.7.1 HPLC conditions

Pre-rinse the HPLC system with eluent A: *n*-hexane (5.3) and eluent B: DCM (5.4) and check system suitability by injection of *n*-hexane. Table 1 shows an example of the gradient programme.

Table 1 — Gradient programme for the separation of MOSH and MOAH on a silica gel column with backflush after 6 min and reconditioning after 15 min

Time min	Hexane %	DCM %	Flow µl/min
0,0	100	0	300
1,5	65	35	300
5,9	65	35	300
6,0	0	100	500 (backflush)
15,0	0	100	500 (reconditioning)
15,3	100	0	500
25,0	100	0	500
25,2	100	0	300
30,0	100	0	300
0,0	100	0	300

After 6,0 min, the flow direction of the column is reversed to remove remaining matrix components of the sample from the column by backflush. This step significantly increases the lifetime of the HPLC column. For operation with an automated online alumina column, it has proven to be best to start the gradient about 0,1 min later.

Figure A.1 shows a typical HPLC-UV chromatogram with the UV signal and in addition the pressure curve. From 5,5 min onwards, a strong increase in the baseline can be seen due to DCM. Perylene from the ISTD mix elutes at 5,6 min. Backflush should decrease the baseline signal to the initial level. The fractionation of the MOSH fraction from 2 min to 3,5 min and the MOAH fraction from 5,7 min to 6,2 min can be detected by slight baseline changes. If necessary, the change in pressure can also be monitored for this purpose; here, attention should also be paid to a possible pressure increase, which indicates a blockage in the system. The times given can vary slightly depending on the system and columns used and should be checked regularly by injecting a diluted ISTD solution.

The capillaries from the fraction transfer switching valve are combined with the carrier gas supply in a T-connector and directed into the pre-column in the GC oven.

8.7.2 GC configuration

Install the columns in the gas chromatograph and check the function of the system by injecting solvent. The baseline should be straight with as little positive drift as possible. If the drift is too high (see 8.7.5), heat the column or, if the stationary phase of the GC separation column is suitable for this procedure, rinse it with solvent or replace the column. In case of a negative drift, check the connections of the columns for tightness. Heat columns which are used for the first time (e.g. 4 h in the oven at a temperature gradient up to least 350 °C with a stream of carrier gas and maintain the final temperature for at least 30 min).

The following working conditions proved to be suitable for the analysis and may be adapted to the requirements of the HPLC-GC-FID system:

— Pre-column uncoated, deactivated, 7 m to 10 m × 0,53 mm (6.8).

— Separation column: 100 % dimethyl polysiloxane, low bleeding (15 m long, 0,25 mm i.d., 0,1 µm to 0,25 µm film thickness) (6.9).

— Oven temperature: Initial temperature 60 °C, 8 min isothermal, programmed with 15 °C/min to 120 °C, further on with 25 °C/min to 370 °C, hold for 6 min.

— Carrier gas: hydrogen, inlet pressure for MOSH 70 kPa, after closing the solvent vapour exit 150 kPa until the MOAH fraction is ready for GC separation, inlet pressure for MOAH 65 kPa, after closing the solvent vapour exit 150 kPa for both channels.

NOTE 1 In two-channel systems, the first fraction can be kept at a lower pressure, e.g. 30 kPa on the pre-column, until the second fraction is ready for separation. Finetuning of the pressure values can be necessary for each individual system.

— Detector temperature: 380 °C. (Very high detector temperatures can lead to a shortened lifetime of the temperature sensor. If lower oven and detector temperatures are used, a higher baseline drift can occur at the end of the chromatograms or the FID nozzle shall be cleaned more often. In addition, check for discrimination of high boiling *n*-alkanes.)

— Fraction volume: 450 µl.

8.7.3 Solvent vapour exit configuration

Discharge most of the evaporating solvent via the solvent vapour exit between the pre-column and the separation column. The best time to close the solvent vapour exit can be determined as follows:

— While the hexane is evaporating from a hexane injection, ignite the escaping solvent vapour at the end of the valve tubing with a lighter and record the stop time until the flame extinguishes. This time minus 3 s ensures that a residual small part of hexane remains in the system to allow the reconcentration of volatile components in the separation column. The time can vary slightly depending on the length of the restriction capillaries.

— The exact parameters should be optimized so that no significant losses are observed for C10.

— In addition, when operating a two-channel system, it has proven to be best to adapt the end of the solvent signals to the same time if possible in order to be able to use the same retention times for *n*-alkanes for the indication of sub-fractions for both channels.

8.7.4 Peak identification

Typical chromatograms can be found in Annex A.

Check the retention times and area ratios of the standards by injecting 50 µl of a solution of 100 µl standard solution (5.17) diluted by adding into 600 µl *n*-hexane. The resolution of *n*-undecane (C11) and the solvent shall be given (see Figure A.3).

Adjust the standard solution concentration as suitable for the system, if necessary.

Using the *n*-alkane standard mixture, ensure non-discriminating detection of all hydrocarbons, check the areas and the retention time up to C50, and determine the retention time limits for MOSH and MOAH fractions.

A very broad peak (of about 5 min to 15 min width, depending on the GC conditions) in sample extracts represents an unresolved complex mixture (UCM) of hydrocarbons, which cannot be resolved with the chromatographic system. It is attributed to petroleum hydrocarbons (see Figure A.7).

Hydrocarbons from mineral oil do not include *n*-alkanes with dominant odd-numbered chain lengths or terpene-like hydrocarbons such as squalene, sterene or carotenoids naturally occurring in edible oils. In the saturated hydrocarbon fraction of sunflower oil, the main peaks correspond to the saturated aliphatic hydrocarbons C27, C29 and C31 (see Figure A.6). For MOSH determination, all sharp signals on the hump shall be subtracted.

Similarly, for MOAH determination, all sharp signals on the hump shall also be subtracted.

In addition, for some purposes a distinction is made between MOSH and oligomeric polyolefins as for POSH (see Figure A.12) or ROSH (see Figure A.14), or synthetic lubricants such as poly-alpha-olefins (PAO) (see Figure A.13). In some cases, these substances cannot be distinguished from hydrocarbons from mineral oil using HPLC-GC-FID.

If POSH or ROSH are recognizable in the MOSH chromatogram due to their typical signal pattern, the riding peaks shall be subtracted from the MOSH content, but their presence is mentioned in the report, as part of their content is counted into the MOSH fraction.

If ROAH are recognizable in the MOAH chromatogram due to their typical signal pattern (see Figure A.15), the riding peaks shall be subtracted from the MOAH content, but their presence is mentioned in the report, as part of their content is counted into the MOAH fraction.

If PAO are recognizable in the MOSH chromatogram due to their typical signal pattern (see Figures A.16 and A.17), the PAO peaks shall be integrated in the MOSH content, and their presence is mentioned in the report.

For petroleum products not obtained by hydrogenation or mixing of different base products, the signals in the MOSH and the MOAH fraction should show the same molecular weight range and both signals should have an approximately Gaussian chain length distribution. In most cases, the MOSH fraction is significantly higher than the MOAH fraction, which exceeds 30 % of the total MOH in special products, only. However, in presence of mixtures of several mineral oil products or other technical processes, these plausibility assumptions are not always correct (see References [8] and [9]).

CYCY is normally not present in MOSH and is used as a quantification standard. The areas of CYCY and C13 should be in a 2:1 ratio, otherwise one of the two standards may be superimposed.

TBB serves as an internal standard for the MOAH fraction. 1-MN and 2-MN are easily recognized as peak pairs. They shall be completely separated from each other and have an area of equal size in order to exclude overlapping (see Figure A.4).

TBB features a higher degree of alkylation than 1-MN and 2-MN and therefore was more comparable to the main compounds of most MOAH fraction constituents found in edible oils. In addition, the integration of the signal peak for TBB was often easier than for 1-MN and 2-MN. Therefore, TBB yielded better repeatability and reproducibility data in collaborative trials and shall be used regularly as quantification standard.

8.7.5 System suitability test

Inject an aliquot of the diluted ISTD 2 solution (5.17) and the series of *n*-alkanes from C10 to C50 (5.27). For the internal standards, this should correspond to an absolute amount, for example, for CYCY in the range of about 1,5 ng (e.g. 20 µl of a 1/150 dilution or 50 µl of a 1/500 dilution).

The following information is provided on the presence or absence of the various substances and their area ratios. If the MOSH and MOAH fractions are completely separated, CYCY, C11, C13 and CHO shall be detected exclusively in the MOSH fraction, while PB, 1-MN, 2-MN, TBB and perylene occur only in the MOAH fraction. If a marker is detected in the wrong fraction, check whether the HPLC column separates the MOSH and MOAH fractions correctly or whether the fraction windows are set correctly. The end of the MOAH fraction can be clearly seen in the UV chromatogram of the HPLC (see Figure A.1). The order of the substance classes in the fractionation is shown schematically in Figure A.2. The gentle blow-off or complete elution of the MOSH fraction can be recognized by the undiminished areas of C11 and CHO, respectively. In the MOAH fraction, TBB and perylene shall be completely recovered to ensure correct fractionation. If problems occur, the column shall be reconditioned, the gradient shall be adjusted or the HPLC column shall be replaced.[10]

C11 should be sufficiently well separated from the solvent signal and its area shall not be discriminated against the other internal standards to ensure correct refocusing after blowing off excess solvent without loss of volatile substances. CHO elutes in the HPLC at the end of the MOSH fraction. In the case of low CHO signals, the fraction limits shall be checked. However, CHO is partially removed with alumina oxide column clean-up. The presence of interfering long-chain *n*-alkanes, such as those found in sunflower oil (see Figure A.6), shall be eliminated by using the alumina column (see 8.4) (see Figure A.7).

Losses of PB indicate poor refocusing or too much blow-off at the SVE and TBB indicates the correct fraction start during transfer. A reduced area of perylene, which elute at the end of the MOAH fraction, indicates

incomplete fraction transfer or can be caused by loss after epoxidation. Successful removal of interfering substances is shown in Figures A.8 and A.9.

The system is ready for use when the fractions of MOSH and MOAH are completely separated according to the standards.

See also the information given in 5.15 for samples containing highly alkylated benzenes and napthenes. High levels of MOSH can result in a carry-over of MOSH into the MOAH faction. In such cases, the sample concentration shall be lowered in order to avoid false-positive results for MOAH.

Using the chromatogram of the series of *n*-alkanes from C10 to C50 (5.27), check that the ratio of the peak areas of C50 to C20 and of C10 to C20 is between 0,8 and 1,2 (see Figure A.5). No other response factors are used.

In order to ensure correct signal evaluation, especially at the level of the LOQ, the offset of the baseline in the area of integration of MOSH and MOAH shall not exceed one quarter of the height of the signal to be integrated (see Figure A.18). In such cases, the chromatographic separation performance of the system shall be improved or the LOQ shall be increased.

In summary, the following conditions should be examined:

— Appropriate straight baseline, blank level ≤ 1/3 of the LOQ.

— Peaks are symmetrical and do not show significant tailing.

— Complete separation of solvent and C10.

— Discrimination C10/C20 and C50/C20 between 80 % ≤ x ≤ 120 %.

— Peak ratio between TBB and 2-MN ≤ 1,25.

— Verification of the LOQ of a matrix by adding a suitable mineral oil product before sample preparation. The signal of the hump at the LOQ should have a relative standard deviation ≤ 20 % and a signal to noise ratio ≥ 10. In addition, the LOQ should be tested with a mineral oil product, whose signal width from start to end is comparable to or higher than the boiling range of the MOH in the sample to be evaluated.

8.8 Blank run

Every working day, a reagent blank run shall be carried out and evaluated like the samples. The blank run should be free of interfering humps and the baseline should be as flat as possible (without excessive drift). This result should be subtracted from the sample result. The blank level shall be less than one third of the LOQ.

8.9 Quality control

At regular intervals, determine the accuracy of the measurements by adding known quantities of a mineral oil mixture to a sample that is preferably uncontaminated.

The addition of a certified material containing both MOSH and MOAH and no other ingredients has proven to be effective. The use of external or internal reference material with a control chart is also recommended.

9 Result of the determination

9.1 Testing the chromatograms for sufficient epoxidation and other relevant parameters

Before the actual calculation of the MOAH fraction, the chromatogram obtained from the epoxidized sample shall be checked for sufficient epoxidation. Sufficient epoxidation is achieved when all interfering compounds, discrete peaks on the hump, have been eliminated to such an extent that all residual peaks are completely resolved and the course of the hump can be identified unambiguously. The size of the hump shall not be affected by poorly resolved peaks. Inspection of the chromatogram should be done at a scale, which shows the hump at a size of about two thirds of the chromatogram. An example of insufficient removal

of interfering substances is shown in the chromatogram in Figure A.8. In contrast, the chromatogram in Figure A.9 allows an unambiguous quantification of the hump.

For chromatograms that indicate overloading, it can also be useful to reduce the injection volume or sample mass.

If a sufficient removal of interfering peaks cannot be achieved, the LOQ shall be increased to such an extent that the non-resolved interfering signals simulate a hump.

Proceed as follows:

— Integrate the unresolved signals as in Figure A.19 as riding peaks and subtract them from the total area underneath, which includes the riding peaks.

— If an unambiguously distinction between unresolved signals and possibly underlying hump can no longer be made, the LOQ shall be raised by the amount that results from the sum of the total area of unresolved signals and any possibly underlying hump.

In addition, the chromatograms should be checked for:

— sufficient recovery of internal standards;

— presence of the verification standards in the correct fractions;

— absence of discrimination for low and high boiling substances.

9.2 Calculation

In order to calculate the content of hydrocarbons from mineral oil in a sample, the range of all hydrocarbons A_1 is determined by manually integrating the total signal of the UCM and all sharp peaks in the range of the UCM (see Figure A.10), starting from the baseline until the baseline is reached again in the elution range of the *n*-alkanes from C10 to C50.

Then determine the sum of the areas of all sharp riding peaks above the UCM to determine area A_2. The baseline in the chromatogram shall be drawn from valley to valley (see Figure A.11).

The difference A_i for determining the area of the UCM is shown by Formula (2):

$$A_i = A_1 - A_2 \tag{2}$$

If the position of the quantification standards (CYCY, TBB or 1-MN) are on the UCM signal, the areas of the standards are also integrated with a valley to valley baseline.

The MOSH and MOAH mass fraction (w_{HC}) in mg/kg is shown by Formula (3):

$$w_{HC} = \frac{A_i \times m_{IS} \times 1000}{A_{IS} \times m} \tag{3}$$

where:

A_i is the peak area of the UCM after deducting all sharp peaks above the UCM (A1 - A2);

A_{IS} is the peak area of the internal standard peak;

m_{IS} is the mass of the added internal standard in mg;

m is the mass of the sample in g.

The result shall be expressed to two significant digits.

In addition to this information, the result can also be divided into fractions corresponding to specific elution ranges of the *n*-alkanes. The fractions given in Table 2 were reported separately in Reference [8].

Table 2 — Fractions according to Reference [8]

MOSH	MOAH
Total MOSH [C10-C50]	Total MOAH [C10-C50]
MOSH ≥ *n*-C10 to ≤ *n*-C16	MOAH ≥ *n*-C10 to ≤ *n*-C16
MOSH > *n*-C16 to ≤ *n*-C20	MOAH > *n*-C16 to ≤ *n*-C25
MOSH > *n*-C20 to ≤ *n*-C25	MOAH > *n*-C25 to ≤ *n*-C35
MOSH > *n*-C25 to ≤ *n*-C35	MOAH > *n*-C35 to ≤ *n*-C50
MOSH > *n*-C35 to ≤ *n*-C40	
MOSH > *n*-C40 to ≤ *n*-C50	

To determine the concentration of the fraction of the UCM in the elution range of the *n*-alkanes from, for example, C10 to C16, determine the retention times from the peak start of C10 and the peak end of C16 from a chromatogram of the standard mixture of *n*-alkanes from C10 to C50 (5.27) and transfer these retention times to the UCM for perpendicular integration. Each of the following fractions begin with the peak end of the specified *n*-alkanes.

The LOQ depends on the respective width and height of the area of the UCM signal. The area value is subject to greater error than for single substances, since small fluctuations in the baseline produce large changes in the area with broad signal peaks. For a reliable quantification, about 50 ng to 100 ng of substance shall arrive at a flame ionization detector.

The LOQ applies to the sum of all petroleum hydrocarbons in the MOSH or MOAH fraction. Information on contents in certain chain length fractions also enables to further characterize the findings and provides indications about possible pathways for carry over. Substances in the elution range below C24 can be transferred via direct contact as well as via the gas phase, whereas the higher-chain substances are only transferred via direct contact or via bound particles.

10 Precision of the method

Details of an interlaboratory test on the precision of the method are summarized in Annex B. It is possible that the values derived from this interlaboratory test are not applicable to concentration ranges and matrices other than those given.

10.1 Repeatability limit

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit, *r*, given in Tables B.2 to B.13, in not more than 5 % of the cases.

10.2 Reproducibility limit

The absolute difference between two single test results found on identical test material reported by two laboratories will exceed the reproducibility limit, *R*, given in Tables B.2 to B.13, in not more than 5 % of the cases.

11 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this document, i.e. ISO 20122:2024;

d) the date of receipt;

e) the date of test;

f) the mass(es) of the test portion(s);

g) the range of the hydrocarbon chain length analysed;

h) all operating details not specified in this document or regarded as optional, together with details of any incidents that can have influenced the test result(s);

i) the test result(s) and the units in which they have been expressed, or, if the repeatability has been checked, the final quoted result obtained;

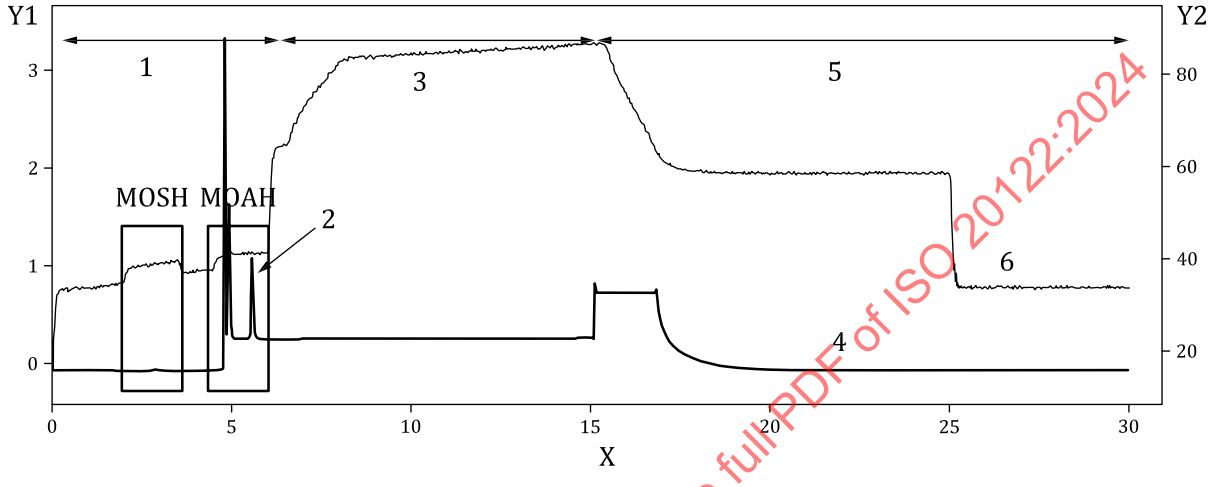
j) information regarding an increased LOQ due the presence of non-resolved interfering signals simulating a hump,

k) information regarding the presence of POSH, PAO, ROSH or ROAH observed in the chromatograms.

Annex A
(informative)

Graphics and chromatograms

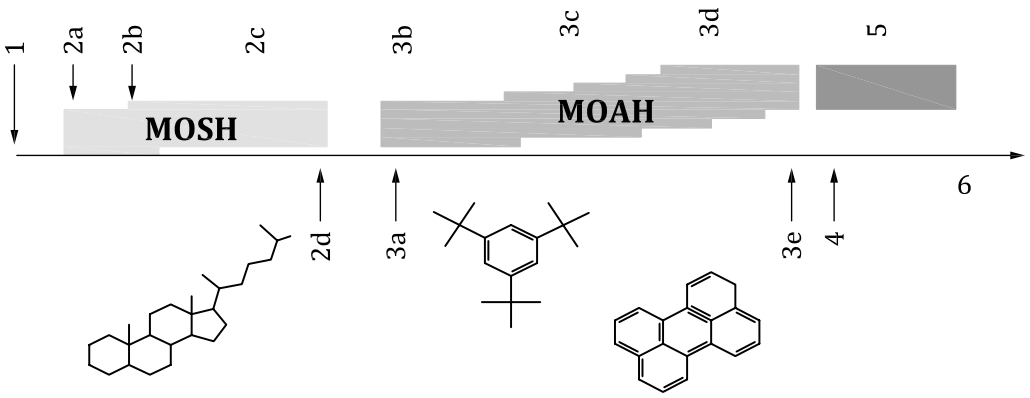
All the chromatograms in this annex are presented with an x-axis related to time in min and y-axis related to abundance.



Key

1 separation
2 perylene
3 backflush
4 UV signal
5 re-equilibration
6 pressure

Figure A.1 — HPLC-UV chromatogram with characteristic signal and pressure channel of a MOSH-MOAH measurement



Key

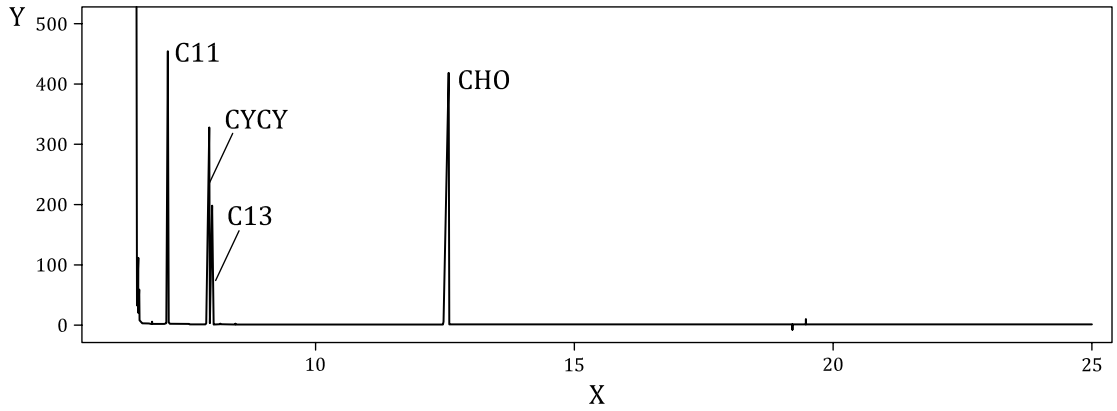
1 injection
2 MOSH
- 2a high mass paraffins
- 2b low mass paraffins
- 2c naphthenes
- 2d cholestane

3 MOAH
- 3a tri-tert-butylbenzene,
- 3b alkylated benzenes
- 3c highly alkylated polycyclic aromatics
- 3d low alkylated polycyclic aromatics
- 3e perylene

4 wax esters
5 saturated wax esters
6 retention time

NOTE Source: Figure A.2 modified from References [3] and [5].

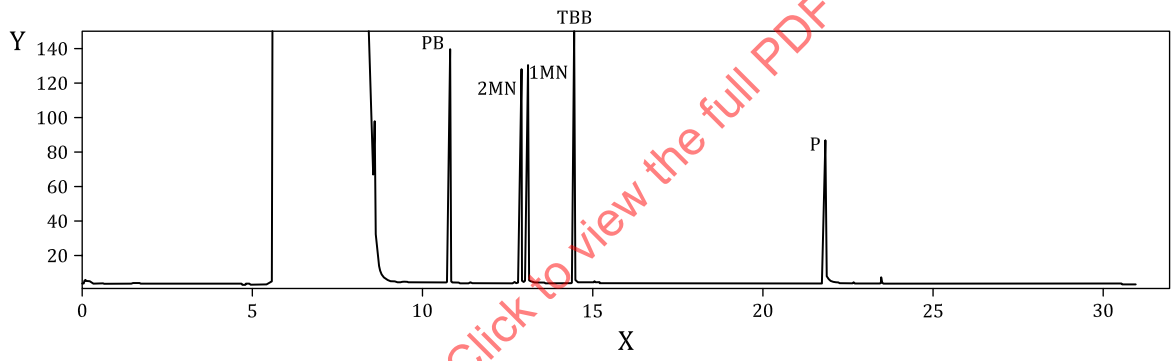
Figure A.2 — Elution scheme of the substance classes of the MOSH and MOAH fraction (upper part) and the verification standards used for the limits of the fraction windows (lower part)



Key

C11 undecane
CYCY bicyclohexyl
C13 tridecane
CHO cholestane

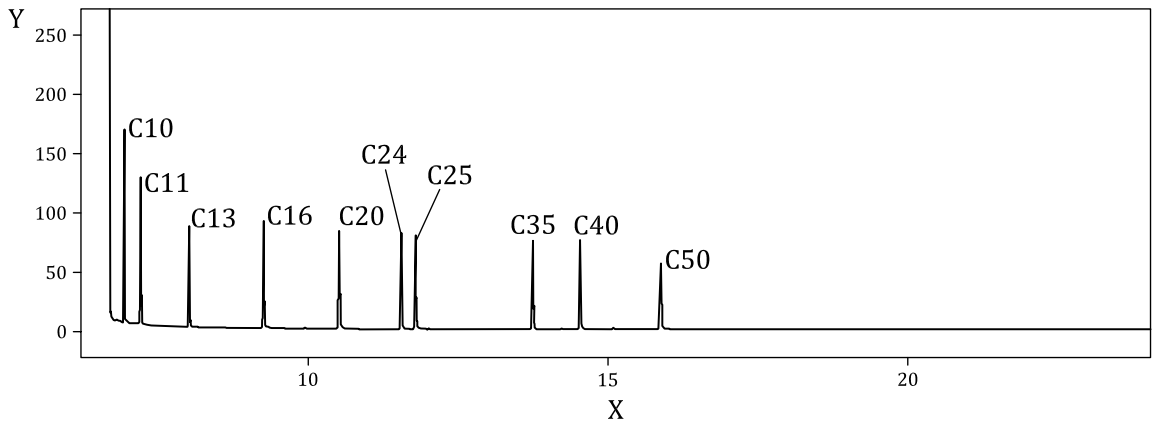
Figure A.3 — HPLC-GC-FID chromatogram of the MOSH fraction of the ISTD mixture



Key

PB pentylbenzene
2-MN 2-methylnaphthalene
1-MN 1-methylnaphthalene
TBB tri-tert-butylbenzene
P perylene

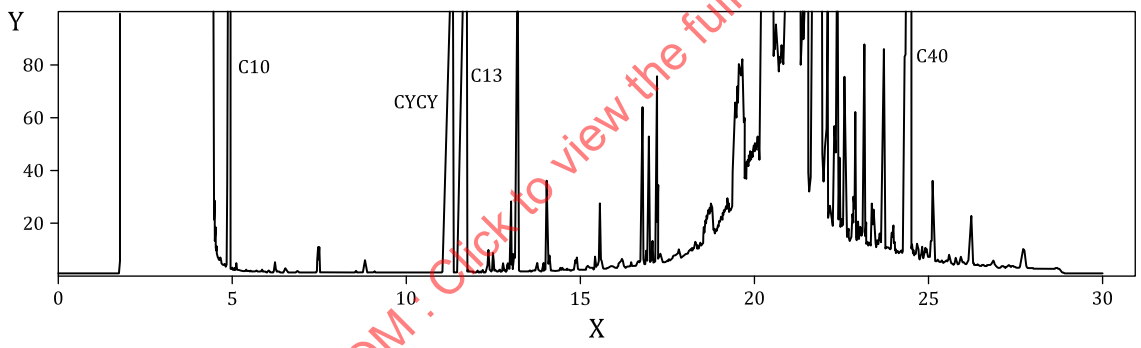
Figure A.4 — HPLC-GC-FID chromatogram of the MOAH fraction of the ISTD mixture



Key

C10 decane
C11 undecane
C13 tridecane
C16 hexadecane
C20 eicosane
C24 tetracosane
C25 pentacosane
C35 pentatriacontane
C40 tetracontane
C50 pentacontane

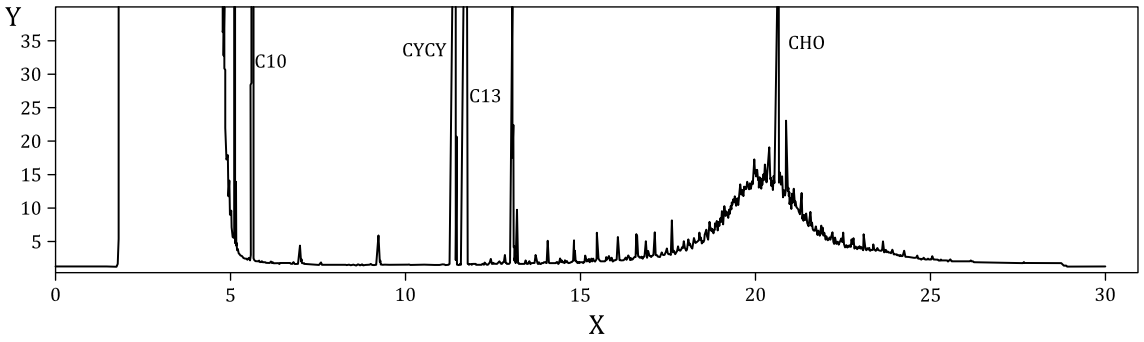
Figure A.5 — HPLC-GC-FID chromatogram of an alkane standard mixture from C10 to C50 for testing non-discriminatory fraction transfer



Key

C10 decane
CYCY bicyclohexyl
C13 tridecane
C40 tetracontane

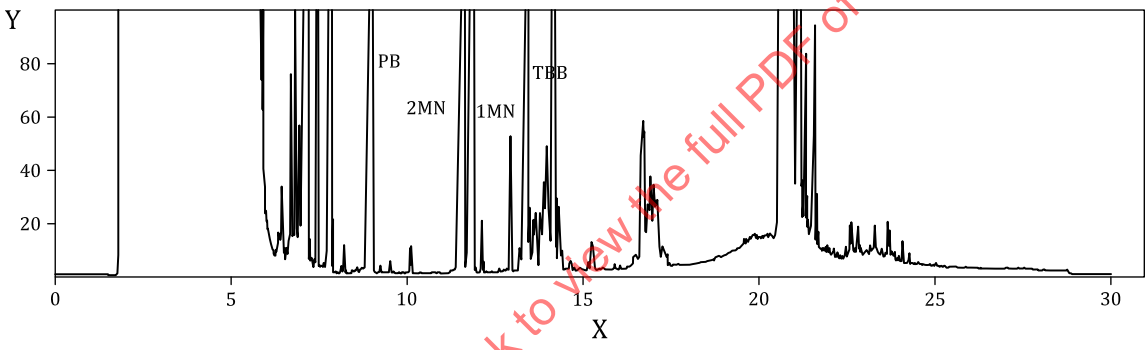
Figure A.6 — HPLC-GC-FID chromatogram of the MOSH fraction of a contaminated sunflower oil with biogenic long-chain *n*-alkanes



Key

C10 decane
CYCY bicyclohexyl
C13 tridecane
CHO cholestane

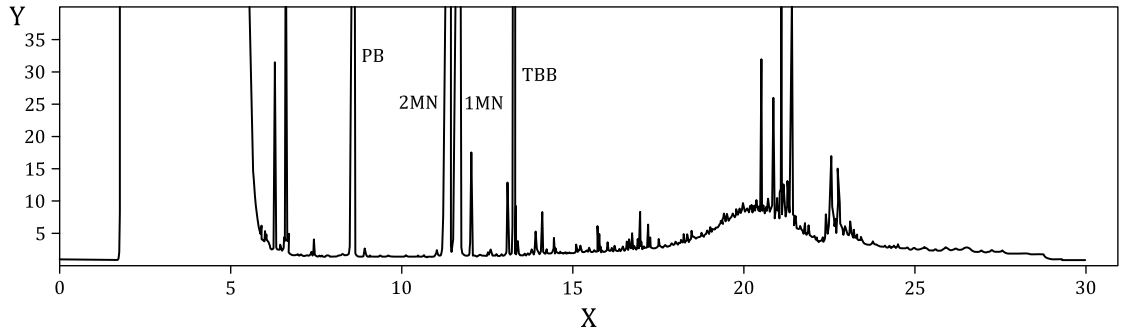
Figure A.7 — HPLC-GC-FID chromatogram of the MOSH fraction of a contaminated sunflower oil (80 mg/kg) after purification with aluminium oxide column



Key

PB pentylbenzene
2-MN 2-methylnaphthalene
1-MN 1-methylnaphthalene
TBB tri-tert-butylbenzene

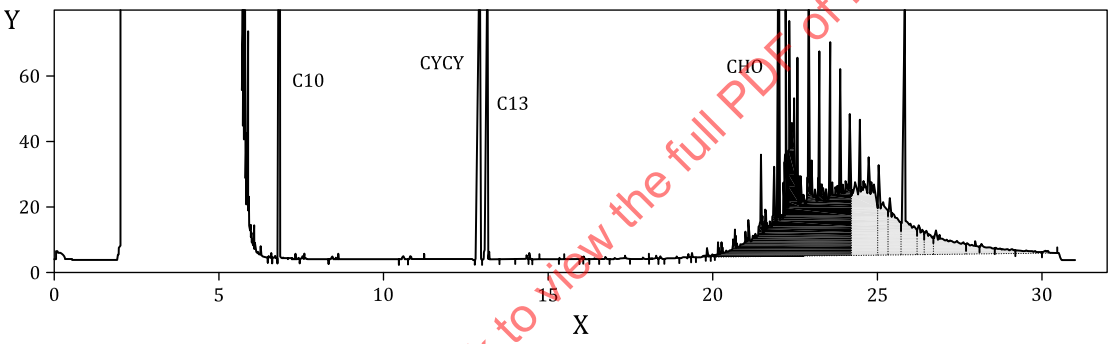
Figure A.8 — HPLC-GC-FID chromatogram of the MOAH fraction of a contaminated sunflower oil



Key

PB	pentylbenzene
2-MN	2-methylnaphthalene
1-MN	1-methylnaphthalene
TBB	tri-tert-butylbenzene

Figure A.9 — HPLC-GC-FID chromatogram of the MOAH fraction of a contaminated sunflower oil (15 mg/kg) after epoxidation

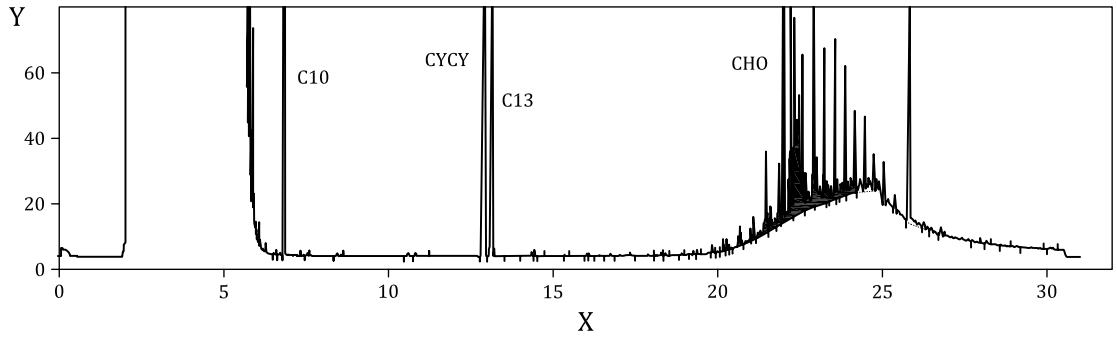


Key

C10	decane
CYCY	bicyclohexyl
C13	tridecane
CHO	cholestane

The area in black up to the retention time of C35 refers to the sum of the UCM, which includes the riding peaks. The C35 retention time corresponds to the end of the black area.

Figure A.10 — HPLC-GC-FID chromatogram of the MOSH fraction of a refined coconut fat

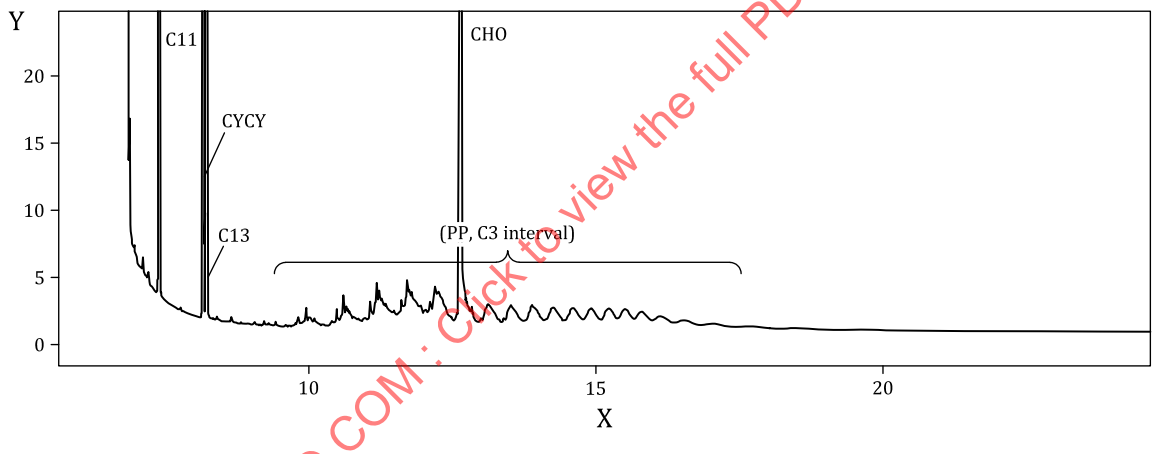


Key

C10 decane
CYCY bicyclohexyl
C13 tridecane
CHO cholestane

The riding peaks shown in black, which have been integrated from valley-to-valley, shall be subtracted from the MOSH fraction.

Figure A.11 — HPLC-GC-FID chromatogram of the MOSH fraction of a refined coconut fat



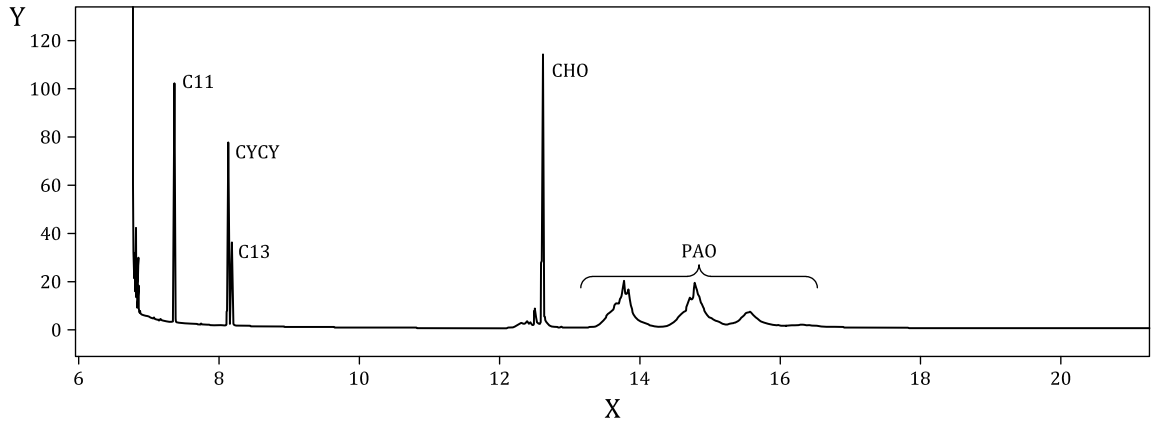
Key

C11 undecane
CYCY bicyclohexyl
C13 tridecane
CHO cholestane
PP, C3 POSH from polypropylene

For POSH from polypropylene, the distance between each peak apex corresponds to three carbon units. For POSH from polyethylene, the distance between each peak apex corresponds to two carbon units.

The POSH peaks shall be subtracted from the MOSH content.

Figure A.12 — HPLC-GC-FID chromatogram of the MOSH fraction with POSH content



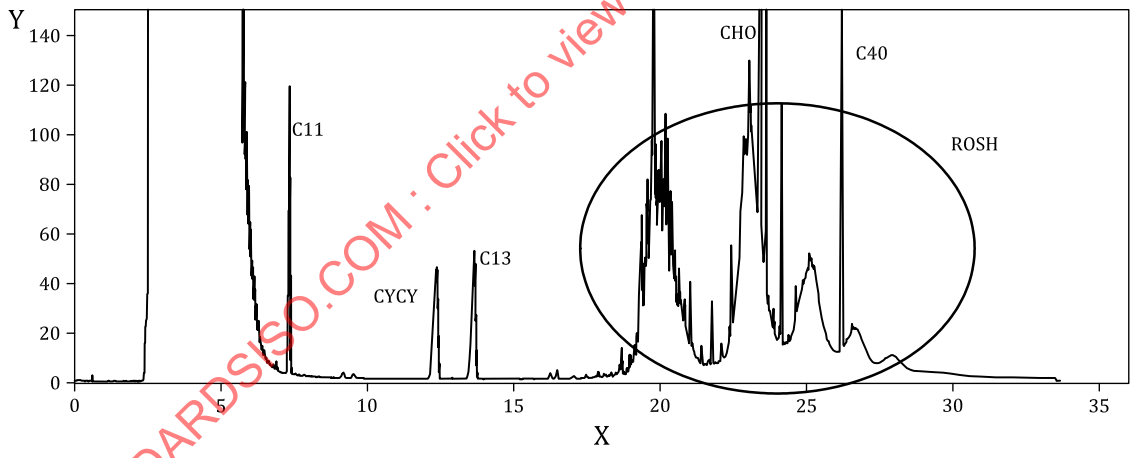
Key

C11 undecane
CYCY bicyclohexyl
C13 tridecane
CHO cholestane
PAO poly-alpha-olefins

The PAO peaks shall be integrated in the MOSH content.

NOTE The height and position of the peak apex varies depending on the product.

Figure A.13 — HPLC-GC-FID chromatogram of the MOSH fraction with PAO contents

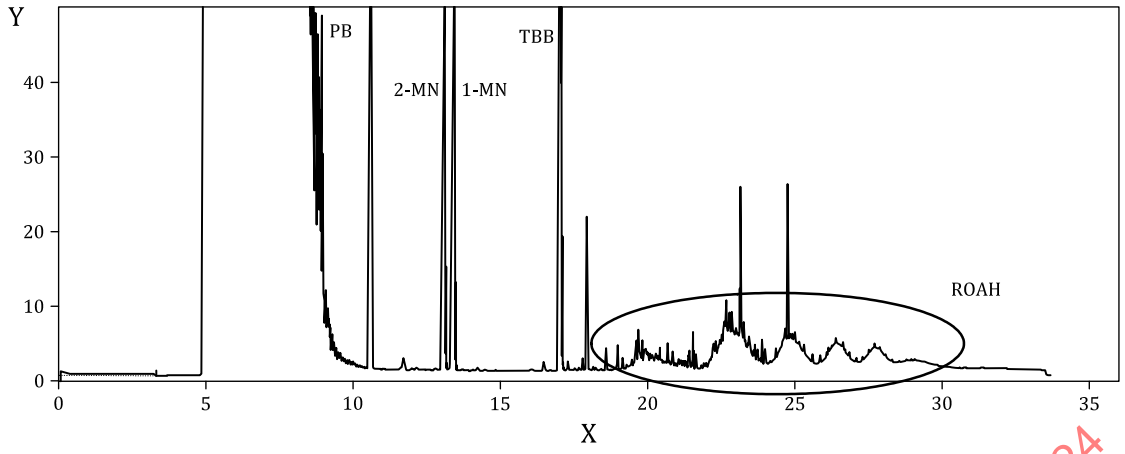


Key

C11 undecane
CYCY bicyclohexyl
C13 tridecane
CHO cholestane
C40 tetracontane
ROSH resin oligomeric saturated hydrocarbons

The ROSH peaks shall be subtracted from the MOSH content.

Figure A.14 — HPLC-GC-FID chromatogram of the MOSH fraction of a sample contaminated with resin oligomers (ROSH)

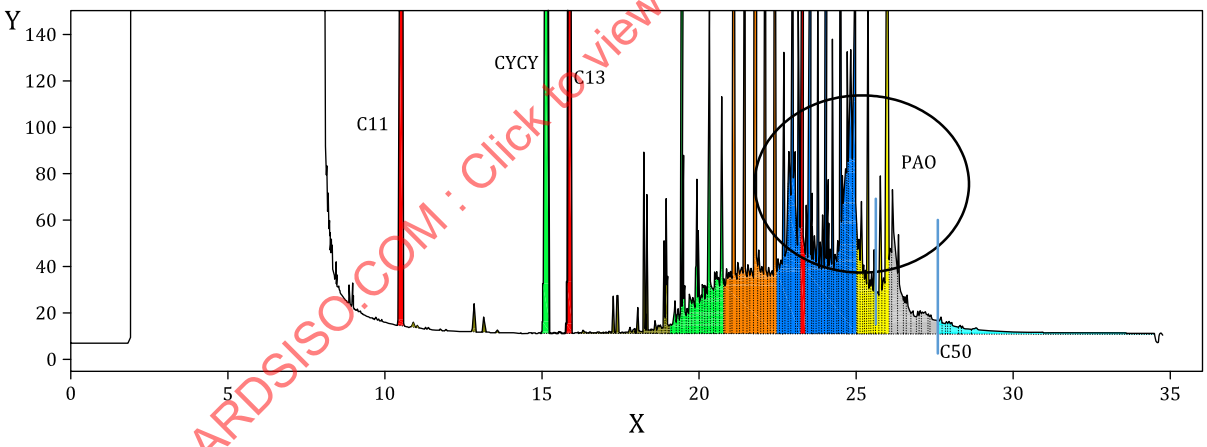


Key

PB pentylbenzene
2-MN 2-methylnaphthalene
1-MN 1-methylnaphthalene
TBB tri-tert-butylbenzene
ROAH resin oligomeric aromatic hydrocarbons

The ROAH peaks shall be subtracted from the MOSH content.

Figure A.15 — HPLC-GC-FID chromatogram of the MOAH fraction of a sample contaminated with resin oligomers (ROAH)

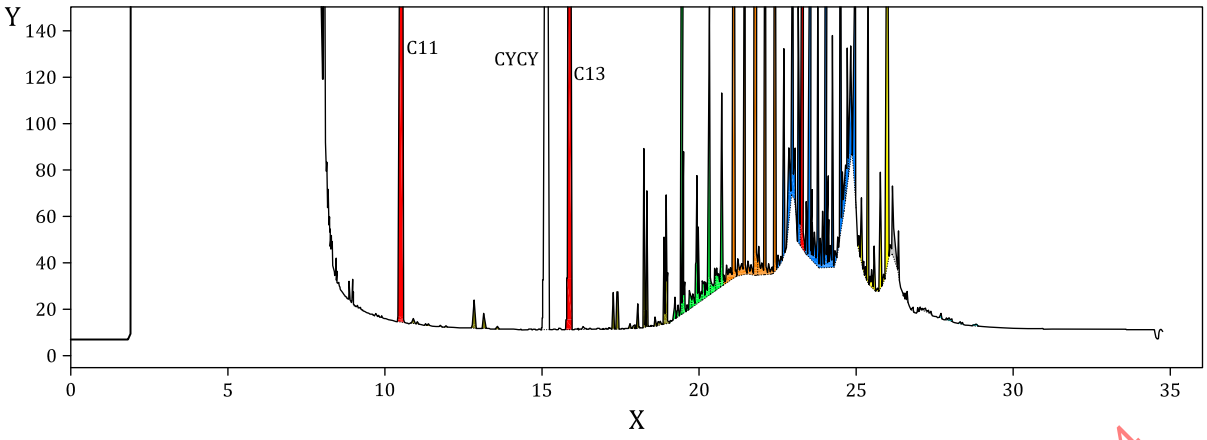


Key

C11 undecane
CYCY bicyclohexyl
C13 tridecane
PAO poly-alpha-olefins
C50 pentacontane

The baseline is traced from C10 to C50 to calculate the total hydrocarbon content.
The PAO peaks shall be integrated in the MOSH content.

Figure A.16 — HPLC-GC-FID chromatogram of the MOSH fraction of a cacao butter sample contaminated with a synthetic lubricant (PAO)

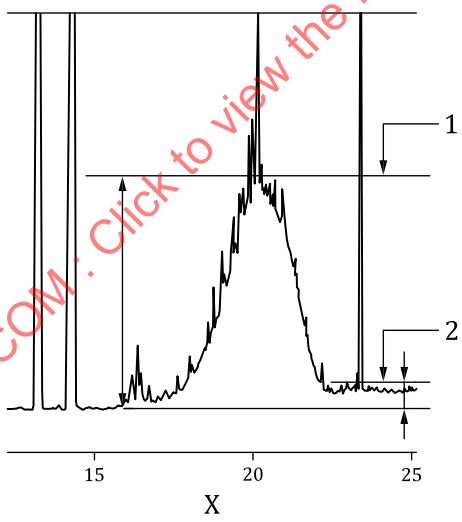


Key

C11 undecane
CYCY bicyclohexyl
C13 tridecane

To quantify the MOSH/MOSH analogues amount, all the sharp peaks above the hump are subtracted but the PAO peaks shall be integrated in the MOSH content.

Figure A.17 — HPLC-GC-FID chromatogram of the MOSH fraction of a cacao butter sample contaminated with a synthetic lubricant (PAO)

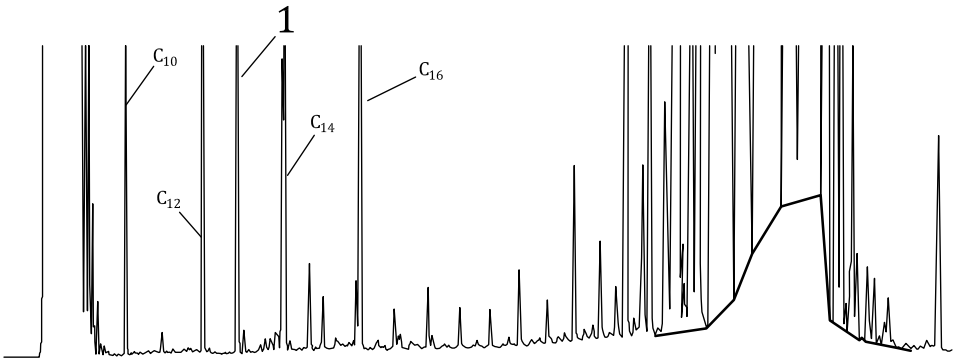


Key

1 signal height
2 baseline shift

The ratio of the height of the baseline offset to the height of the MOH signal in the integration area should not exceed one quarter.

Figure A.18 — HPLC-GC-FID chromatogram of MOH (MOSH and MOAH) – Limit for the ratio of signal/noise



Key

C10	decane
C12	dodecane
C14	tetradecane
C16	hexadecane
1	bicyclohexyl

Figure A.19 — HPLC-GC-FID chromatogram of the MOSH fraction — Integration of unresolved interfering substances

Annex B
(informative)

Precision data

The data given in Tables B.2 to B.13 were obtained in an interlaboratory study, organized by ITERG (France) from 2021 to 2022, in accordance with ISO 5725-2 for collaborative study procedures, to validate characteristics of a method of analysis. Values were calculated from the results of the participants after removal of outliers. Table B.1 gives the identification of the samples.

Table B.1 — Identification of samples

Sample number	Identification
Sample 1	Commercial refined grapeseed oil
Sample 2	Commercial refined palm olein
Sample 3	Commercial virgin coconut oil
Sample 4	Commercial refined rapeseed oil
Sample 5	Commercial extra virgin olive oil
Sample 6	Commercial cold pressed rapeseed oil, spiked at 7 mg/kg with a naphthenic oil containing both MOSH and MOAH (70:30)
Sample 7	Sunflower oil, refined, spiked at 18 mg/kg with a naphthenic oil containing both MOSH and MOAH (70:30)
Sample 8	Commercial virgin coconut oil (Sample 3) spiked at 5 mg/kg with a naphthenic oil containing both MOSH and MOAH (70:30)

Table B.2 — Validation data for MOSH [C10-C50] in different vegetable oils

MOSH [C10-C50] (mg/kg)	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**
No. of participating laboratories, n_P	36	36	36	36	36	36	36	36
No. of laboratories retained after eliminating outliers, n_p	33	33	34	34	35	35	35	36
No. of test results in all laboratories, n_t	66	66	68	68	70	70	70	72
Mean value, $\bar{x}$, mg/kg	74,60	10,23	1,35	3,23	8,45	7,97	15,76	4,78
Repeatability standard deviation s_r, mg/kg	3,65	0,48	0,25	0,31	0,55	0,54	1,11	0,45
Coefficient of variation of repeatability, $C_{V,r}$, %	4,9	4,7	18,2	9,7	6,5	6,8	7,0	9,5
Repeatability limit r [$r = 2{,}8 \times s_r$], mg/kg	10,22	1,34	0,69	0,88	1,55	1,51	3,10	1,27
Reproducibility standard deviation s_R, mg/kg	8,52	1,85	0,68	0,78	1,25	1,30	1,95	0,83
Coefficient of variation of reproducibility, $C_{V,R}$, %	11,4	18,0	50,4	24,1	14,8	16,3	12,4	17,4
Reproducibility limit R [$R = 2{,}8 \times s_R$], mg/kg	23,84	5,17	1,90	2,18	3,51	3,63	5,46	2,33
HorRat value	*1,4*	*1,6*	*3,3*	*1,8*	*1,3*	*1,4*	*1,2*	*1,4*
NOTE The HorRat value limit of 2[12] was considered to be not relevant for this determination as MOSH and MOAH are typical ill-defined analytes that lead to unresolved peaks. A value of 25 % for the reproducibility relative standard deviation was preferred for the quality assessment of the precision data.								

Table B.3 — Validation data for MOAH [C10-C50] in different vegetable oils

MOAH [C10-C50] TBB (mg/kg)	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**
No. of participating laboratories, n_P	35	35	35	35	34	35	35	35
No. of laboratories retained after eliminating outliers, n_p	31	32	34	32	33	34	34	33
No. of test results in all laboratories, n_t	62	64	68	64	66	68	68	66
Mean value, $\bar{x}$, mg/kg	7,06	3,06	0,42	0,99	2,37	2,29	6,12	1,99
Repeatability standard deviation s_r, mg/kg	0,29	0,24	0,14	0,18	0,15	0,17	0,42	0,09
Coefficient of variation of repeatability, $C_{V,r}$, %	4,1	7,8	33,5	18,0	6,3	7,6	6,9	4,4
Repeatability limit r [$r = 2{,}8 \times s_r$], mg/kg	0,82	0,66	0,39	0,50	0,42	0,49	1,19	0,25
Reproducibility standard deviation s_R, mg/kg	1,02	0,85	0,37	0,54	0,66	0,49	0,70	0,34
Coefficient of variation of reproducibility, $C_{V,R}$, %	14,5	27,9	87,6	54,1	27,7	21,2	11,5	17,1
Reproducibility limit R [$R = 2{,}8 \times s_R$], mg/kg	2,87	2,39	1,03	1,50	1,84	1,36	1,97	0,95
HorRat value	*1,2*	*2,1*	*4,8*	*3,4*	*2,0*	*1,5*	*0,9*	*1,2*
NOTE The HorRat value limit of 2[12] was considered to be not relevant for this determination as MOSH and MOAH are typical ill-defined analytes that lead to unresolved peaks. A value of 25 % for the reproducibility relative standard deviation was preferred for the quality assessment of the precision data.								

Table B.4 — Validation data for MOSH [C10-C16] in different vegetable oils

MOSH [C10-C16] (mg/kg)	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**
No. of participating laboratories, n_P	36	36	36	36	36	36	36	36
No. of laboratories retained after eliminating outliers, n_p	34	33	33	33	33	35	34	31
No. of test results in all laboratories, n_t	68	66	66	66	66	70	68	62
Mean value, $\bar{x}$, mg/kg	1,53	< 0,1	< 0,1	< 0,1	< 0,1	0,82	0,29	0,17
Repeatability standard deviation s_r, mg/kg	0,16	—	—	—	—	0,07	0,03	0,02
Coefficient of variation of repeatability, $C_{V,r}$, %	10,1	—	—	—	—	8,2	10,2	13,9
Repeatability limit r [$r = 2{,}8 \times s_r$], mg/kg	0,44	—	—	—	—	0,19	0,08	0,07
Reproducibility standard deviation s_R, mg/kg	0,59	—	—	—	—	0,20	0,11	0,07
Coefficient of variation of reproducibility, $C_{V,R}$, %	38,4	—	—	—	—	24,7	38,7	38,1
Reproducibility limit R [$R = 2{,}8 \times s_R$], mg/kg	1,65	—	—	—	—	0,57	0,31	0,18
HorRat value	*2,6*	—	—	—	—	*1,5*	*2,0*	*1,8*

Table B.5 — Validation data for MOSH [C16-C20] in different vegetable oils

MOSH [C16-C20] (mg/kg)	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**
No. of participating laboratories, n_P	36	36	36	36	36	36	36	36
No. of laboratories retained after eliminating outliers, n_p	35	34	36	33	35	34	33	34
No. of test results in all laboratories, n_t	70	68	72	66	70	68	66	68
Mean value, $\bar{x}$, mg/kg	4,24	< 0,1	0,20	0,13	0,30	3,04	3,66	1,24
Repeatability standard deviation s_r, mg/kg	0,46	—	0,05	0,03	0,04	0,18	0,15	0,09
Coefficient of variation of repeatability, $C_{V,r}$, %	10,8	—	27,2	23,7	14,6	5,9	4,1	7,0
Repeatability limit r [$r = 2,8 \times s_r$], mg/kg	1,29	—	0,15	0,08	0,12	0,50	0,42	0,24
Reproducibility standard deviation s_R, mg/kg	1,29	—	0,12	0,09	0,11	0,37	0,48	0,17
Coefficient of variation of reproducibility, $C_{V,R}$, %	30,3	—	60,8	73,5	35,0	12,1	13,1	13,4
Reproducibility limit R [$R = 2,8 \times s_R$], mg/kg	3,60	—	0,34	0,26	0,30	1,03	1,34	0,46
HorRat value	*2,4*	—	*3,0*	*3,4*	*1,8*	*0,9*	*1,0*	*0,9*

Table B.6 — Validation data for MOSH [C20-C25] in different vegetable oils

MOSH [C20-C25] (mg/kg)	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**
No. of participating laboratories, n_P	36	36	36	36	36	36	36	36
No. of laboratories retained after eliminating outliers, n_p	34	33	35	35	35	35	34	35
No. of test results in all laboratories, n_t	68	66	70	70	70	70	68	70
Mean value, $\bar{x}$, mg/kg	5,89	0,61	0,24	0,64	1,46	1,50	7,60	2,18
Repeatability standard deviation s_r, mg/kg	0,46	0,07	0,04	0,08	0,13	0,20	0,31	0,13
Coefficient of variation of repeatability, $C_{V,r}$, %	7,9	11,1	16,5	11,9	9,0	13,2	4,0	6,1
Repeatability limit r [$r = 2{,}8 \times s_r$], mg/kg	1,30	0,19	0,11	0,21	0,37	0,55	0,86	0,37
Reproducibility standard deviation s_R, mg/kg	1,44	0,21	0,17	0,20	0,25	0,35	0,99	0,31
Coefficient of variation of reproducibility, $C_{V,R}$, %	24,4	34,2	67,8	31,1	17,5	23,4	13,0	14,1
Reproducibility limit R [$R = 2{,}8 \times s_R$], mg/kg	4,02	0,58	0,46	0,55	0,71	0,98	2,77	0,86
HorRat value	*2,0*	*2,0*	*3,5*	*1,8*	*1,2*	*1,6*	*1,1*	*1,0*

Table B.7 — Validation data for MOSH [C25-C35] in different vegetable oils

MOSH [C25-C35] (mg/kg)	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**
No. of participating laboratories, n_P	36	36	36	36	36	36	36	36
No. of laboratories retained after eliminating outliers, n_p	34	33	35	36	35	34	35	34
No. of test results in all laboratories, n_t	68	66	70	72	70	68	70	68
Mean value, $\bar{x}$, mg/kg	32,79	4,98	0,51	1,88	5,06	1,20	3,20	0,77
Repeatability standard deviation s_r, mg/kg	1,69	0,27	0,12	0,20	0,34	0,25	0,34	0,13
Coefficient of variation of repeatability, $C_{V,r}$, %	5,2	5,4	24,0	10,4	6,6	21,2	10,5	17,3
Repeatability limit r [$r = 2{,}8 \times s_r$], mg/kg	4,74	0,76	0,34	0,55	0,94	0,71	0,94	0,37
Reproducibility standard deviation s_R, mg/kg	4,10	0,79	0,34	0,43	0,69	0,47	0,70	0,33
Coefficient of variation of reproducibility, $C_{V,R}$, %	12,5	15,9	66,7	22,7	13,6	38,8	22,0	43,4
Reproducibility limit R [$R = 2{,}8 \times s_R$], mg/kg	11,49	2,21	0,95	1,19	1,92	1,31	1,97	0,94
HorRat value	*1,3*	*1,3*	*3,8*	*1,6*	*1,1*	*2,5*	*1,6*	*2,6*