Analysis of MOSH and MOAH in food by GC×GC

Guidance on analysis, interpretation and data reporting

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Authorship

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Contents

1 Introduction

Mineral oil hydrocarbons (MOH) are saturated (MOSH) and aromatic hydrocarbons (MOAH) containing 10 to about 50 carbon atoms which are naturally present in crude oil and refined mineral oil fractions, or are synthetically derived from coal, natural gas and biomass. Contamination of foods with MOH can occur from several sources including environmental contamination, harvesting and processing machinery, processing aids (e.g. release agents, dust binders), food or feed additives, and food contact materials (FCM) [1]. MOH contamination in foods was first reported in the late 1980s and increasing evidence of occurrence of MOH contamination in a wide variety of foods including cereals, edible oils, bakery products, cacao and coffee beans, and baby foods has been collected since then [2]. In 2012, following the request of the European Commission, the Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) determined that the presence of total MOAH in food raises potential concerns for human health [3]. In 2017, the European Commission adopted the recommendation that member states together with food business operators should be involved in the monitoring of MOH in foods, and that efforts should be taken to ensure a harmonised quantification of MOH in foods and food contact materials, and the identification of the sources of contamination [4].

In 2019, in the frame of the EU recommendations, the European Union Reference Laboratory for Food Contact Materials (EURL-FCM) at the Joint Research Centre of the European Commission (JRC) published a guidance document to harmonise the sampling, analysis and data reporting of MOH in food and food in contact materials [5]. In 2023, the JRC published a second edition of the guidance on MOH analysis [6] while EFSA published an updated risk assessment of MOSH and MOAH in foods in which it concluded that the current exposure to total MOAH in the diet raises health concerns due to genotoxicity and carcinogenicity. MOSH can be accumulated in various organs, but the current exposure does not raise health concerns, although the margin for a safe exposure is very limited [1]. Since 2023 the European Union Reference Laboratory for Processing Contaminants (EURL-PC) is responsible of promoting harmonisation in the analysis of MOSH/MOAH in food performed at the National Reference Laboratories (NRLs), and of disseminating knowledge on MOSH/MOAH within the European framework.

2 Scope

This guidance document was developed to support the implementation of the amendment of the Regulation (EC) No 333/2007 concerning the methods of sampling and analysis for the control of levels of mineral oil hydrocarbons in foods [7]. The document is intended to support stakeholders in the characterisation of the saturated (MOSH) and aromatic (MOAH) fractions of MOH present as contaminants in foods by using comprehensive two-dimensional gas chromatography (GC×GC). The main objective of this guidance is to promote the harmonisation of GC×GC analysis, chromatogram interpretation and reporting of results for qualitative characterisation of MOSH and MOAH.

The document aims to provide guidelines for tentative identification of analytical interferences that affect quantification of MOSH and MOAH by LC-GC-FID, to provide insights for the identification of the source of contamination, and to support the characterisation of MOAH compounds based on the number of aromatic rings and their degree of alkylation [1, 3]. This document introduces the state of play in regard to the use of GC×GC-FID for the quantification of MOSH and MOAH and their respective subclasses, however it focusses in the qualitative interpretation of MOH fractions using GC×GC as a complementary technique to LC-GC-FID, the reference method for quantification of total MOSH and total MOAH in foods [5]. For a quantitative determination of MOH by GC×GC, further development in harmonisation of procedures for chromatogram integration and evaluation of quantitative reproducibility is still required.

3 GC×GC as complement to quantification of total MOSH and MOAH by LC-GC-FID

MOH are classified into MOSH and MOAH and include compounds with a volatility range between that of alkanes from *n*-C_{10} to *n*-C_{50}, although compounds with higher molecular mass can be present in some foods and food contact materials depending on the source of contamination. MOSH consist of linear, branched, cyclic and alkyl substituted cyclic hydrocarbons while MOAH comprise alkyl-substituted (polycyclic-)aromatic molecules (**Figure 1**). Although MOH encompass only a limited number of

compound classes, the individual compounds within these classes exhibit remarkable variability and diversity [8–10].

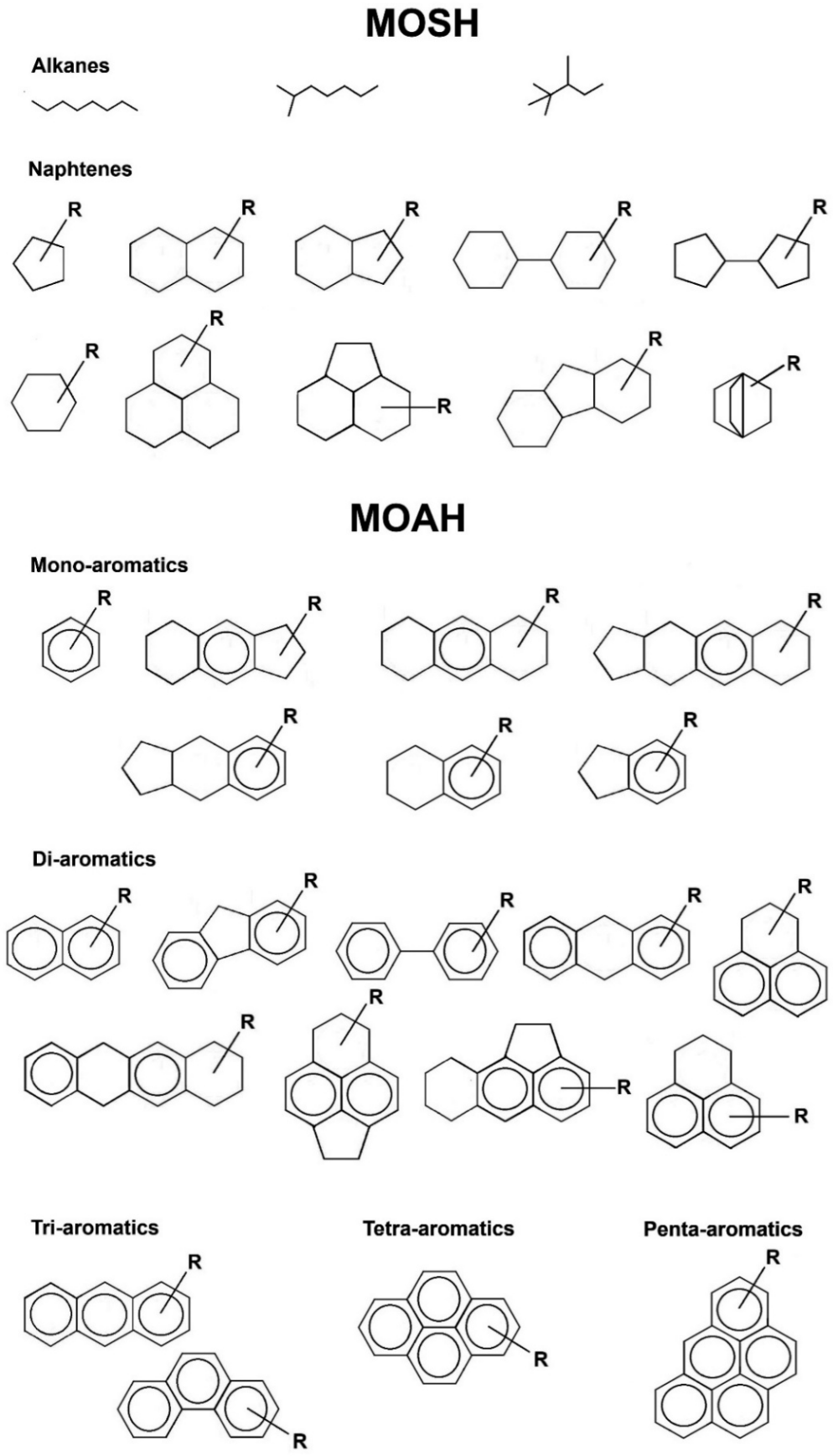


Figure 1. *Examples of mineral oil saturated (MOSH) and aromatic hydrocarbons (MOAH) found in crude mineral oils. Adapted from EFSA Scientific opinion on Mineral oil hydrocarbons in food (2012). In displayed chemical structures, "R" denotes the presence of an alkyl-substitution of variable length.* ***Source:*** [1, 3].

The routine instrumental technique for the analysis of MOSH/MOAH in food and food contact materials is on-line coupled liquid chromatography-gas chromatography with flame ionization detection (LC-GC-FID). In the LC-GC-FID system, the MOSH and MOAH fractions are individually collected after their separation from the more polar components by means of normal phase LC, and later, both fractions are injected separately into the GC-FID system for quantification. In the LC-GC-FID system the two-step chromatography is fully automated, and the LC column can be used several hundred times [11]. On-line coupling of the LC and GC steps reduces sample manipulation and the risk of contamination, and solvent consumption compared to manual solid phase extraction (SPE) followed by GC-FID [12].

In cases when the interpretation of LC-GC-FID chromatograms is uncertain due to the presence of interferences, the use of GC×GC is advised as described in the "*Figure 5"* and *"Annex I"* of the "*Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials"* [6]. In samples with low content of interferences (e.g. rice) where integration of LC-GC-FID chromatograms and removal of riding peaks on the humps can be done confidently and more straightforward (**Figure 2A**), further GC×GC confirmation is not required. In samples, where interferences are present, even after sample pretreatment methods such as epoxidation (**Figure 2B**), GC×GC will be required for confirmation of MOH contamination [6].

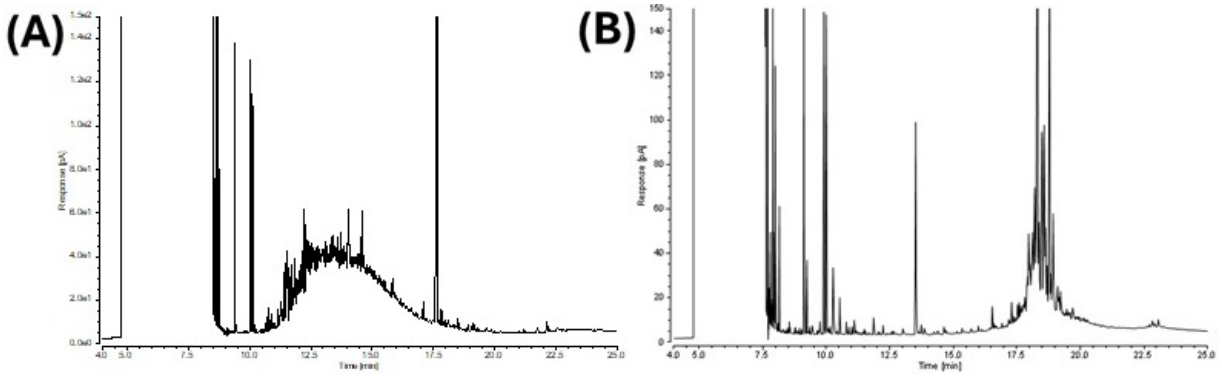
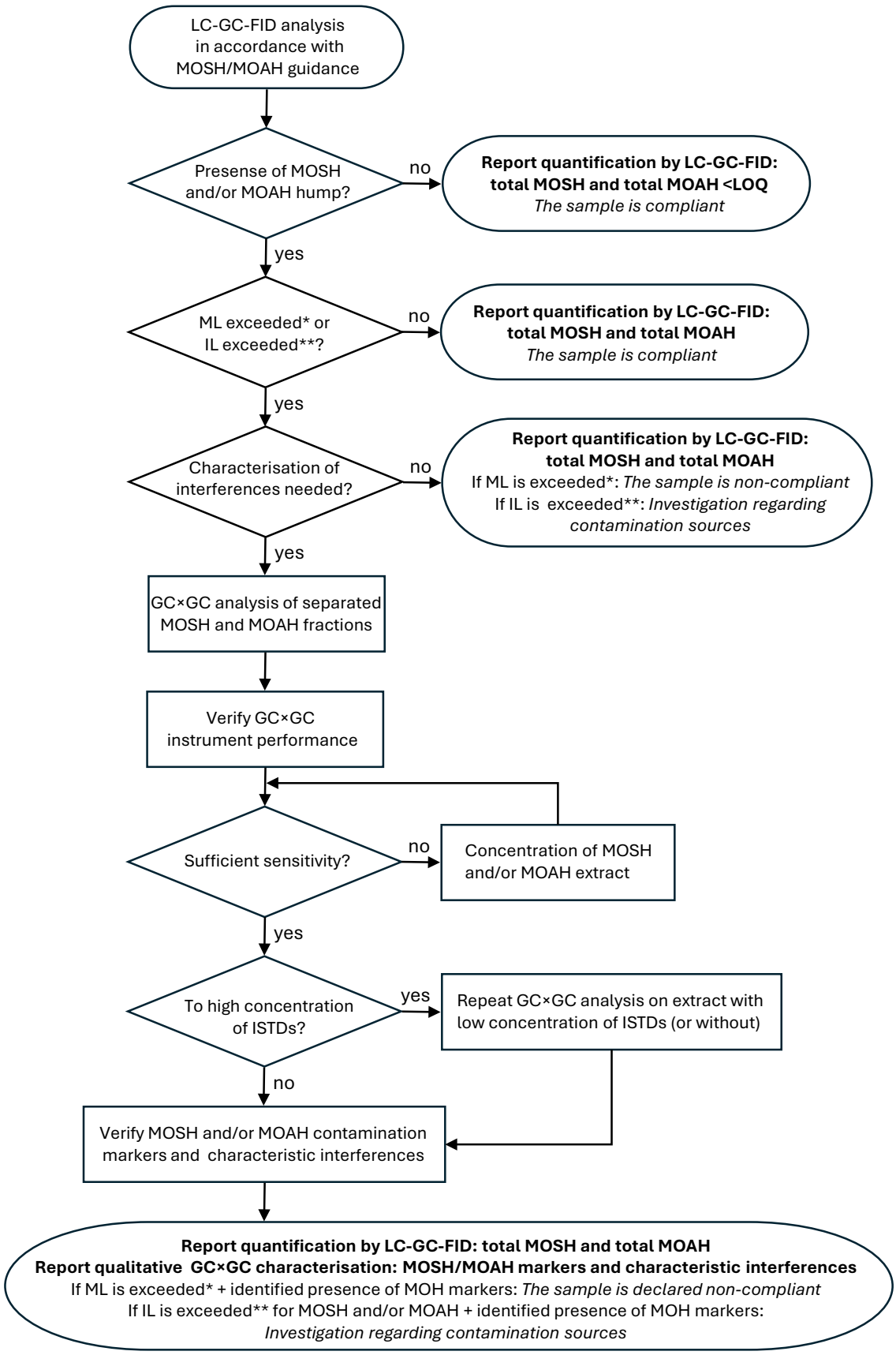


Figure 2*.Representative LC-GC-FID chromatograms of MOH fractions with low (rice MOAH fraction without epoxidation)* ***(A)*** *and high content of analytical interferences (wheat germ oil MOAH fraction after epoxidation)* ***(B)****.* ***Source:*** [6].

GC×GC has several applications within the field of MOH analysis. Specifically, for the enforcement of maximum levels (ML) and the control of indicative levels (IL) of MOH, GC×GC allows the qualitative characterisation of MOSH and MOAH fractions, since characterisation of MOH fractions permits the identification of characteristic compounds and compound families that can be used as general markers of MOH contamination, or as specific markers for determining the possible sources of contamination. For the purpose of this guidance GC×GC characterisation is performed in samples where analytical interferences are so substantial that they hinder the confirmation of MOH contamination and it must be considered after following the decision tree on the use of auxiliary methods of the "*Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials"* [6].

In the routine practice at experienced laboratories, approximately 5 to 10% of samples showing MOH contamination is expected to require further characterisation by GC×GC for the confirmation of contamination. A flowchart illustrating the decision-making steps in the MOH analysis by GC×GC is presented in **Figure 3**. In the case when a sample analysed by LC-GC-FID exceeds a ML (analytical result is higher than ML taking into account the expanded analytical uncertainty) or an IL (analytical result is higher than IL) but its result indicates that a confirmation of MOH contamination by GC×GC is required, the sample can only be declared non-compliant (in case of exceeding ML), if GC×GC characterisation confirms the presence of MOH markers. When a sample exceeds an IL investigation must be carried out in order to identify the potential source of contamination. Reporting of total MOSH and total MOAH must follow the recommendations for data reporting using LC-GC-FID [6]. When a sample is analyzed by GC×GC, results from LC-GC-FID should be accompanied by a report of the results obtained from the GC×GC analysis (see section **Reporting of results**).



*Maximum level (ML) is exceeded if analytical result is higher than ML taking into account the expanded analytical uncertainty.
**Indicative level (IL) is exceeded if analytical result is higher than IL.

Figure 3. *Flowchart for decision-making during MOH analysis by GC×GC considering respective maximum levels (ML) and indicative levels (IL) for MOSH and MOAH in foods.*

4 Introduction to GC×GC

Comprehensive two-dimensional gas chromatography (GC×GC) allows the analysis of complex matrices by sequentially separating compounds on two GC columns with different selectivity. The columns are connected via a modulation device (modulator), which periodically transfers small fractions of the sample from the first column (first dimension or ^{1}D) to the second column (second dimension or ^{2}D) for further separation. Some general advantages of GC×GC include the increase in peak capacity, resolution, and sensitivity, and the separation of compounds into structurally related groups, facilitating their characterisation [10, 13]. In the context of MOH analysis, GC×GC allows, for example, the determination of MOAH subfractions based on their number of aromatic rings, a parameter of toxicological relevance [1]. Additionally, GC×GC allows the identification of elution patterns of characteristic MOH contamination and improves the identification of interfering compounds coeluting with MOSH or MOAH by increasing the resolution capability.

4.1 Basic GC×GC instrumentation for MOSH and MOAH analysis

A GC×GC system is typically composed of an inlet (injection system), a carrier gas provider, a first-dimension column (^{1}D), a modulator, a second-dimension column (^{2}D) with or without a secondary oven, and a detection system (**Figure 4**). The specific configuration of the GC×GC system, such as the choice of the type of inlet, modulator or detector, can be adapted to meet the requirements of the intended application. The following sections discuss general system configurations relevant to MOSH/MOAH analysis.

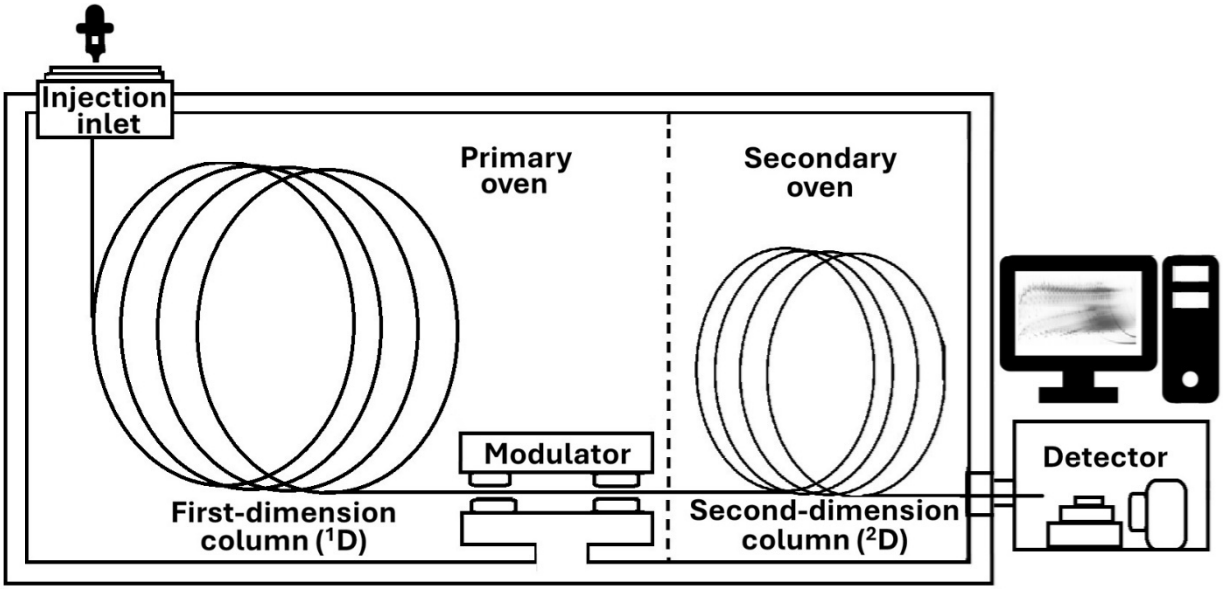


Figure 4. *Schematic representation of a typical GC×GC system instrumentation.* ***Source:*** [14].

4.1.1 Injection inlet

MOSH and MOAH comprise compounds spanning a wide volatility range (*n*-C_{10} to *n*-C_{50}). Therefore, injection techniques that minimise volatility-based discrimination are preferred. On-column (OC) and programmed temperature vaporizer (PTV) injection are the most suitable options, with OC offering the lowest discrimination during sample introduction. To achieve limits of detection and quantification (LOD/LOQ) comparable to those obtained with LC-GC-FID, large volume injection (LVI) should be considered when using FID detection. LVI is best implemented via OC injection using a long retention gap (*i.e*. uncoated and deactivated pre-column) and a solvent vapor exit (SVE), as used in LC-GC-FID. The use of a 10 m × 0.53 mm pre-columns allows injection volumes of up to 80 µL without using SVE. Injection to GC×GC can also be coupled to a preceding liquid chromatography separation (LC-GC×GC). When using mass spectrometric (MS) detection, the sensitivity of detectors allows lower injection volumes while maintaining adequate detection performance, but it must be noted that the MS detector itself may cause discrimination of the less volatile compounds.

4.1.2 Columns

Different column configurations can be employed for the determination of MOSH and MOAH by GC×GC. Currently, the most recommended configuration is the so-called *reverse column* configuration or *reverse orthogonality*, which uses a mid-polar column in ^{1}D and a non-polar column in the ^{2}D (mid-polar×non-polar configuration). In this configuration, compounds are primarily separated by volatility (with a slight influence of polarity) in the ^{1}D, and by polarity in the ^{2}D.

The advantages of the mid-polar×non-polar configuration include:

- Relatively high resolution of MOSH in the ^{2}D, facilitating the identification of *n*- and *iso*-paraffins, naphthenes, and steranes/hopanes (the latter used as markers of MOH contamination) [15].
- Good separation of MOSH from interferences such as polyolefin oligomeric hydrocarbons (POH), mainly multibranched oligomers from polypropylene [15] [16].
- Good separation of MOAH into distinct homologous series according to the number of aromatic rings.
- Narrow retention time distribution and narrower peaks in ^{2}D, which tends to provide higher sensitivity, particularly relevant for ≥3-ring MOAH (MOAH with 3 or more aromatic rings in the chemical structure).

In terms of composition, the mid-polar ^{1}D column usually consist of 50% phenyl/50% dimethyl polysiloxane or another equivalent stationary phase. The main requirement during column selection is the resistance of the mid-polar column to high temperatures (350-360 °C) necessary to elute the high boiling MOSH and MOAH compounds [17, 18]. Non-polar columns used for the ^{2}D most commonly employ 100% dimethylpolysiloxane as stationary phase. In some specific cases, mainly when using LV on-column injection, a pre-column can be used before the ^{1}D. For this purpose, uncoated columns with phenyldimethyl-silylation deactivation can be used [15].

In general, the film thickness of ^{1}D column should not exceed 0.15 µm, since this allows that the elution of *n*-C50 still occurs within the gradient of the GC oven temperature program typically used in GC×GC. If no secondary oven is used the length and film thickness (retention power) of the ^{2}D column must be adjusted such that the most retained components (e.g. saturated POH) still elute within the time range of a modulation period. If a secondary oven is present, too much retention on ^{2}D column can be compensated by an increase in the temperature offset between ^{1}D and ^{2}D.

The *normal column* configuration (non-polar×mid-polar configuration) [10] brings some advantages in the characterisation of MOH contamination such as a larger retention time distribution of MOAH, allowing a better separation between 1-2-ring MOAH and ≥3-ring MOAH in comparison to the mid-polar×non-polar configuration. However, the non-polar×mid-polar configuration reduces the capability of a detailed characterisation of MOSH fractions [19]. For the purpose of this guidance document, recommendations are based on the use of the mid-polar×non-polar configuration.

4.1.3 Modulator

The modulator regulates the transfer of the sample from the ^{1}D to the ^{2}D column at regular intervals and improves the sensitivity of analysis by refocussing the analytes before the separation in the ^{2}D [15]. Given the wide boiling point range covered by MOSH/MOAH, the modulation device must efficiently focus and remobilise both volatile and high-boiling compounds during modulation. Cryogenic modulators are generally the most suitable for this purpose but other systems such as flow modulator can be also used with appropriate results. Modulation time must be defined as a compromise. If too long it causes a loss in resolution in the separation at ^{1}D, while if too short, "*wrap-around*" may occur [20]. Typical modulation times in MOSH and MOAH determination varies from 5 to 10 seconds [15, 21, 22]

4.1.4 Secondary oven

A secondary oven may be used for the ^{2}D column usually applying a temperature program with slightly higher temperatures than those at the main oven to avoid the "*wrap-around*" of signals. *Wrap-around* occurs when the retention time in the ^{2}D column for a compound or group of compounds is longer than the modulation time (which determines the duration of the ^{2}D run), causing the compound or group of

compounds to appear in the following modulation cycle [10]. By increasing the temperature offset in the secondary oven, ^{2}D retention times are reduced and *wrap-around* can be reduced, minimized or prevented [23]. If no secondary oven is present, *wrap-around* is prevented by optimizing the length and the film thicknesses of the two columns [15] and by optimising the oven temperature ramp.

4.1.5 Detector

Common detection systems for GC×GC in MOSH and MOAH analysis are Flame Ionization Detector (FID) and Mass Spectrometry (MS). FID detection is based on the formation of ions during the combustion of organic molecules under a hydrogen/air flame and is valuable in MOSH and MOAH analysis due to its virtually consistent mass response to hydrocarbons. This provides similar responses across different hydrocarbon structures, reducing the need for compound-specific standards and allowing quantification without the requirement of defining specific response factors for different compounds [18].

MS detection, in contrast, separates ions based on their mass-to-charge ratio (*m/z*) and tends to have higher sensitivity compared to FID. Adequate acquisition frequency must be applied for proper reconstruction of the very fast ^{2}D eluting peaks. Generally, time-of-flight (ToF)-MS systems are preferred over other MS analysers due to their high spectral acquisition speed, but other MS-systems can also be used, providing sufficient acquisition speed. MS spectral data enables their comparison to databases providing additional information for the tentative identification of MOH beyond elution patterns and characteristic retention times as it occurs with FID detection.

4.2 Characteristic MOSH and MOAH elution patterns in GC×GC

For a reverse column set, comprising a mid-polar column in the ^{1}D and a non-polar column in the ^{2}D (mid-polar×non-polar), elution occurs as follows:

- In the ^{1}D, compounds are eluted by decreasing volatility and increasing polarity. Compounds with lower volatility, but the same polarity show higher ^{1}D retention times. Typically, for compounds with similar volatility, the more polar compound will exhibit a higher ^{1}D retention time.
- In the ^{2}D, separation is based on decreasing polarity, with less polar compounds eluting at higher ^{2}D retention times.

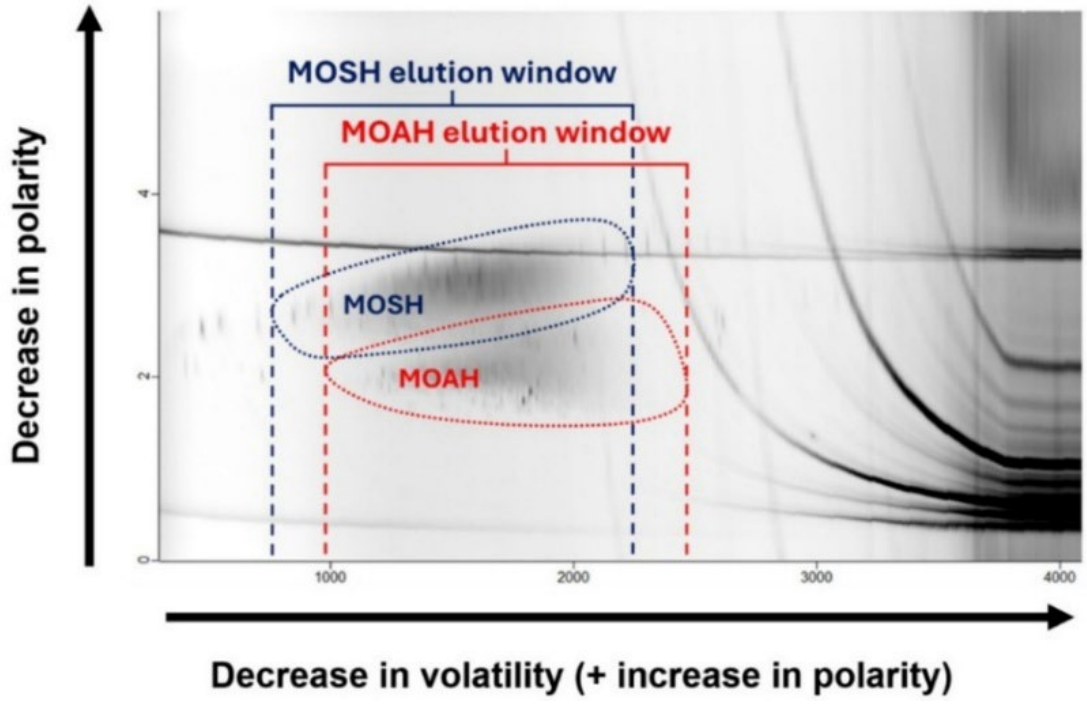


***Figure 5** . GC×GC chromatogram of a MOSH and MOAH from a process oil showing the shift of retention time window between MOSH and MOAH. **Source:** [24].*

Consequently, MOSH elute at the mid and top part of the chromatogram (i.e., at higher ^{2}D retention times), while MOAH elute at the mid and bottom part (i.e., at lower ^{2}D retention times) slightly deviated to the right (higher ^{1}D retention times) when compared to the corresponding MOSH hump (i.e., originated from the same mineral oil). If MOSH and MOAH are not physically separated before GC injection, parts of both humps may coelute in the mid part of the chromatogram (**Figure 5**). In addition, **Figure 6** shows the overlap of the retention times of the MOSH standard cholestane (a sterane) together with the monocyclic alkylbenzenes (MOAH).

4.3 Fit for Purpose: GC×GC-MS and GC×GC-FID

GC×GC-MS approach is valuable for its high sensitivity and the capability of tentative identification of compounds [10] that can be used for the confirmation of MOH contamination, the evaluation of the potential sources of contamination, and the identification of specific interferences that should be excluded from the quantification of total MOSH and total MOAH [17]. Nevertheless, it is not possible to use GC×GC-MS with quantification purposes since the peak intensity in this detection system depends on the ionisation and fractionation of ions in the ion source which will differ among MOH subclasses. A wide variety of proper authentic standards along the mass range and chemical diversity of MOH compounds will be then required for an accurate quantification in that case.

In this sense, GC×GC-FID has the advantage over MS approaches of having a mass response virtually equal across the different classes of hydrocarbons and only marginally higher for aromatic compounds, which implies that there is no requirement to define response factors for all analysed compounds [18]. However, the transference of information from MS to FID systems is possible, when both systems are available and after precise optimisation of the conditions of analysis to match GC×GC retention times on both systems [25].

4.4 Quantitative MOH determination by GC×GC-FID

As analytical tool, GC×GC-FID has the potential for a quantitative determination of MOSH and MOAH fractions, both for the quantification of total content MOSH and MOAH and the quantification of the content of selected subclasses, as for example MOAH classified in terms of the number of aromatic rings or degree of alkylation. However, for the time being further steps must be taken to ensure harmonisation in chromatogram integration and data processing to ensure reproducible quantitative interlaboratory comparisons independently of software availability. Chromatogram processing strategies, integration, and algorithms used for trimming of interfering peaks or for hump smoothening have an influence on the capacity for detection of specific signals and be therefore considered in quantitative approaches.

In the future, the guidance for analysis of MOSH and MOAH in food by GC×GC can be further developed to include a quantitative determination of MOH fractions. Nevertheless, it is strongly recommended to perform a quantitative determination of the total MOAH hump. For monitoring purposes, a quantification of MOAH subclasses based on the number of aromatic rings and the degree of alkylation can also be considered. The quantitative approach can be performed by relating the area of the hump to that of the peak of the quantification internal standard, typically 2-methylnaphthalene (2-MN). In a similar direction, quantification of MOSH humps can be calculated by using the internal standard cyclohexyl-cyclohexane (CyCy) as previously described for LC-GC-FID [6].

5 Sampling and sample pretreatment

This guidance document focuses on characterisation of MOSH and MOAH fractions by GC×GC. For guidelines on sampling, sample management and sample pretreatment, strategies for reduction of analyte loss and analytical interferences as well for guidelines on quantification of total MOSH and total MOAH in foods by LC-GC-FID, the reader must refer to the "*Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials*" [6].

As discussed later, sample pretreatment steps such as epoxidation can be used to eliminate biogenic compounds that overload LC-GC-FID chromatograms and interfere with MOH analysis [26]. However, while epoxidation allows the removal or reduction of carotenoids, terpenoids and olefins frequently observed as interferences in MOAH fractions [27], it also impacts the MOH profile, particularly reducing

the ≥3-ring MOAH subfraction [21]. Therefore, sample pretreatment should only be used when strictly necessary [6]. When samples require pretreatment for their LC-GC-FID analysis, it is recommended that in addition to the sample prepared for LC-GC-FID, further samples without pretreatment are also prepared for GC×GC analysis, for a comprehensive understanding of the type and source of MOH contamination. However, in samples containing large amounts of interferences such as food-derived olefines, it is not recommended to use GC×GC for extracts that has not being epoxidised, since GC×GC columns would be severely overloaded by the interferences.

6 Interpretation of GC×GC chromatograms for MOH analysis

6.1 Reference GC×GC chromatogram for MOSH and MOAH analysis

Figure 6 shows an annotated GC×GC chromatogram that illustrates the characteristic elution pattern of MOSH and MOAH compounds when using a mid-polar×non-polar column configuration. A description of the different MOSH and MOAH sub-classes is given in the sections below. **Figure 6** shows the results using a P50-mix as described by Biederman *et al* [21], but similar results can be obtained using other mineral oil mixtures either prepared by individual laboratories [28] or supplied commercially in the future.

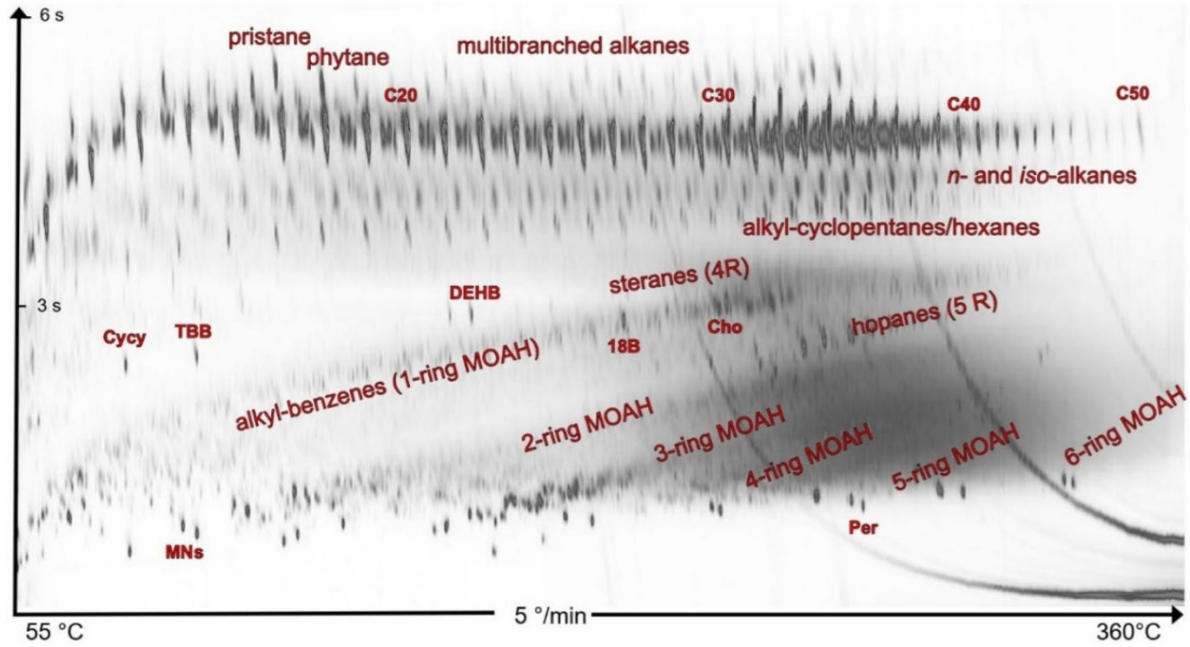


Figure 6. *Representative GC×GC-FID chromatogram of the P50-mix of mineral oils containing diesel oil, batching oil, heavy distillate crude oil, medium distillate crude oil, paraffin wax, distillate aromatic extract, DAE, EPA PAH standard mix, MOSH/MOAH internal standard mix, octadecylbenzene, DEHB, coronene, benzo[rst]pentaphene and pentacontane (composition described in Biedermann et al. 2022). Chromatogram kindly provided by M. Biedermann, further details in* [21].

The framing of the elution zones of MOSH and MOAH humps and corresponding subclasses can be done by using a mixture of standards (**Figure 7**), for instance:

1. The **MOSH/MOAH standard mix** used in LC-GC-FID quantification containing *n*-C11, *n*-C13, cyclohexyl cyclohexane (CyCy), and 5-*α*-cholestane (Cho) as MOSH standards; and *n*-pentyl benzene (5B), 1-methyl naphthalene (1-MN), 2-methylnaphthalene (2-MN), tri-*tert*-butylbenzene (TBB) and perylene (Per) as MOAH standards. As for LC-GC-FID, MOSH/MOAH standard mix can be used for quantification in GC×GC-FID, if necessary (See **Section 4.4**).
2. A ***n*-alkane standard mix** (e.g. C10 to C50 alkane mix) used to frame the retention times of MOSH.
3. A **polycyclic-aromatic hydrocarbon (PAHs) standard mix** covering a wide range of aromaticity (e.g., the 16 EPA PAHs, with 2–7 aromatic rings), to support the identification of different MOAH subclasses according to their number of aromatic rings.
4. **A poorly hydrogenated MOH mixture** containing compounds with different number of aromatic rings (wide elution range in ^{1}D), and degree of alkylation (wide ^{2}D elution range) that must be used

for determining the elution pattern of the different aromatic sub-classes in the MOAH fraction. This is particularly useful to define the cut between e.g. 1-2 ring MOAH and ≥3-ring MOAH (**Figure 7**), and for estimating the degree of alkylation of MOAH (Section **6.4.2**, **Figure 21**) [21, 29]

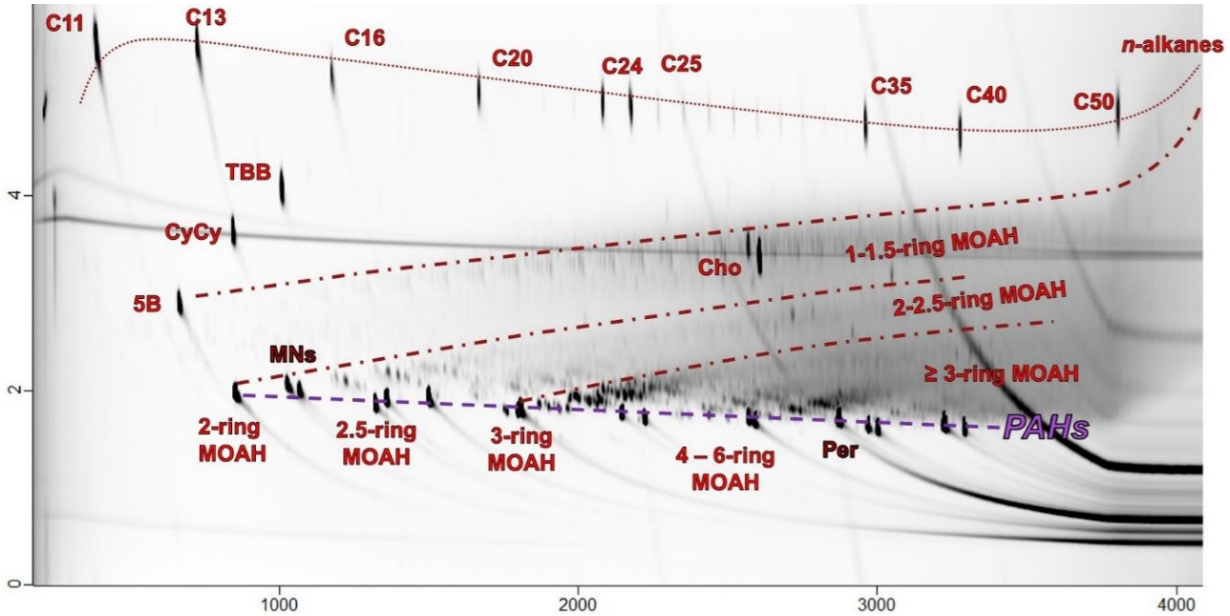


Figure 7. *GC×GC-ToF chromatogram including MOH standard, a C7-C40 alkane mix, and a PAH standard mix. Commercial standards of MOH (n-C11, n-C13, cyclohexyl-cyclohexane (CyCy), cholestane (Cho), n-pentyl benzene (5B), 1-methyl naphthalene (1-MN), 2-methylnaphthalene (2-MN) tri-tert-butyl benzene (TBB), and perylene (Per)), linear alkane C10-C50 mix (n-C10, n-C11, n-C13, n-C16, n-C20, n-C24, n-C25, n-C35, n-C40, n-C50) and PAH (acenaphthene, acenaphtylene, anthracene, benzo[α]anthracene, benzo[κ]fluoranthene, benz[α]pyrene, benzo[b]fluoranthene, benzo[ghi]perylene, chrysene, dibenzo, benzo[α,h]anthracene, fluorene, indeno[1,2,3-cd]pyrene, naphthalene, phenanthrene and pyrene) were used.* ***Source:*** [24].

6.2 Elution of compounds in the MOSH fraction

MOSH can be divided into main sub-classes:

1. Linear alkanes
2. Branched and multibranched alkanes
3. Alkyl cycloalkanes and alkyl polycyclic alkanes

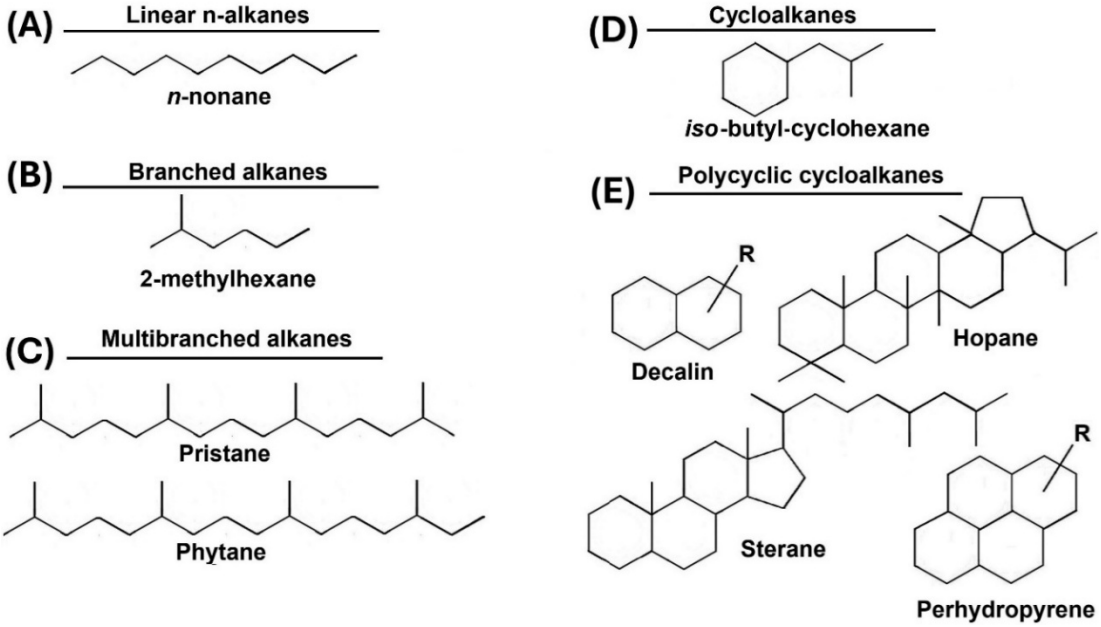


Figure 8. *Chemical structure of representative mineral oil saturated hydrocarbons (MOSH).*

A list of chemical structure of representative MOSH is shown in **Figure 8**, and the elution pattern of MOSH subclasses in GC×GC will be explained in the following sub-sections, along with comments related to potential interferences which are not of mineral oil origin.

6.2.1 Linear alkanes

Linear alkanes (*n*-alkanes) form discrete and regular peaks at the top of chromatograms. They are (more or less) horizontally aligned, depending on the chromatographic conditions. Their elution zone can be easily located by injecting a commercial MOH retention time standard (**Figure 6** and **Figure 7**). Linear alkanes are not present in all mineral oil products, but when they are, they can be easily recognised by their equidistant distribution in the ^{1}D. The alkanes of mineral oil origin do not show any prevalence for even- or odd carbon-numbered *n*-alkanes, and the proportion of individual alkanes (hump center) depends on the type of contamination. **Figure 9A** shows the GC×GC chromatogram and reconstructed ^{1}D chromatogram of a MOSH contamination showing the presence of *n*-alkanes from C21 to C35, with the highest intensity of the signal for C27 and no prevalence of either even- or odd carbon-numbered compounds.

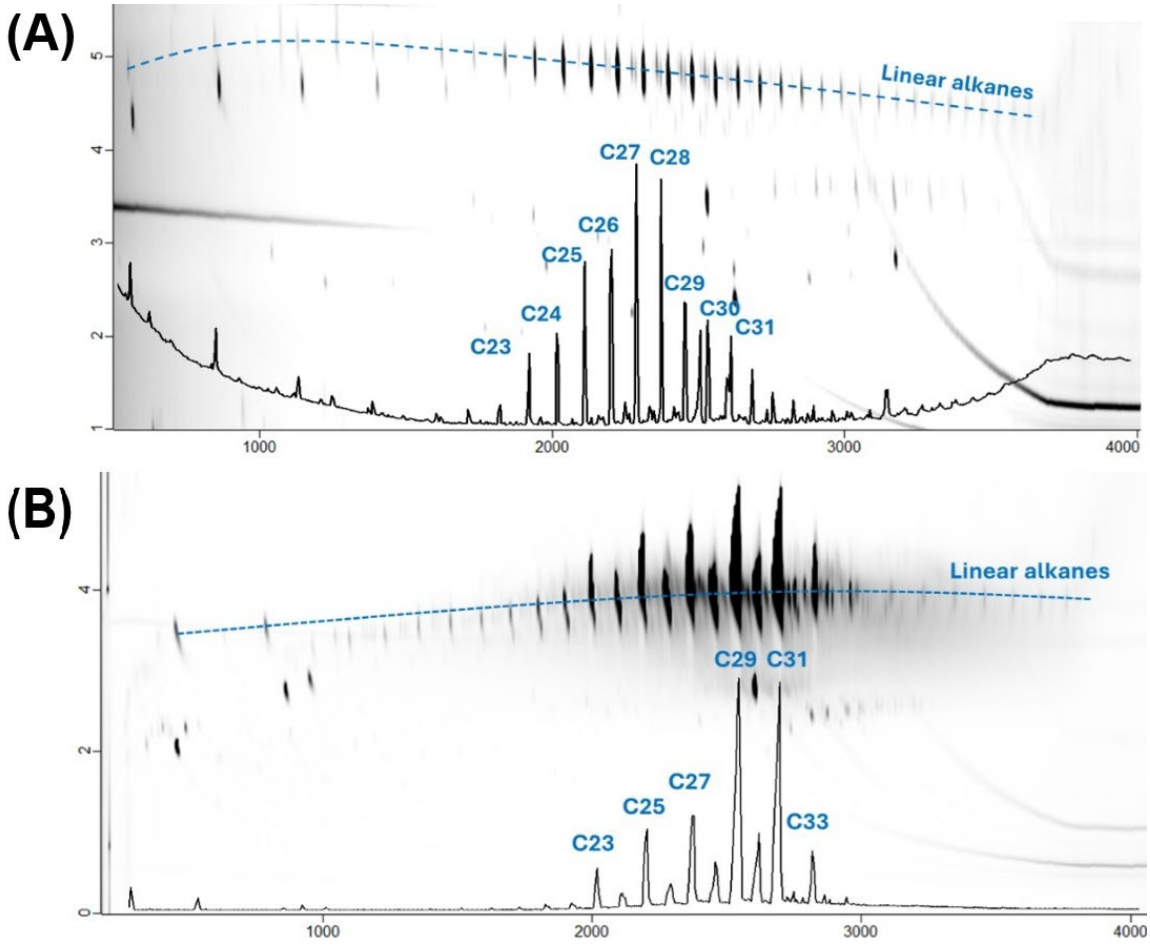


Figure 9. *GC×GC chromatogram and reconstructed ^{1}D chromatogram showing the pattern of n-alkanes. Panel **(A)** shows the MOSH fraction of a plant extract where there is no prevalence of even or odd carbon number linear alkanes confirming they are from mineral oil origin. Panel **(B)** shows the MOSH fraction of a plant extract where there is visible prevalence of odd carbon number linear alkanes.* ***Source:*** [24]**.**

If a prevalence for odd carbon numbers is visible, the linear alkanes are most probably of plant origin and should not considered MOSH. In fact, *n*-alkanes from plant waxes are composed of mainly odd-numbered chains ranging from C21 to C35, with higher peaks typically at C29 or C31 (**Figure 9B**). With some exceptions such as cocoa butter, which shows the highest peak at C23. Even-numbered alkanes

are much less abundant, and minor peaks follow a repeating pattern [30]. In edible oils, natural paraffins can be particularly abundant, and if they overload the GC column, activated aluminium oxide can be used to remove them [6]. If a prevalence of even carbon-numbered alkanes is observed, it could indicate a contamination by saturated hydrocarbons (POSH) from high density polyethylene (HDPE) produced with metallocene catalysts. Further details and illustration are available in **section 6.3.3** and in the work of Biedermann and Grob (2012)[31].

6.2.2 Branched alkanes (*iso*-alkanes) and multibranched alkanes

Above the *n*-alkanes, as well as in between them, elute the *iso*-alkanes (branched alkanes). In fact, for a same carbon number, an increase in the branching degree increases the volatility of the compound causing a decrease in the retention time in ^{1}D. In consequence, a branched alkane enters the ^{2}D column at lower GC oven temperature, and therefore, the retention in ^{2}D is increased compared to a corresponding linear *n*-alkane. In summary, this behavior causes that compared to linear alkanes, the branched *iso*-alkanes appear shifted to the left and the upper part of the chromatogram (**Figure 10**).

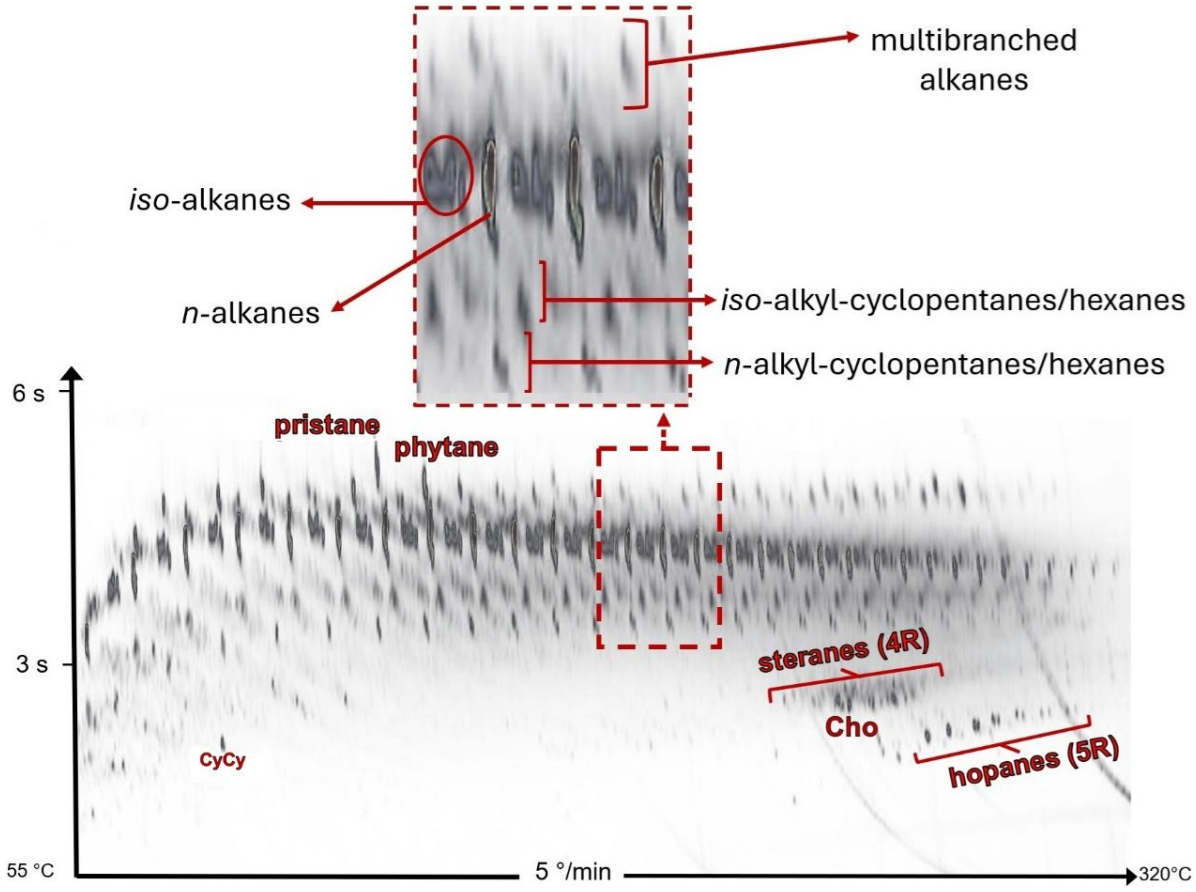


Figure 10. *Elution zone of n-alkanes, iso-alkanes, multibranched alkanes, iso-alkyl-cyclopentanes/hexanes and n-alkyl-cyclopentanes/hexanes in a representative GC×GC-FID chromatogram. Chromatogram kindly provided by M. Biedermann, further details in* [21]**.**

Iso-alkanes may be abundantly present in mineral oils but are not specific to them apart from pristane, phytane and longer chain multibranched alkanes which, when present, are considered as markers of MOH contamination (see **section Summary of contamination markers**). Pristane ($C_{19}H_{40}$) and phytane ($C_{20}H_{42}$) (**Figure 8**) are isoprenoid alkanes derived from the degradation of chlorophyll or the lipid membranes of ancient biological organisms. In consequence, they are exclusively derived from petroleum and their detection in the sample is considered a confirmation marker of MOH contamination [31]. On the GC×GC chromatogram, pristane elutes slightly before and above *n*-C_{17}, while phytane slightly before and above *n*-C_{18} (**Figure 6**, **Figure 10**). Some *iso*-alkanes derive from biological sources, although their carbon number range is more restricted (typically C_{15}-C_{35}) and they have more specific branching

patterns. Migration of chemicals from plastic packaging may also be a cause of *iso*-alkanes contamination in the sample, as will be discussed in **section 6.3.**

6.2.3 Alkyl cycloalkanes and alkyl polycyclic alkanes

Cycloalkanes (commonly referred in MOH literature as napthenes) elute below the line of *n*-alkanes, and the higher the number of saturated rings they contain, the lower their ^{2}D retention times (**Figure 10**). The addition of alkyl chains to the cyclic structure decreases compound volatility, as well as polarity. Therefore, the alkylated derivatives of a cycloalkanes elute at higher ^{1}D and ^{2}D retention times. Among cycloalkanes, two groups of MOH contamination markers are hopanes (pentacyclic triterpenes) and steranes (**Figure 8**). These two groups of alkyl polycyclic cycloalkanes are formed from biological materials over geological time and are highly stable and abundant in petroleum products. Their detection by LC-GC-FID is difficult, but , they are well separated and have a typical elution pattern in GC×GC using a mid-polar×non-polar configuration (**Figure 10**). Other polycyclic cycloalkanes such as decalins, perhydroanthracenes/-phenanthrenes (perhydro 3R) and perhydropyrens (**Figure 11**) show a characteristic pattern within intermediate ^{1}D retention times and low to middle ^{2}D retention times, eluting in consequence in the middle part of the two-dimensional chromatogram. Such polycyclic cycloalkanes are mainly present in partial or fully hydrogenated mineral oil fractions.

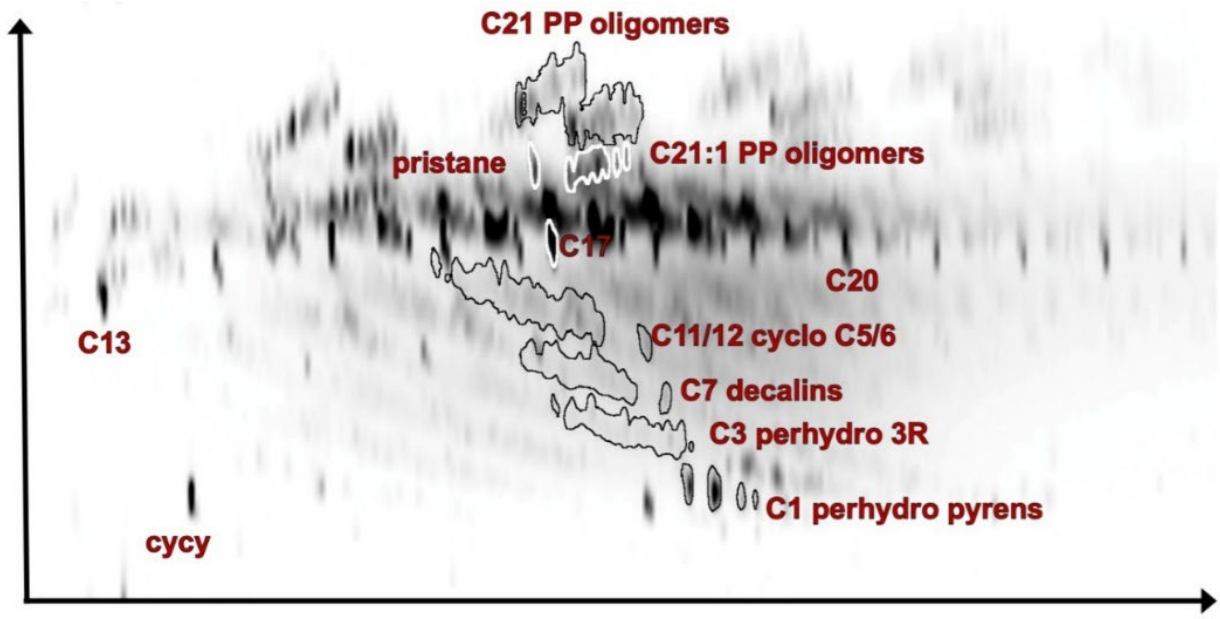


Figure 11. *Representative GC×GC chromatogram showing elution of polycyclic cycloalkanes. Chromatogram kindly provided by M. Biedermann, further details in* [15]**.**

6.3 Compounds co-eluting with MOSH fraction

Characterisation of MOSH using GC×GC is challenging due to the presence of co-eluting compounds not classified as MOH. Common interferences found in MOSH fractions are compounds naturally present in food matrices, or polysiloxanes from silicon septa and column bleeding. Other compounds that are not MOSH but are quantified by LC-GC-FID together with total MOSH, such as synthetic hydrocarbons (e.g. polyolefin oligomeric hydrocarbons (POH) and polyalpha olefins (PAO)), can also co-elute with MOSH fractions and be observed during GC×GC. **Figure 12** shows the chemical structure of representative compounds co-extracted with MOSH.

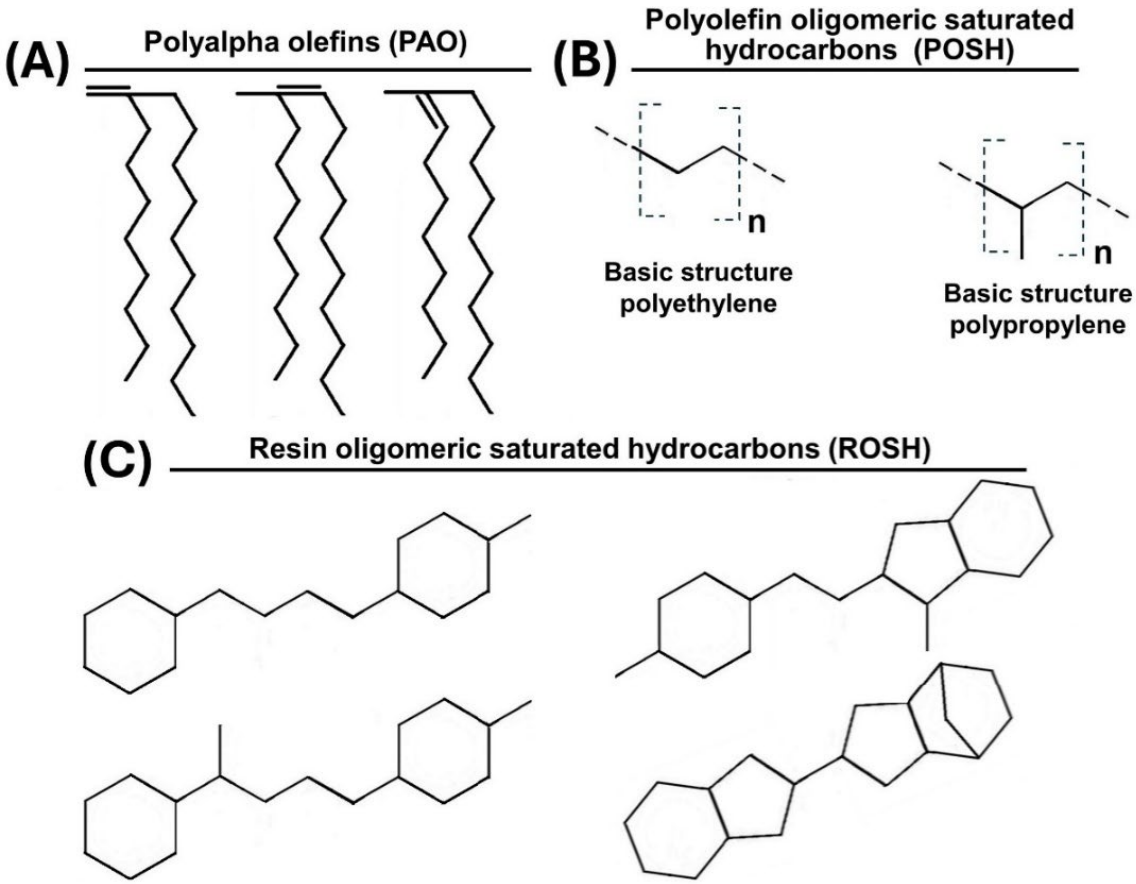


Figure 12**. Chemical structure of representative compounds co-extracted with MOSH.* ***Source [32, 33]

6.3.1 Terpenes and derivatives

Terpenic compounds derived from biological matrices can be coextracted with MOSH/MOAH. The most non-polar terpenic compounds can be collected in the MOSH fraction and coelute with the MOSH hump in the GC×GC chromatogram. They form very typical, diagonal elution patterns in the GC×GC plot when using the mid polar×non-polar column configuration (**Figure 13**).

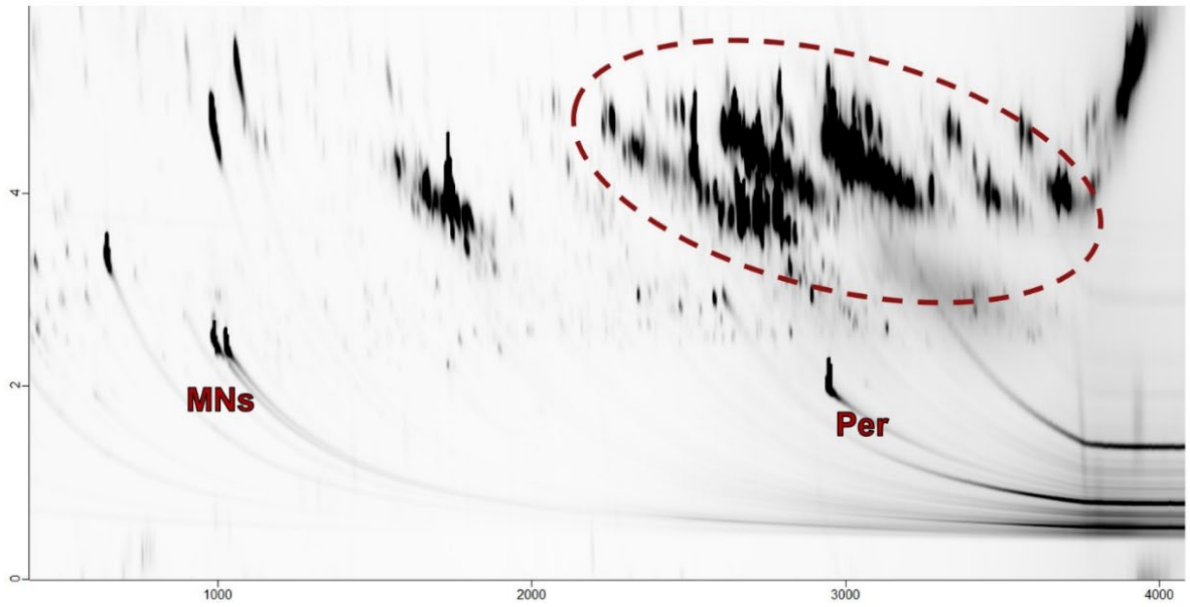


Figure 13.** GC×GC chromatogram of rapeseed oil showing terpenic compounds (red-dashed). as interferences during MOSH determination.* ***Source: [24]**.**

6.3.2 Polyalpha olefins (PAO)

Polyalpha olefins (PAO) are synthetic isoparaffins [5] used as lubricating materials and mainly formed by *α*-olefins with chain length between 8 and 12 carbon atoms [32]. Due to chemical properties, they can be co-extracted with MOSH and be present in the upper part of the GC×GC chromatogram using mid-polar×non-polar configuration (**Figure 14**). However, some PAO components might elute together with the MOAH [31].

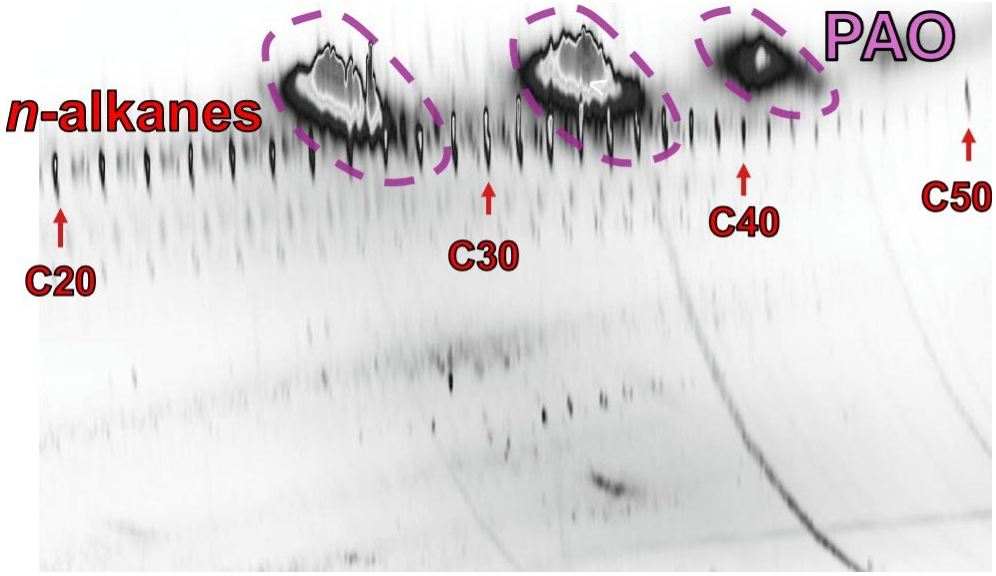


Figure 14. *Detail of the GC×GC chromatographic fingerprint of interfering polyalpha-olefins (PAO)(doted circle) above the n-alkanes at the region of multibranched alkanes and polyolefin oligomeric hydrocarbons from propylene (PP POH) Chromatogram kindly provided by M. Biedermann, further details in* [34]**.**

6.3.3 Polyolefin oligomeric hydrocarbons (POH)

Polyolefin oligomeric hydrocarbons (POH) are polyolefin-derived saturated (POSH) and unsaturated (mainly mono-unsaturated) hydrocarbons (POMH) which are not considered as MOSH but may interfere with their analysis. POH are oligomers present in polypropylene and polyethylene [5] that tend to form specific patterns in GC×GC chromatograms **(Figure 15)**.

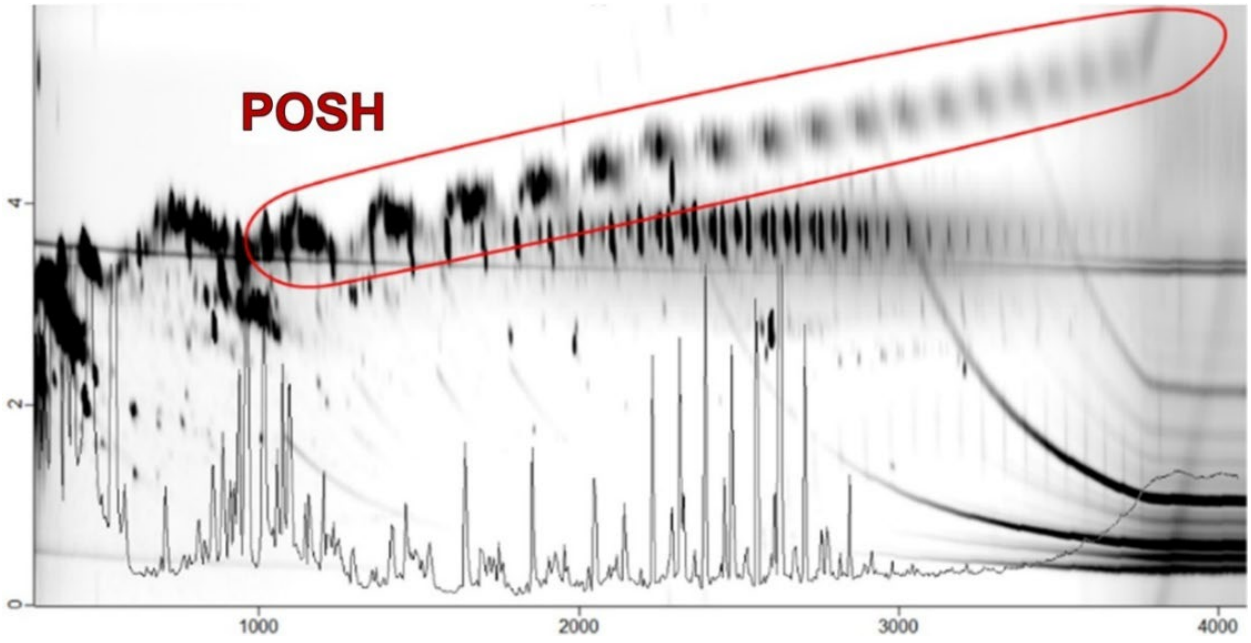


Figure 15. *GC×GC and reconstructed GC (1D) chromatograms of a MOSH fraction of a plant extract with visible POH deriving from polypropylene.* ***Source:*** [24]**.**

POH can migrate from plastic containers, bags or heat-sealable layers (*e.g* in aluminium bags), adhesives, or plasticisers [31] during food storage or as consequence of sample processing and/or manipulation [16]. The POH profile from high density polyethylene (HDPE) is dominated by even-numbered *n*-alkanes (formed by ethylene units and corresponding POMH) while low density polyethylene (LDPE) shows higher complexity in the mixture with dominance of even-numbered open chain (POSHoc) and cyclic (POSHcy) alkanes, but with the presence of minor odd-number alkanes and corresponding POMH [15, 16]. **Figure 16** shows characteristic GC×GC fingerprints of POH contamination from some representative plastic materials.

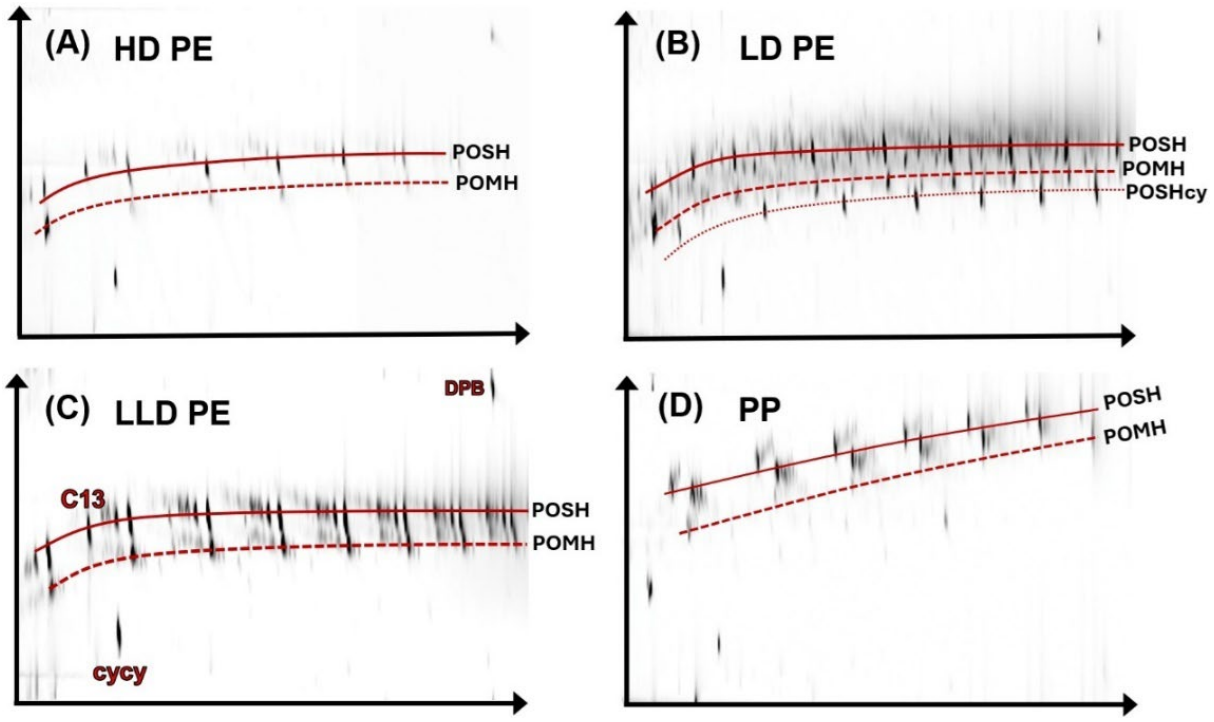


Figure 16. *Characteristic chromatographic fingerprint of interfering POH from different type of plastic materials. High-density polyethylene (HDPE)* ***(A)****, low-density polyethylene* ***(B)****, linear low-density polyethylene (LLD PE)* ***(C)*** *and polypropylene (PP)* ***(D).*** *Chromatogram kindly provided by M. Biedermann, further details in* [16]***.***

6.3.4 Saturated resin hydrocarbons

Resin hydrocarbons are industrial polymers used in hot-melt adhesives, and are produced from unsaturated compounds derived from petroleum cracking such as styrene, cyclopentene, indene and cyclopentadiene [33]. Hot-melt adhesives are used in multilayer food packaging made of paper, cardboard, plastic or glass, and can be a source of contamination with resin hydrocarbons, even if they are not directly in contact with foods [35]. The profile of resin hydrocarbons in GC×GC depends on the degree of hydrogenation applied during their production, with higher content of resin oligomeric aromatic hydrocarbons (ROAH) in non-hydrogenated or partially hydrogenated adhesives, and higher proportion of resin oligomeric saturated hydrocarbons (ROSH) in fully hydrogenated ones [33]. ROSH are extracted together with MOSH fractions but are not considered MOSH and should be excluded from analysis, however the presence of ROAH indicates the possible migration of volatile hydrocarbons from resins used in sealing of FCM. **Figure 12** shows the chemical structure of representative ROSH potentially co-eluting with MOSH while **Figure 17** shows the characteristic GC×GC elution pattern of resin-derived hydrocarbons.

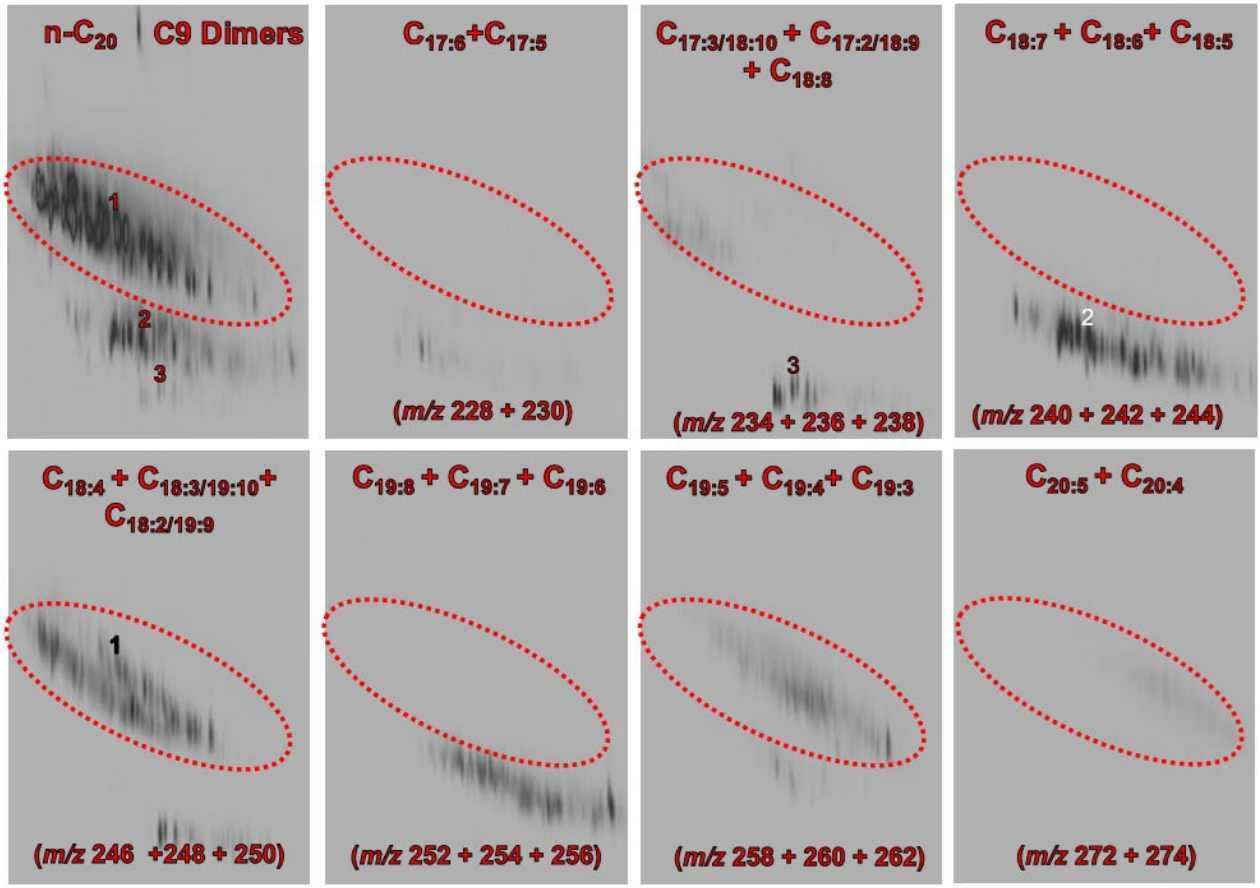


Figure 17. *Representative GC×GC-MS chromatograms showing the elution pattern of dimers from a C9 resin including saturated and monoaromatic hydrocarbons. Chromatograms kindly provided by M. Biedermann, further details in* [33]

6.3.5 Polysiloxanes

Siloxanes are a wide variety of industrial polymers which predominantly have −Si–O–basic backbone. They differ in the degree and type of polymerization and the amount and length of alkyl groups in the chemical structure and are used for numerous industrial applications [36, 37]. In MOSH and MOAH analysis, polysiloxanes can be released from silicon septa and labware caps, or from bleeding of GC columns. The intensity of polysiloxane signals from column bleeding depends on different factors including the stability of the columns, the temperature ramp and the ageing of the column. Signals from column bleeding (**Figure 18A**), may cause an overestimation of the MOSH and MOAH fractions and must be excluded from the analysis by subtracting signals from corresponding blanks [22]. Polysiloxane contamination from septa and vial caps shows a different elution pattern from that of column bleeding. **Figure 18B** shows the elution pattern of polysiloxanes migrated from vial caps to an LC-GC×GC blank solution. This type of contamination can be identified by the presence of vertical bands along ^{1}D. Contamination from labware polysiloxanes (silicones) must be prevented during sample handling and extraction, e.g. by using silicone-free vial caps. In a worst-case scenario, where this sort of contamination cannot be prevented by optimization of experimental protocols, the signals from silicone oligomers could be subtracted after manual or automatically integration (*e.g.* "*trimming*" of riding signals). Nevertheless, the subtraction of the signals using those of a corresponding contaminated blank, requires the assurance of having the exact same amount of contaminants (peak volumes) in the blank and in the sample. The use of "*trimming*" strategies must be carefully defined and described, since the outcome of the degree of elimination of peak areas during chromatogram processing may differ depending on the software used.

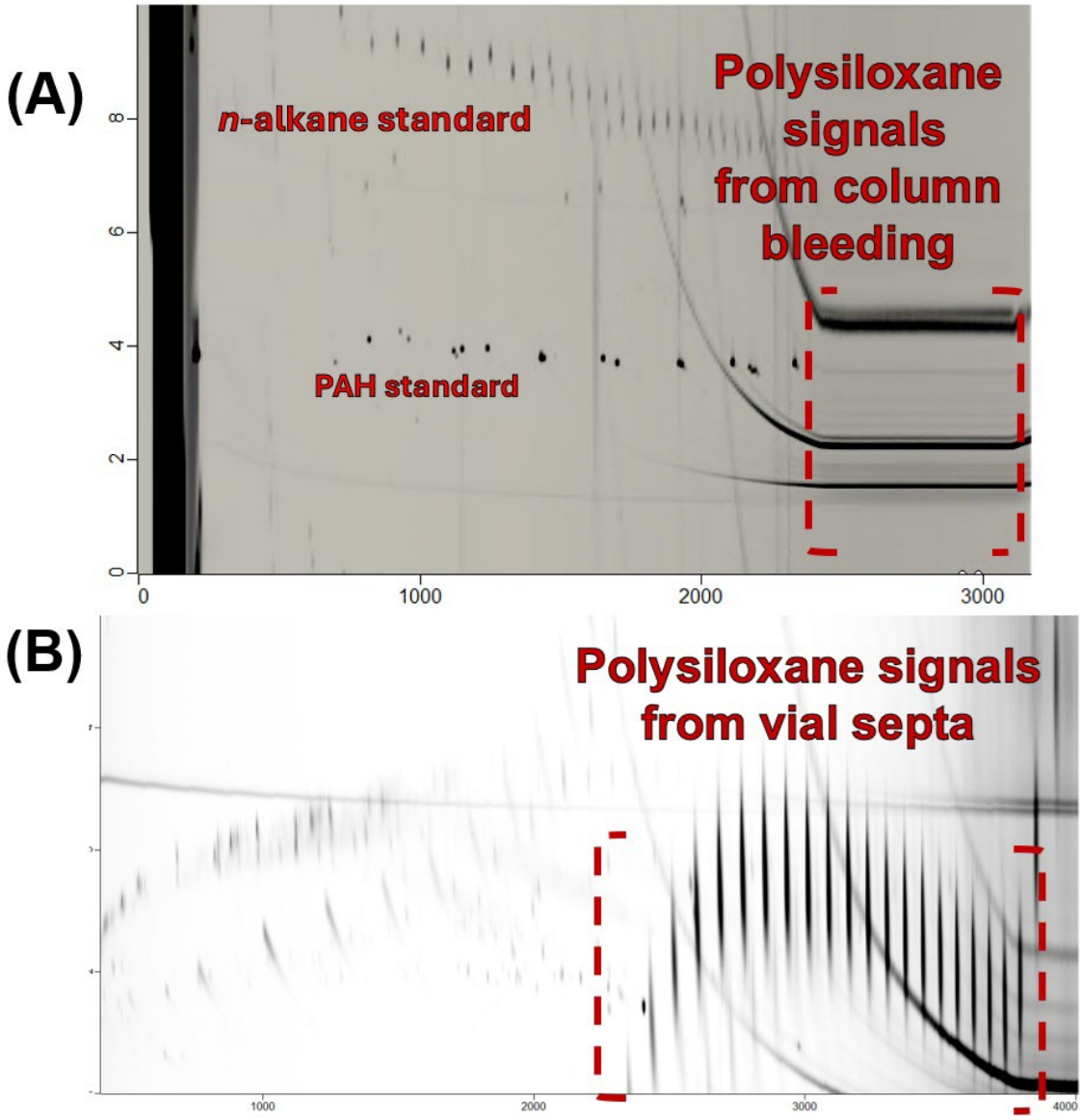


Figure 18. *Characteristic GC×GC showing the characteristic signals of polysiloxanes from column bleeding* ***(A)*** [38] *and contamination from vial septa* ***(B)*** [24].

6.4 Elution of compounds in the MOAH fraction

MOAH consist of molecules containing one to seven fused aromatic rings (with the possibility of having some rings that are partially saturated) (**Figure 1**) and that are alkylated to a various extent. **Figure 19** shows the chemical structure of representative MOAH from different subclasses. The number of aromatic rings and the alkylation degree influence the retention time of MOAH compounds. Weakly alkylated MOAH will be found at low ^{2}D retention times (more polar), while highly alkylated are located at higher ^{2}D retention times (less polar).

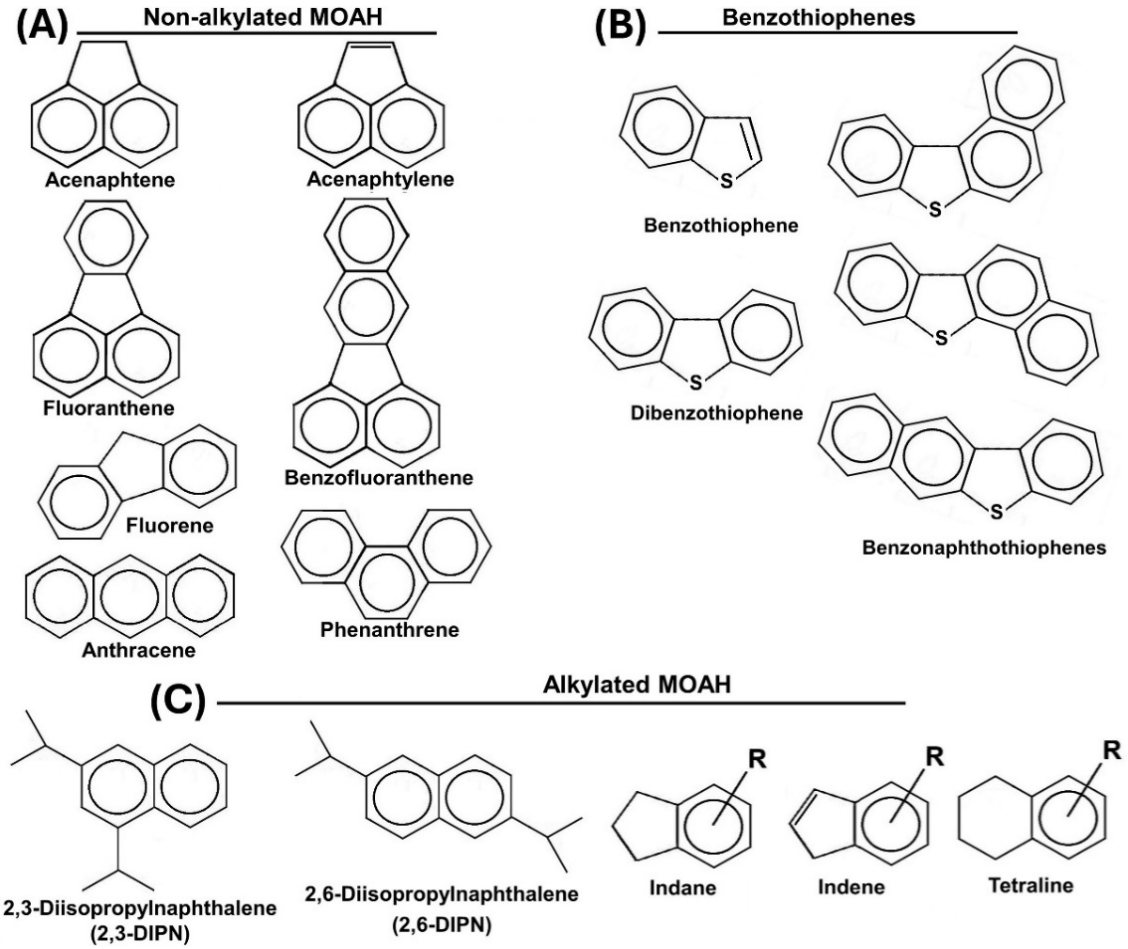


Figure 19. *Chemical structure of representative mineral oil aromatic hydrocarbons (MOAH) subclasses.*

6.4.1 Classification of MOAH by number of aromatic rings

When using a mid-polar×non-polar column configuration, non-alkylated 1- and 2-ring MOAH compounds elute at low retention time in both ^{1}D and ^{2}D while ≥3-ring MOAH elute at intermediate to late retention times in ^{1}D and low retention times in the ^{2}D. Therefore, non-alkylated 1- and 2-ring MOAH appear at the left corner of the two-dimensional chromatogram and ≥3-ring MOAH show a characteristic pattern in lower right region of the MOAH hump (**Figure 7**).

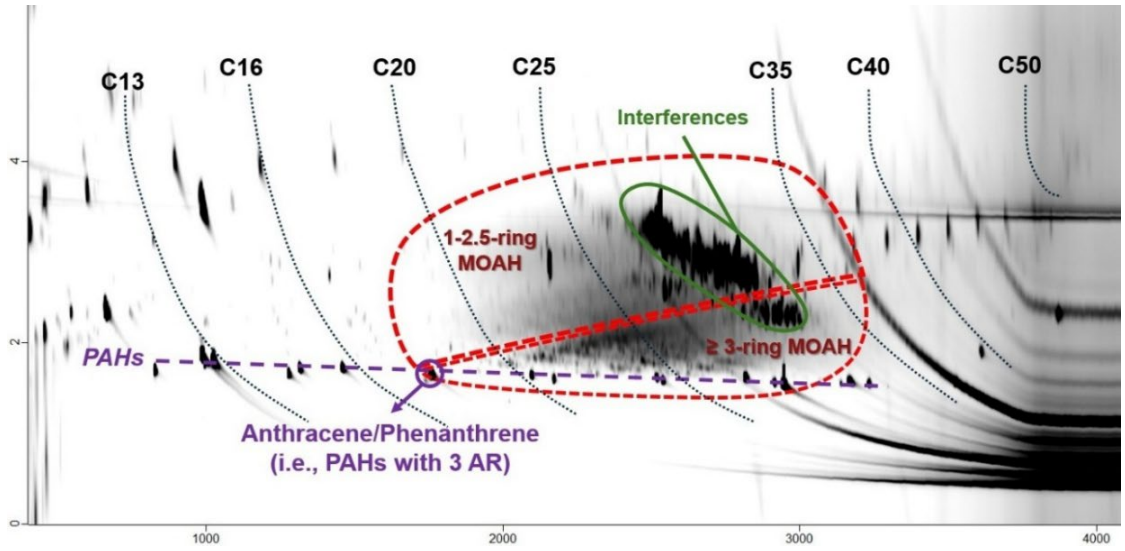


Figure 20*. Identification of MOAH subfractions based on number of aromatic rings. Characteristic MOAH hump showing the cut for identification of aromatic compounds with ≥3-ring MOAH.* ***Source****:* [24].

The elution zone of ≥3-ring MOAH is typically defined by retention time of anthracene/phenanthrene and other standard compounds such polycyclic aromatic hydrocarbon (PAHs) (**Figure 20**). The inclusion of standard compounds such as 1,4-didodecylbenzene; (2-(*tert*-butyl)anthracene; 4,4'-bis(trans-4-propylcyclohexyl)biphenyl; 9-methylanthracene; 9,10-diphenylanthracene, and 9-(2-naphthyl) anthracene has been also suggested for a better estimation of the border line of ≥3-ring MOAH [29].

Alkylated 1- and 2-ring MOAH have higher retention times in ^{1}D compared to non-alkylated ones and show also higher retention times in ^{2}D compared to ≥3-ring MOAH, tending to appear at the upper right region of the MOAH hump (**Figure 21**). MOAH with the same number of rings elute forming characteristic bands of homologue series. The presence of a five-carbon ring or a six-carbon cycloalkane is considered as 0.5 increase in the number of total aromatic ring count of the MOAH compound, and it influences retention times of MOAH. Acenaphthene, acenaphtylene and fluorene (**Figure 19**) are 2.5-ring MOAH and their retention times tend to be intermediate between that of 2-ring and 3-ring MOAH compounds in GC×GC (**Figure 7**) [18, 21]. A similar trend applies for fluoranthene and benzofluoranthene, compounds with 3.5 and 4.5 aromatic rings, respectively.

Sample pre-treatment has an effect over the composition of MOAH fractions; therefore, any sample pre-treatment step should be carefully considered while interpreting and reporting GC×GC results. Epoxidation of samples during MOH determination may cause a reduction in the amount of MOAH compounds [21, 26, 39], particularly of ≥3-ring MOAH and thiophenes [21]. Sample pre-treatment should only be used when strictly necessary [27]. Also, it is strongly recommended to evaluate the MOAH fractions by GC×GC before and after application of sample pre-treatment protocols to properly identify the presence of interferences and to accurately identify the presence of ≥3-ring MOAH.

6.4.2 Determination of the degree of alkylation in MOAH

The degree of alkylation of a MOAH compound increases its retention time in ^{1}D [40] and ^{2}D since a higher alkylation reduces polarity of the MOAH [21]. Therefore, MOAH compounds showing the same aromatic core and differing from the degree of alkylation form characteristic diagonal bands from bottom-left to middle-right in the GC×GC chromatograms using mid-polar×non-polar configuration (**Figure 6**). The degree of alkylation can be determined by the fragmentation pattern observed in GC×GC-MS, however, an estimation of alkylation degree can be done using GC×GC-FID as described by Biedermann and colleagues (2022) [21]. When using a GC×GC-FID with mid-polar×non-polar configuration, the alkylation degree of a compound can be estimated based on its retention time, using as coordinates the intersection of the homologue series to which the MOAH compound belongs (homologue series by number of aromatic rings) and the projection of the elution pattern of *n*-alkane standards (**Figure 21**).

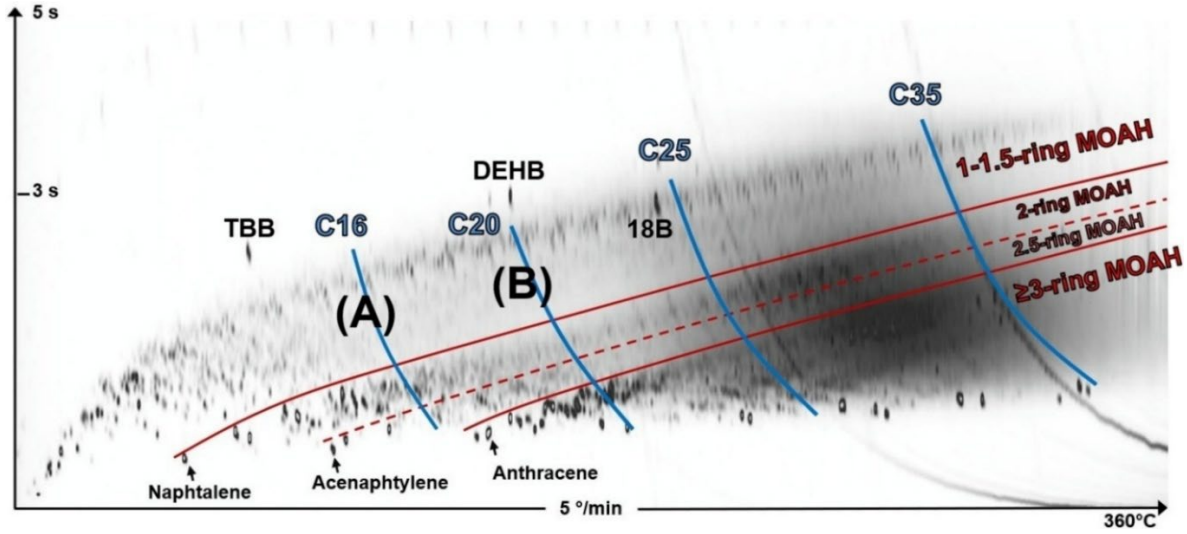


Figure 21. *GC×GC chromatogram showing the elution range of alkylated MOAH based on number of aromatic rings and indicating the intersection of the 1-ring MOAH homologue series with the elution pattern of the n-C16 (expected elution region of a C10-alkylated 1-ring MOAH)* ***(A)*** *and n-C20 (expected elution region of a C14-alkylated 1-ring MOAH)* ***(B)****. Red lines indicated the borders between the elution zones of MOAH with different number of aromatic rings. Chromatogram kindly provided by M. Biedermann, further details in* [21].

Figure 21 shows the intersection of the 1-ring MOAH homologue series with the elution pattern of the *n*-alkanes C16 **(A)** and C20 **(B).** For monocyclic MOAH, the intersection with C16 allows to estimate an alkyl substitution of 10 carbons, while the intersection with C20 allows to estimate a substitution of 14 carbons [21]. Since the volatility and polarity of compounds varies between homologue series, the variation in retention time based on the degree of alkylation is not linear. **Table 1** shows the estimation of number of carbons in the alkyl-substitution of a MOAH compound (numbers in parenthesis correspond to ranges in carbon number) based on the elution pattern of the specific homologue series in GC×GC.

Table 1. *Matrix for estimated number of carbons in the alkyl-substitution of a MOAH (Numbers in parenthesis correspond to ranges in carbon number) based on their elution pattern in GC×GC.* ***Source:*** *Adapted from:* [21].

Homologue series (Number of aromatic rings)	***n*-alkane co-elution range**				
	C10	**C16**	**C20**	**C25**	**C35**
1	4	10	14	19 (19-20)	29 (29-30)
2	0	4 (4 to 5)	9 (9-10)	15 (14-16)	25 (24-26)
Benzothiophenes	0	5 (4 to 5)	9 (9-10)	15 (14-15)	25 (25-26)
2.5	0	1	5 (5-6)	11 (10-12)	22 (21-23)
3	-	0	3	9 (8-10)	20 (19-22)
Dibenzothiophenes	-	0	4 (3-4)	9 (9-10)	19 (18-20)
3.5 / 4	-	-	0	5	16 (15-28)
4	-	-	0	2	14 (13-15)
4.5 / 5	-	-	-	0	9 (8-11)
5	-	-	-	0	5 (4-6)
5.5 / 6	-	-	-	0	3 (3-4)
6	-	-	-	-	0
7	-	-	-	-	0

6.4.3 DIPN

Among alkylated naphtalenes, diisopropylnaphthalenes (DIPN) (**Figure 19**) are relevant compounds that can be used as markers of MOH contamination [25]. Using MS detection, the presence of DIPN can be confirmed by the presence of the characteristic signals: *m/z 155, 197 and 212* [41]. **Figure 22** shows the characteristic elution pattern of DIPN.

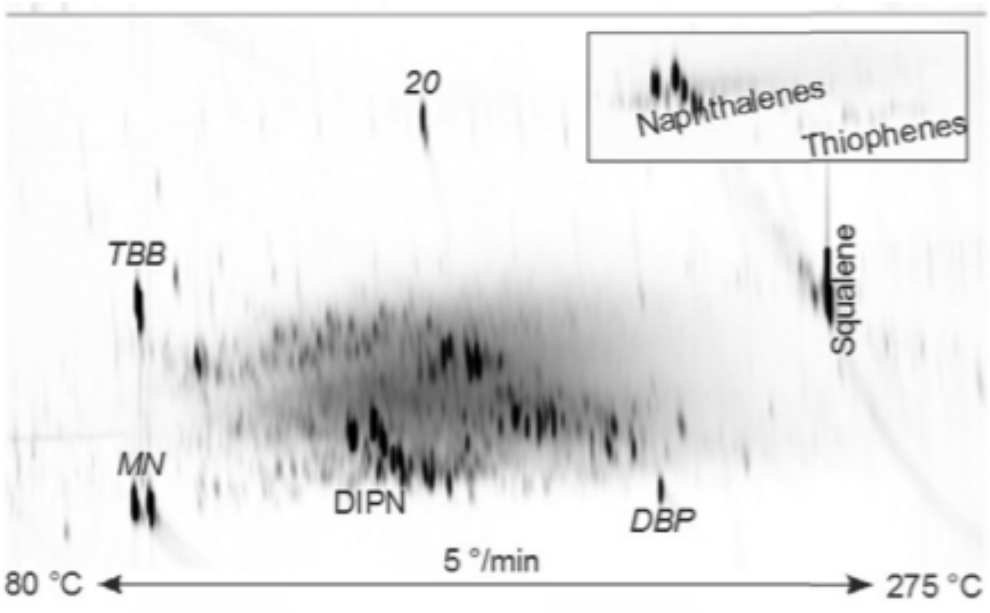


Figure 22. *GC×GC chromatogram showing the elution pattern of DIPN. Figure used with permission of the author.* ***Source****:* [15]

6.4.4 Effect of Hydrogenation on MOAH compounds

Hydrogenation process applied during petroleum refining influences the identity and elution pattern of MOAH. Hydrogenation generates partially saturated MOAH with a mixture of aromatic and saturated rings in the structure that elute as unresolved clouds in GC×GC (**Figure 23**) [15, 40]. Partially saturated MOAH should be included as total MOAH.

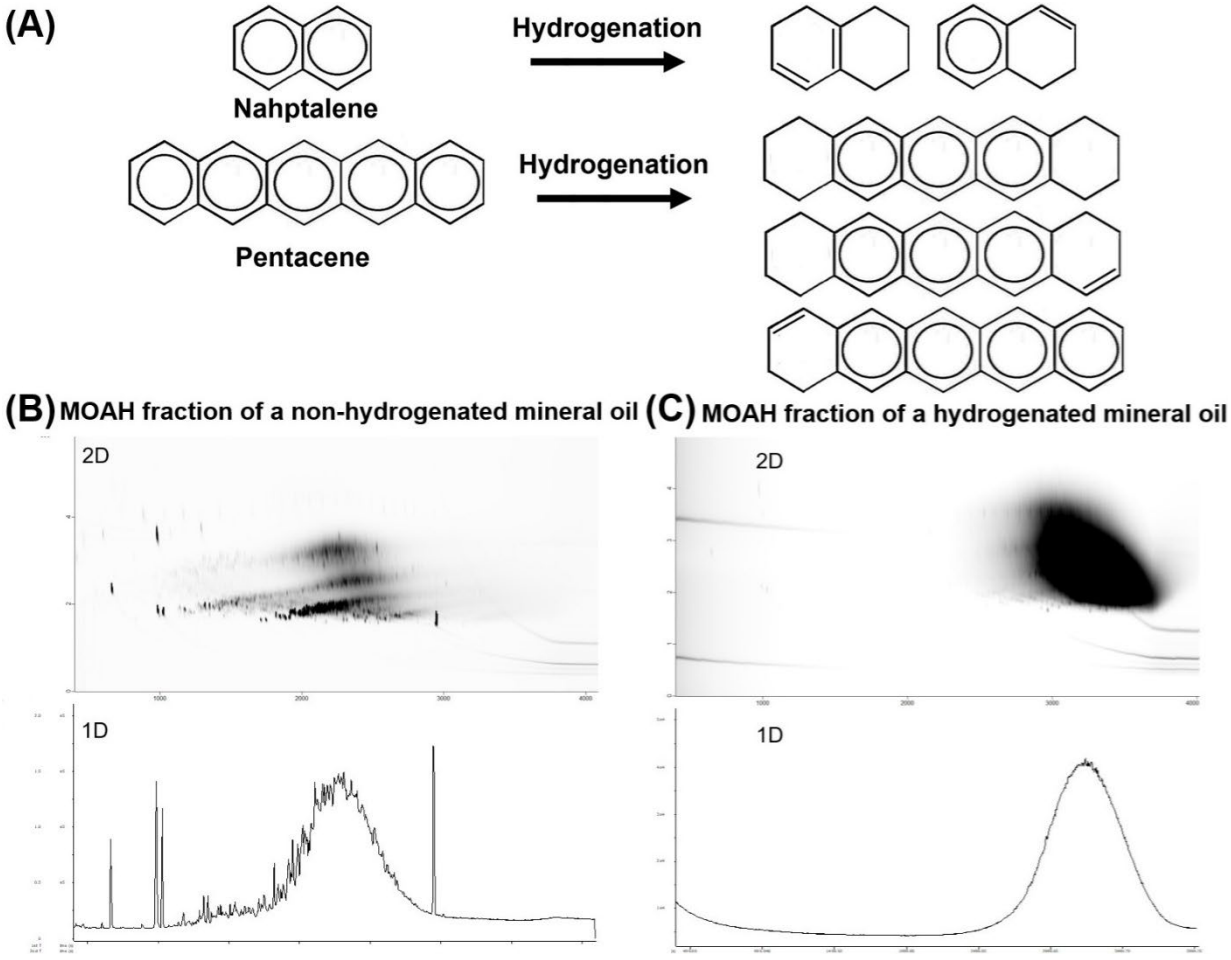


Figure 23. *Chemical structure of partially hydrogenated MOAH compounds derived from naphthalene and pentacene* ***(A)*** [42]*, and representative GC×GC (2D) and corresponding reconstructed 1D chromatograms of a non-hydrogenated MOAH contamination* ***(B)*** *and a hydrogenated MOAH contamination* ***(C)*** [24].

6.4.5 Thiophenes

Polycyclic aromatic sulphur-containing compounds such benzothiophenes, dibenzothiophenes, benzonaphthothiophenes and their alkylated homologues (**Figure 19**) are characteristic of crude mineral oils and can be used as markers of MOH contamination [43]. Similar to MOH, sulphur containing compounds have an FID response proportional to the carbon number [44]. However, since the presence of atoms different than carbon reduces the detector response [45], and having that the presence of sulphur-containing compounds in crude oils is quite low compared to MOH [46], the intensity of the signal of sulphur containing aromatic compounds such as thiophens tend to be low compared to MOAH. In addition, epoxidation reduces significantly the content of sulphur-containing compounds [21], almost eliminating, for example, the presence of dibenzothiophenes in food samples. Thiophens cannot be distinguished from the rest of the MOAH when using FID detection, and GC×GC-MS is then required for their tentative identification. **Figure 24** shows the characteristic elution pattern of dibenzothiophenes (**Figure 19**) in relation to 3-ring MOAH (anthracenes/phenantrenes).

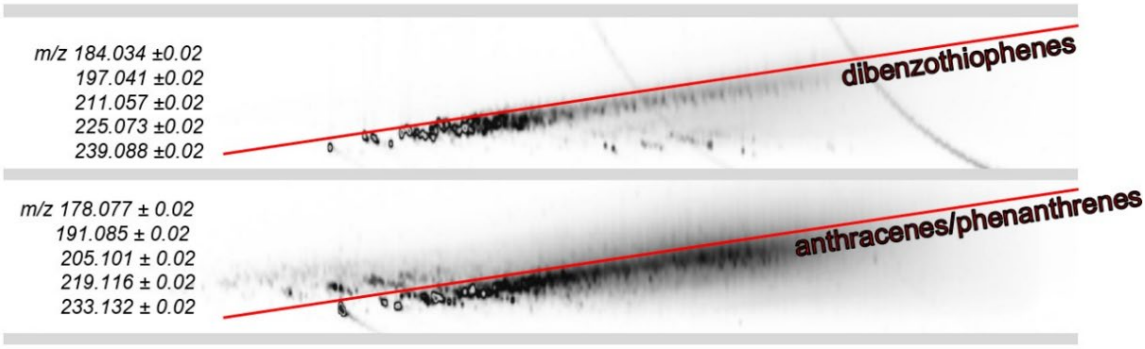


Figure 24. *Detail of GC×GC-MS chromatogram showing the elution pattern of dibenzothiophenes and in relation to anthracenes and phenanthrenes. Depicted red line refers to the border of the elution of ≥3-ringMOAH as indicated in* ***Figure 21****. Chromatogram kindly provided by M. Biedermann, further details in* [21].

6.5 Compounds co-eluting with MOAH fraction

Terpenic olefins, abietic acid and its derivatives, polysiloxanes, and resin aromatic oligomeric hydrocarbons (ROAH) (**Figure 25**) are among the main compounds co-eluting with MOAH.

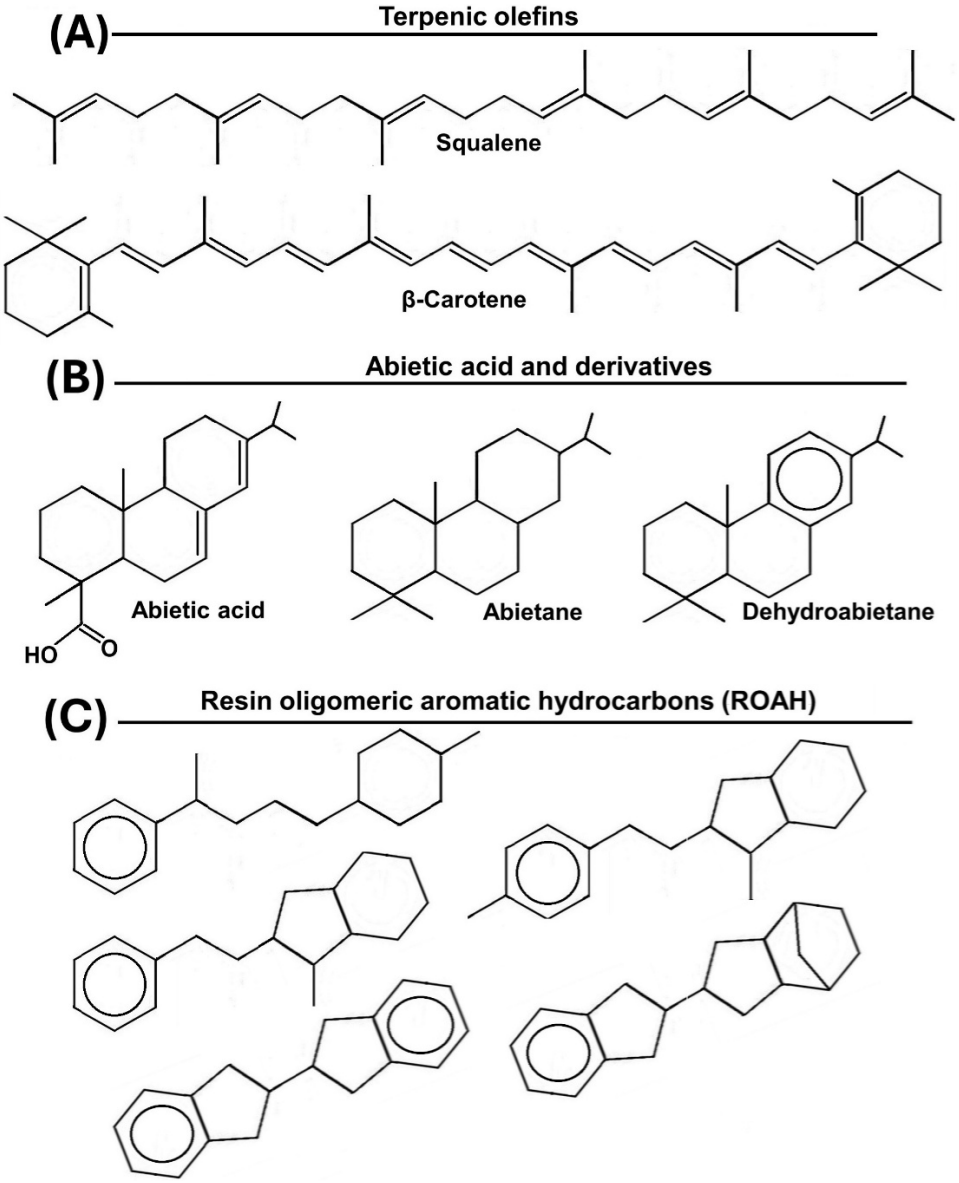


Figure 25. *Chemical structure of representative compounds co-extracted with MOAH.* ***Source****:* [32, 33]

6.5.1 Terpenic olefins

Terpenic olefins are a diverse family of terpenoid compounds with double bounds in their chemical structure, naturally occurring and characteristic of food matrixes. Terpenic olefins include compounds such monoterpenes, diterpenes, sesquiterpenes, carotenes, squalene and sterenes [18, 47]. Small molecules such as terpenes and mono unsaturated terpenes co-elute with the MOSH fraction, while polyunsaturated terpenes such sterenes, squalene, and carotenes co-elute with the MOAH fraction (**Figure 26**) [26, 48] Terpenes can be reduced from extracts using epoxidation, but these treatments can eliminate also some of the MOAH [27]. Specific food types are characterized by specific terpenic fingerprint. For example, within vegetables oils, rapeseed samples show intense signals of several terpenic interferences (**Figure 26A**) while refined palm oil samples show intense squalene and carotene signals (**Figure 26B**) interfering with MOAH characterisation.

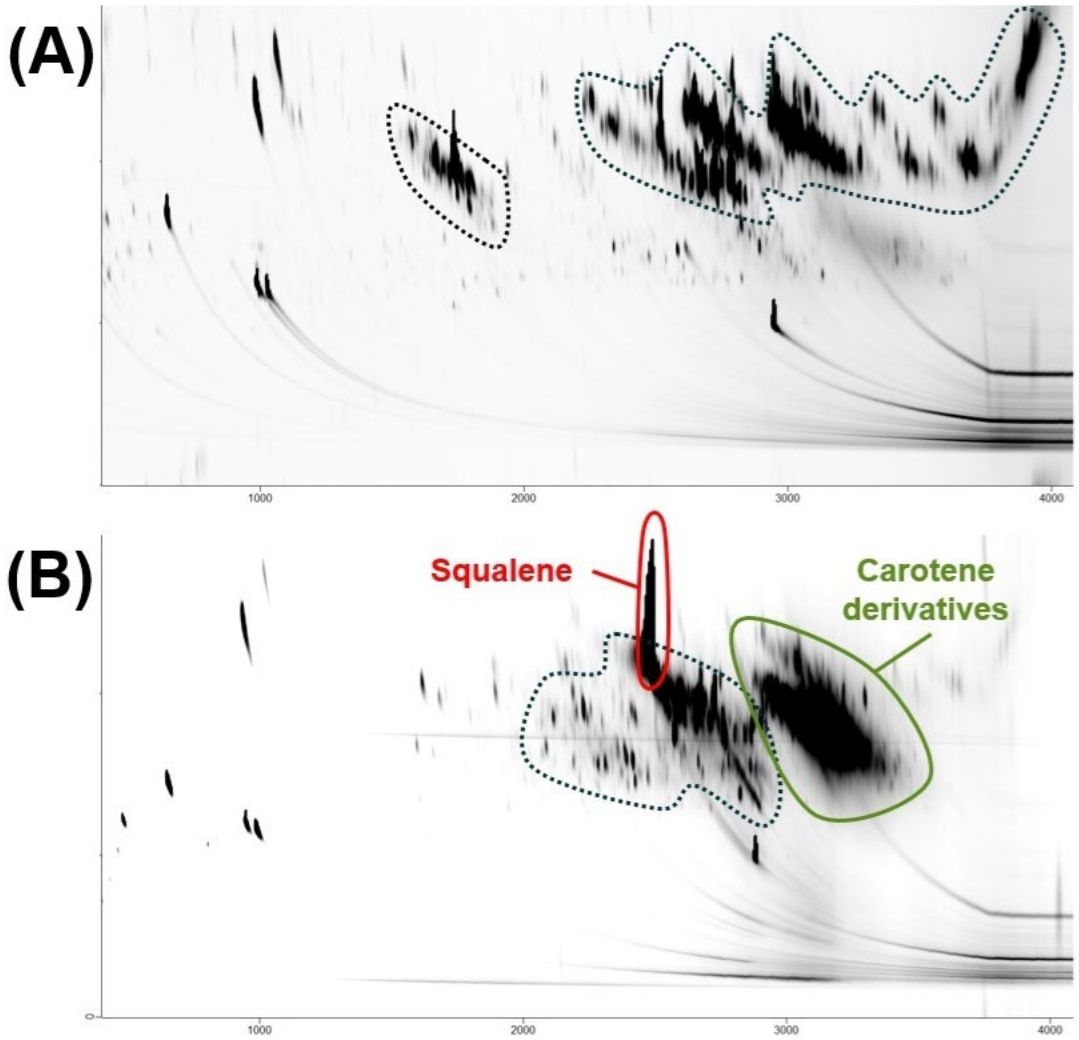


Figure 26. *GC×GC chromatograms showing interferences in rapeseed oil* ***(A)*** *and refined palm oil* ***(B)*** *during characterisation of MOH. Rapeseed sample shows signal of un-defined terpenic interferences, while palm oil shows characteristic signals for squalene (red circle) and carotene derivatives (green circle)* ***Source:*** [24].

6.5.2 Abietic acid derivatives

Abietic acid and its derivatives - such as abietane, dehydroabietane (**Figure 25**), and other diterpenoid hydrocarbons - originate from pine resin components like colophony (rosin) and tall oil, commonly used in paper sizing, adhesives, and printing inks. In GC×GC, abietic acid derivatives appear in the chromatograms of the MOAH fraction, forming a small hump around *n*-C17 to *n*-C19. Their presence can indicate contamination from wood-derived materials, particularly recycled paperboard or packaging containing rosin-based additives.

6.5.3 Polysiloxanes

As described in **section 6.3.5**, polysiloxanes from column bleeding, septa and labware caps, are interferences and should be excluded from analysis by subtraction of corresponding signals from the corresponding blanks (**Figure 18**).

6.5.4 Resin oligomeric aromatic hydrocarbons

Resin oligomeric aromatic hydrocarbons (ROAH) (**Figure 25**) are synthetic aromatic compounds that can migrate to foods from non-hydrogenated and partially hydrogenated hot-melt adhesives, which chemical structure allows them to be extracted together with the MOAH fraction. ROAH can be found in the bottom of mid-polar×non-polar GC×GC and their content is reduced in hydrogenated samples since full hydrogenation eliminates aromatic resin hydrocarbons (**Figure 27**) [33]. ROAH are not considered MOAH and should be excluded from analysis, however the presence of ROAH indicates the possible migration of aromatic compounds from resins used in sealing of cardboard FCM.

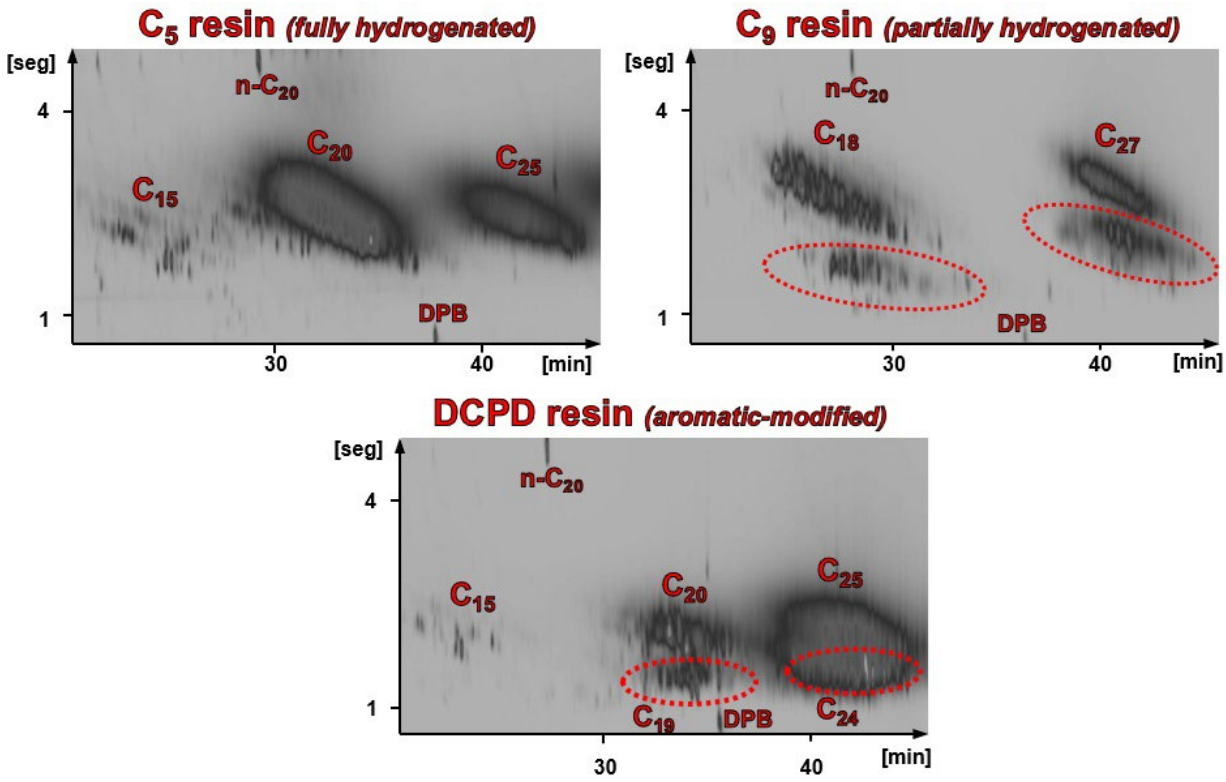


Figure 27. *Representative GC×GC chromatograms showing the elution pattern of aromatic resin hydrocarbons in three resins with different degrees of hydrogenation. Chromatograms kindly provided by M. Biedermann, further details in* [33].

6.5.5 Naphthenic hydrocarbons (MOSH)

Aliphatic naphthenic hydrocarbons (polycyclic cycloalkanes) with a high number of rings like hopanes and steranes (**Figure 8**), and which are part of MOSH, could show similar retention times to MOAH compounds with high alkylation degree in GC×GC (**Figure 6**), when MOSH and MOAH fractions are not pre-separated [9]. To avoid these interferences, it is recommended to evaluate MOH contamination only after fractioning of respective MOSH and MOAH fractions [49].

7 Verification of GC×GC instrumental method performance and results

Method performance can be evaluated by inspection of the chromatographic behaviour of internal standard mixes. Commercial standards of MOH including *n*-C11, *n*-C13, CyCy, Cho, 5B, 1-MN, TBB, and

Per (See **Figure 7**) are used in GC×GC as described for LC-GC-FID. In the case of GC×GC-FID, the relative signal intensity between the different compounds can be used as a quality assessment of the instrumental method performance. The ratio of the signal intensity between the area of the peak of the different internal standards (Per/TBB, 2-MN/1-MN, and 5B/TBB for MOAH; and CyCy/C13 and CyCy/Cho for MOSH) should be aligned with the ratio of their concentrations in the standard mix [6]. Retention time reproducibility for internal standards is also a method performance parameter to be evaluated. In the case of GC×GC-MS, signal intensity and retention time reproducibility from successive injections of internal standards can be used as performance parameters.

Routine analysis of chromatographic blanks is required since non-polar solvents used for extraction and separation of MOSH and MOAH fractions may contain interferences or MOH to varying degrees. The inclusion of several solvent blanks within a single GC×GC batch/sequence is strongly recommended to evaluate also the effect of successive sample injections in column performance and/or bleeding. For the qualitative analysis, a detection capability allowing the identification of the different families of compounds is required. Conditions to achieve sufficient detection can be defined by using MOH mixes commercially available or developed in-house [21]. In the absence of suitable reference materials due to the given diversity of samples, a standard should be analysed to verify that the instrument has the proper settings for measuring in the appropriate concentration range for each sample.

The concentration range for determination of MOH in GC×GC varies depending on the type of sample (food matrix), as it happens with the LOQ when using LC-GC-FID. The detection capabilities for MOH by GC×GC are strongly dependent on the food matrix, as consequence of the presence/absence of naturally occurring interfering compounds, and the possible incorporation of interferences from the environment and/or food packaging.

Due to the complex nature of MOH composition and the extraordinary variability of chemical compounds present in MOSH and MOAH, it is unfeasible to determine specific LOD for specific compounds. It is also not possible to determine LOD for the MOSH and MOAH humps, as done for LC-GC-FID. Nevertheless, for harmonisation of the qualitative characterisation of MOH by GC×GC, it is necessary to report an estimation of performance that would permit the assessment and interpretation of results. Therefore, for reporting GC×GC results, instead of the LOD, the specified amount of the standard is stated in the documentation. This helps to ensure that the absence of substances in the samples is not detected as a false negative due to too small amount during injection without having to determine an LOD for each individual substance. For instance, each laboratory must calculate the total injected amount based on the sample preparation and sample matrix for a defined analysis.

Alternatively, by measuring a standard mixture with a low concentration close to the detection capability of the GC×GC equipment, the absolute content required for one injection to achieve a positive result can also be calculated. This allows a laboratory to check whether the sample preparation for LC-GC-FID is sufficient for analysis of the sample directly by GC×GC, or whether concentration of the fractions is necessary before their injection into the GC×GC system.

GC×GC is generally more sensitive than LC-GC-FID. Nevertheless, if extensively lower oven temperature ramps are applied in GC×GC, the increase of sensitivity can be hampered. When working in GC×GC, the objective is typically to maximize the resolution between signals in the two-dimensional space that can be achieved by reducing the rate of temperature increase during the oven ramp (°C/min) compared to the conditions used for LC-GC. However, the increase in resolution causes that MOH humps are distributed over a longer retention time window in the GC×GC chromatogram, with the consequence that, despite the theoretical gain in sensitivity provided by GC×GC, this increase might not be observed in practice because the lower temperature ramp leads to signal dispersion over time.

On a general base, the amount required for reaching the LOQ in LC-GC determinations (i.e., ~50 ng into the GC column) is sufficient to reach the required sensitivity for MOSH and MOAH characterisation by GC×GC. If the detection capability needs still to be improved, a pull of fractions from multiple collections from the LC concentrated to the required injection volume can be used as sample for GC×GC [49]. During concentration of samples, caution must be taken during the evaporation to avoid losing the most volatile compounds in MOH fractions.

Consideration must be taken during selection of keepers for this purpose [50, 51] If a sample is to be further characterised with GC×GC after preparation for LC-GC-FID, the use of e.g. bis(2-ethylhexyl) maleate keeper might interfere with GC×GC characterisation, since it is a non-volatile compound normally added at very high concentrations compared to MOH [51]. Other keeper agents such as isooctane or toluene can be used to evaporate the sample in order to retain the volatile components in the concentrated fraction without interfering with the GC×GC characterisation. However, these keepers should not be used for sample preparation if the same extract will be used for LC-GC determinations, since toluene, for example, can cause a shift in the MOH fractions on the LC-GC system.

Chromatogram processing strategies such as trimming or hump smoothening should be used only when strictly necessary and for quantification purposes or for the elimination of clearly identified interferences such polysiloxanes from silicones. If possible, they should be applied in defined subregions where clear interferences are observed and not as global chromatogram processing techniques to avoid the loss of minor peaks.

8 Mass spectrometry signals for GC×GC-MS interpretation

8.1 GC×GC-MS signals (*m*/*z*) for MOSH identification

Characteristic GC×GC-MS signals (*m*/*z*) used for tentative identification of MOSH and frequent co-eluting compounds are presented in **Table 2**. Reported nominal *m*/*z* values are approximations and may vary in different systems.

Table 2. *Characteristic GC×GC-MS signals (m/z) for identification of MOSH compounds and co-eluting compounds.* ***Source****:* [33, 52].*(Values reported as (X/Y) refer to alternatives depending on the rounding strategy)*

Class	Compound		*m*/*z*
Linear and branched MOSH	Paraffins (*n*-alkanes and *iso*-alkanes)		43/44 + [43/44 + (14**n*)]
Cyclic MOSH	Monocycloparaffins		67 + 68 + 69 + 81 + 82 + 83 + 96 + 97
	Dicycloparaffins		123/124 + [123/124 + (14**n*)]
	Decalins		
		Decalin	138
		C1-Decalins	152
		C2- Decalins	166
		C3- Decalins	180
		C4- Decalins	194
	Tricycloparaffins		149/150 + [149/150 + (14**n*)]
	Hopanes		191
	Steranes		217/218
Frequent MOSH co-eluting compounds	POSH/POMH		85
	PAO		85
	ROSH		234 + 236 + 246 + 248 + 250 + 258 + 260 + 272 + 274 (+0 DBE*)

**DBE: Double bond equivalent.*

However, tentative identification of contamination MOSH markers based on characteristic *m*/*z* must be carefully evaluated, since food matrix-derived compounds can, for example, show the same ions characteristic of steranes (*m*/*z* 217) and hopanes (*m*/*z* 191) (**Figure 28**). Tentative identification of contamination markers must be always evaluated as a combination of retention time and characteristic *m*/*z* ions. **Figure 29** shows a representative chromatogram indicating the characteristic elution patterns of common co-eluting compounds in MOSH fractions.

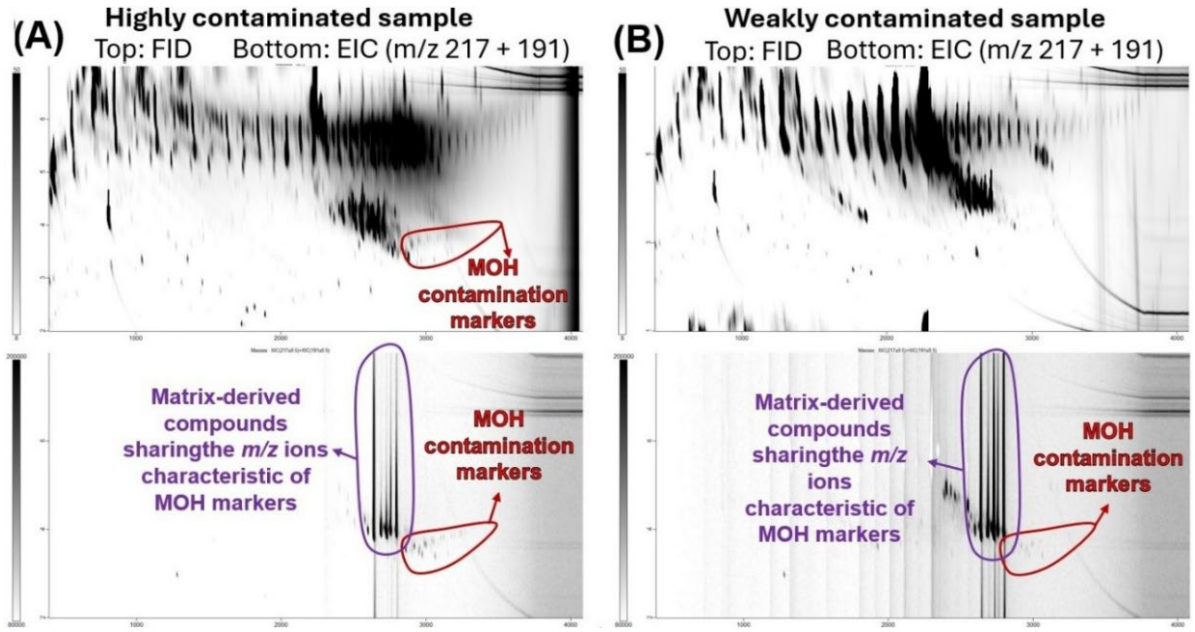


Figure 28**. GC×GC chromatograms of food additive samples (E471) showing naturally occurring food compounds sharing the ions m/z 217 and 191 characteristic of the MOH markers steranes and hopanes.* ***Source: [24].

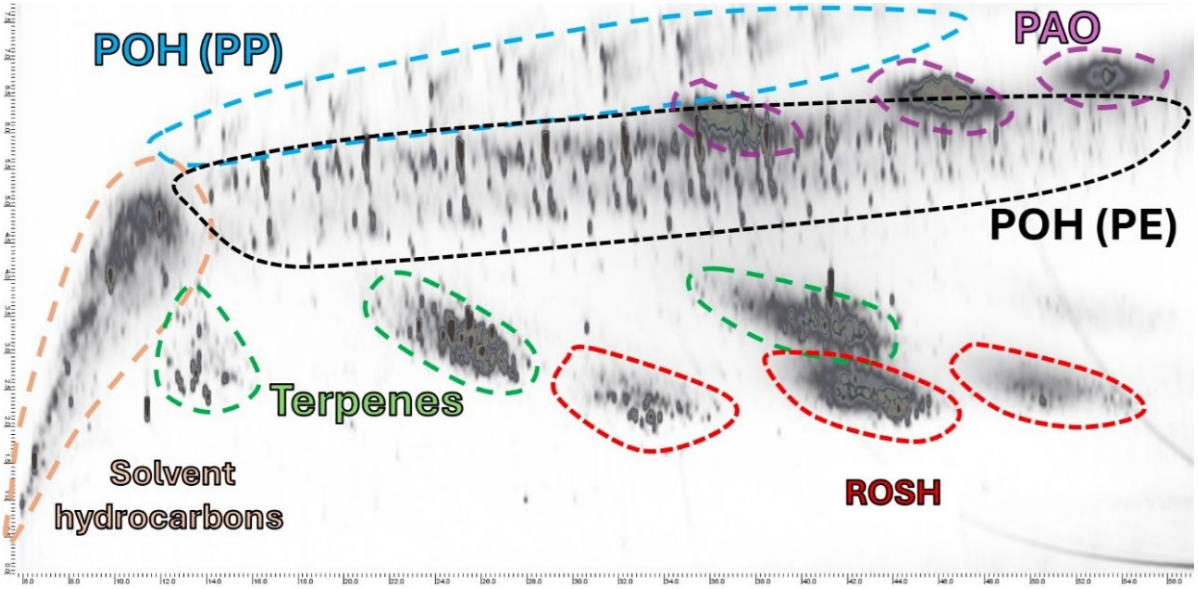


***Figure 29**. Elution pattern of co-eluting compounds in MOSH fractions: terpenes; polypropylene (PP) and polyethylene (PE) polyolefin oligomeric hydrocarbons (POH), polyalpha olefins (PAO), resin oligomeric saturated hydrocarbons (ROSH) and solvent-derived hydrocarbons. Chromatogram kindly provided by M. Lommatzsch, further details in* [15, 33, 53].

8.2 GC×GC-MS signals (*m*/*z*) for MOAH identification

Characteristic GC×GC-MS signals (*m*/*z*) used for tentative identification of MOAH and MOAH interferences are shown in **Table 3**. Reported nominal *m*/*z* values are approximations and may vary in different systems.

Table 3. *Characteristic GC×GC-MS signals (m/z) for identification of MOAH compounds and frequent interferences.* ***Source****:* [33, 52, 54, 55] *(Values reported as (X/Y) refer to alternatives depending on the rounding strategy).*

Class	Compound		m/z
MOAH	Alkylbenzenes		91/92 + [91/92 + (14*n)]
	Indanes and tetralines		103/104 + [103/104 + (14*n)]
	Indenes		115/116 + [115/116 + (14*n)]
	Naphtalenes		141/142 + [141/142 + (14*n)]
		C1-naphtalenes	142
		C2-naphtalenes	156
		C3-naphtalenes	170
		C4-naphtalenes	184
	Acenaphtenes		153/154 + [153/154 + (14*n)]
	Acenapthylenes		151/152 + [153/154 + (14*n)]
	Fluorene		166
		C1-fluorenes	180
		C2-fluorenes	194
		C3- fluorenes	208
	Diisopropylnaphtalene (DIPN)		155 + 197 + 212
	Alkylated mono-/diaromatic MOAH		119 + 128 + 142 + 156 +170 + 184 + 198, 226 +240
	Alkylated tri-aromatic MOAH		178, 192, 206, 220, 234
	Alkylated tetra-aromatic MOAH		202, 216, 230, 244, 258
Frequent MOAH co-eluting compounds	Abietic acid		41 + 43 + 91 + 285 + 302
	Squalene		81 + 93 + 95 + 107 + 121
	Carotenes	β-carotene	43 + 69 + 235 + 536
		α-carotene	41 + 69 + 105 + 144
		Lycopene	69 + 91 + 106 + 196
	ROAH	Monoaromatic	228 + 230 + 240 + 242 + 252 + 254 + 256 (+3 DBE*)
		Diaromatic	234 + 236 + 238 + 248 + 250 (+6 DBE*)

**DBE: Double bond equivalent.*

9 Tentative identification of contamination source

The fingerprint of the MOSH and MOAH humps obtained by GC×GC can be useful for the tentative identification of the contamination source. For example, batching oils linked to contamination of jute bags are characterised by a MOSH fraction with *n*-alkanes and other compounds with lower carbon length of those observed in motor oils, where the *n*-alkane fraction can be observed to be centered at higher retention times in both ^{1}D and ^{2}D. Differences in the distribution of *n*-alkanes due to the differences in the distillation fraction used for oil preparation can be also observed in the GC×GC profile of MOH in different mineral oils (**Figure 30**) [56]. Generation of local databases with potential MOH contaminating sources, the comparison of GC×GC fingerprints to reported data on MOH and the identification of contamination markers (see **Summary of contamination markers**) are strategies for the tentative identification of the MOH contamination source [15].

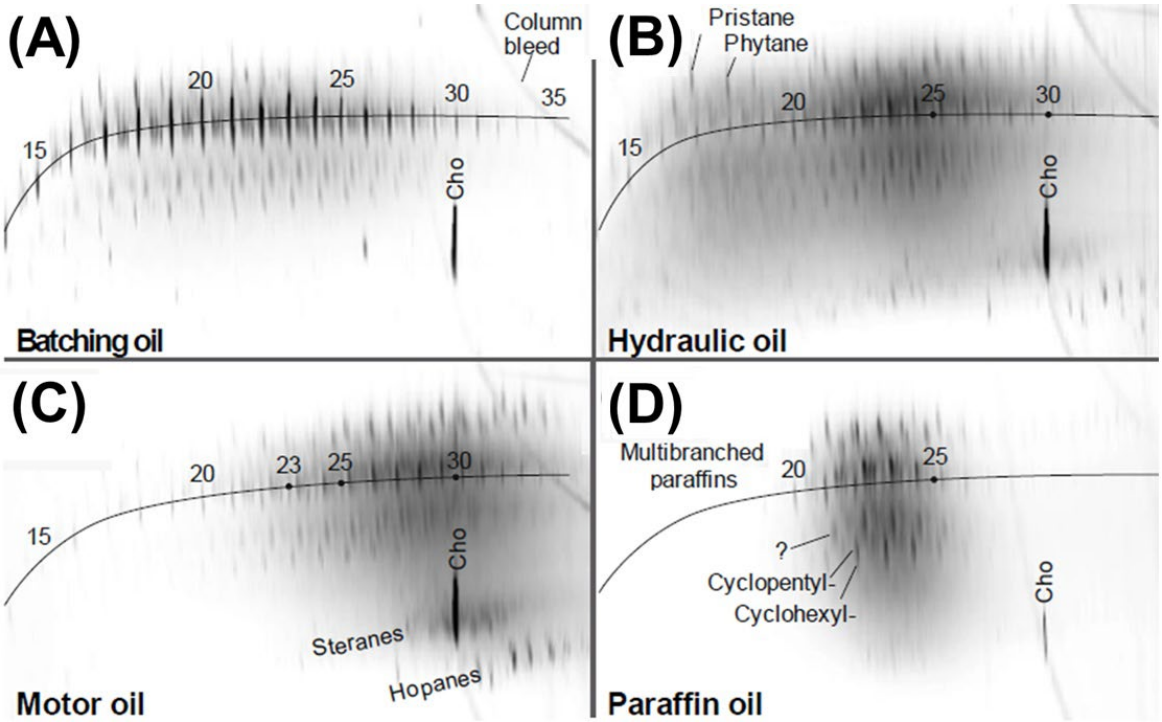


Figure 30. *GC×GC chromatograms showing characteristic fingerprints of MOH from different type of industrial oils: Batching oil* ***(A)****, hydraulic oil* ***(B)****, batching oil* ***(C)*** *and paraffin oil* ***(D)****.* ***Source:*** [56] *based on the work of Bidermann et al. (2015)*[57].

10 Summary of contamination markers and co-eluting compounds

Table 4 shows a list of compounds and compound families recommended to be analysed when using GC×GC for the confirmation of MOH contamination.

Table 4. *Summary MOSH and MOAH markers for confirmation of in MOH contamination using GC×GC.* ***Source:*** [15].

Characteristic compound(s)	Indication
Linear *n*-alkane series with repeated units differing in one atom carbon without prevalence for odd (food matrix) or even carbon number (POH)	MOSH contamination
Presence of prystane and phytane	MOSH contamination
n-alkyl cyclopentanes / *n*-alkyl cyclohexanes	MOSH contamination
Diisopropyl naphtalenes (DIPN)	MOAH contamination from inks (recycled paper board)
Thiophenes	MOAH contamination Slightly or non-refined MOH
Diffuse signals forming a cloud in the region of the MOAH fraction	Hydrogenated MOAH fraction
Perhydropyrene	Hydrogenated MOH
Hopanes	MOSH contamination
Gray cloud, slanted bands of naphtalenes	Hydrogenated MOAH

Table 5 shows a list of compounds and compound families frequently found as interferences and co-eluting compounds during MOSH and MOAH analysis.

Table 5. *Summary of characteristic co-eluting compounds and interferences in MOSH and MOAH analysis.* ***Source:*** [15, 33].

Characteristic compound(s)		Indication
MOSH		
	Polyolefin hydrocarbons with repeated units differing in two atom carbons (ethylene units)	Presence of POH from plastic
	Cluster of peaks above *n*-alkane homologue series with repetitive carbon units	Presence of POH from plastic
	ROSH	Migration of hydrocarbons from hot-melt adhesives
MOAH		
	ROAH	Migration of hydrocarbons from hot-melt adhesives
	Carotenes and squalene	Interferences from food matrix
	Abietic acid	Contamination from wood-derived materials

11 Reporting of results

Harmonisation in reporting of results is recommended for accurate assessment of the risk related to MOH contamination. Recommendations for data reporting include general sample information as described in *"Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials"* [6], for example:

- Detailed sample description (food item, packaging material, origin).
- Details of sample pretreatment before MOH analysis (e.g. saponification, epoxidation, AlOx treatment).
- Total MOSH content (mg/kg) from LC-GC-FID quantification
- Total MOAH content (mg/kg) from LC-GC-FID quantification

A detailed description of MOH contamination markers (**Table 4**) and co-eluting compounds (**Table 5**) tentatively identified in the sample with reference to the absolute amount of substance injected to evaluate the threshold for the determination of the presence/absence for each specific compound/class of compounds is recommended for reporting of data from MOH analysis by GC×GC (**Table 6**). For each sample, it is strongly recommended to document, the specific ions used for tentative identification of contamination markers and co-eluting compounds together with the evidence of chromatograms used for confirmation of the respective retention times.

Table 6. *Recommended report of results from MOH analysis by GC×GC.*

General Sample Information		
Sample:	Sample pretreatment	
Total MOSH (mg/kg) from LC-GC-FID:	( )	Saponification
Total MOAH (mg/kg) from LC-GC-FID:	( )	Epoxidation
	( )	Alox
	( )	Other:
Observations:		
Results from GC×GC characterisation		
A) MOSH Fraction		
Total amount of sample used, volume of final extract, volume injected for GC×GC analysis =		
Tentative identification of MOSH contamination markers		
MOH *n*-alkanes	( )	
cycloalkanes	( )	
Prystane	( )	
Phytane	( )	
Hopanes	( )	
Steranes	( )	
Decalins	( )	
Prystane	( )	
Others (specify):	( )	
Tentative identification of MOSH co-eluting compound		
Matrix-derived olefins	( )	
Resin oligomeric saturated hydrocarbons (ROSH)	( )	
POH	( )	
Polyalpha-olefins (PAO)	( )	
Others (specify):	( )	
Detection capability for MOH by GC×GC		
Based on specified standard(s) or by other means =		
Print of GC×GC contour plot		
Indication of identified MOSH markers and co-eluting compounds and boundaries between compound subclasses within the GCxGC contour plot		
Compounds or compounds mixtures used to locate elution patterns and define the boundaries of subclasses =		

The table continues on the next page.

Table 6 continued. *Recommended report of results from MOH analysis by GC×GC.*

A) MOAH Fraction	
Total amount of sample used, volume of final extract, volume injected for GC×GC analysis =	
Tentative identification of MOAH contamination markers	
Alkylated-monoaromatics	( )
Alkylated-di-aromatics	( )
Alkylated-tri-aromatics	( )
Alkylated-tetra-aromatics	( )
Alkylated-polycyclic aromatics (4-7 rings)	( )
Thiophenes	( )
Diisopropyl naphtalenes (DIPN)	( )
Hydrogenated MOAH	( )
Others (Specify):	( )
Tentative identification of MOAH co-eluting compounds	
Matrix-derived terpenic olefins	( )
Resin oligomeric aromatic hydrocarbons (ROAH)	( )
Abietic acid	( )
Others (specify):	( )
Detection capability for MOH by GC×GC	
Based on specified standard(s) or by other means =	
Print of GC×GC contour plot	
Indication of identified MOSH markers and co-eluting compounds and boundaries between compound subclasses within the GCxGC contour plot	
Compounds or compounds mixtures used to locate elution patterns and define the boundaries of subclasses =	
Conclusion:	

12 List of abbreviations

^{1}D: First Dimension
1-MN: 1-Methyl naphthalene
^{2}D: Second Dimension
2-MN: 2-Methyl naphthalene
1-ring MOAH: Mineral oil aromatic hydrocarbons with one aromatic ring
2-ring MOAH: Mineral oil aromatic hydrocarbons with two aromatic rings
≥3-ring MOAH: Mineral oil aromatic hydrocarbons with three or more aromatic rings
5B: *n*-Pentyl benzene
Cho: 5-*α*-cholestane
CyCy: Cyclohexyl-cyclohexane
DIPN: Diisopropyl naphtalenes
DBE: Double bond equivalent
EFSA: European Food Safety Authority
EURL-PC: European Reference Laboratory for Processing Contaminants
FCM: Food contact material
FID: Flame ionisation detector
GC: Gas Chromatography
GC×GC: Comprehensive two-dimensional gas-chromatography
IL: Indicative level
ISTD: Internal standard
LC: Liquid chromatography
LC-GC-FID: Coupled liquid – gas chromatography with flame ionization detection
LOD: Limit of detection
LOQ: Limit of quantification
LVI: Large volume injection
ML: Maximum level
MOAH: Mineral oil aromatic hydrocarbons
MOH: Mineral oil hydrocarbons
MOSH: Mineral oil saturated hydrocarbons
m/z: Mass to charge ratio.
PAH: Polycyclic aromatic hydrocarbon
PAO: Polyalpha olefins
PASH: Polycyclic aromatic sulphur-containing hydrocarbons
Per: Perylene
POH: Polyolefin oligomeric hydrocarbons
POMH: Polyolefin oligomeric mono-unsaturated hydrocarbons
POSH: Polyolefin oligomeric saturated hydrocarbons
POSHoc: Polyolefin oligomeric saturated open chain hydrocarbons
POSHcy: Polyolefin oligomeric saturated cyclic hydrocarbons
RT: Retention time
ROAH: Resin oligomeric aromatic hydrocarbons
ROSH: Resin oligomeric saturated hydrocarbons
SPE: Solid phase extraction
SOP: Standard operating method
TBB: Tri-*tert*-butyl benzene
ToF-MS: Time-of-Flight Mass Spectroscopy

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