

# A Fully Automatic Workflow for the Determination of Mineral Oil Hydrocarbons in Food and Food Packaging

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## 1. Introduction

Mineral oil contamination is common nowadays and is often in the news, from unsafe sunflower oil in 2008 and the Foodwatch International report on hazardous infant formula in 2019 to the latest ÖKOTEST Report from 2023 about mineral oil findings in most of the traditional German butter brands, where 19 from 20 tested butter samples were heavily contaminated with mineral oils residues [1]



Figure 1: MOSH/MOAH contamination in butter

In 2012, the European Food Safety Authority (EFSA) published a scientific opinion on the health effects of mineral oil contamination in food.[2] That report identified food packaging, different packaging additives and lubricants as the main sources of contamination. Today, it's known that contamination can occur throughout the entire production cycle, not simply through packaging. A distinction is also made between actual food contamination and the permitted use of certain mineral oil products as food additives, e.g. microcrystalline waxes (E905). In either case, the presence of mineral oil hydrocarbons (MOH) in food means that they will be metabolized by the body during digestion. MOH are complex mixtures of hydrocarbons that originate in crude mineral oil. During laboratory analyses, an initial separation into fractions of Mineral Oil Saturated Hydrocarbons (MOSH) and Mineral Oil Aromatic Hydrocarbons (MOAH) is possible due to their chemical structure. MOSH consist of branched and unbranched open-chain hydrocarbons and saturated cyclic hydrocarbons. Although MOSH accumulate in the human body, they are not associated with major negative effects. MOAH, on the other hand, are made up of highly alkylated aromatic substances and are considered to be potentially mutagenic and carcinogenic due to the possible presence of 3-7 ring polycyclic aromatic compounds (PACs).

Type of Food sample	Concentration Level Food [mg/kg]
Edible oils	6000
Bread and baked goods	2800
Chocolate and cocoa	1300
Fish	1200

Table 1: MOSH/ MOAH concentrations in food and packaging [2]

The concentration of MOH in paper products is up to 1000 mg/Kg of which 70% can migrate into the packed product through direct contact or via the gas phase. An overview of MOSH/MOAH concentrations in food and food packaging reported by EFSA are listed in table 1 [2]. The analytical procedure is described in the International Standard ISO 20122:2024 Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis [3].



Figure 2: Shimadzu MOSH/MOAH Analyzer

## 2. MOSH/MOAH System Configuration

The system configuration consists of a Shimadzu Nexera system with 1 LC-40BXR pump, CBM-40A LITE system controller, SPD-40A UFLC UV Detector coupled with GC-2030 and 2 FID detectors. For online aluminum oxide purification 1 LC-40D pump is needed. The PAL autosampler and the user-friendly CHRONOS software (AXEL SEMRAU, Sprockhövel) in combination with LabSolutions software platform allow a high level of automation. An automated sample preparation and pre-separation is realized by normal phase HPLC. MOSH and MOAH fractions are separated from one another and simultaneously from the interfering components like paraffin or wax esters. Unfortunately, some food material like rice or chocolate contain natural occurring (odd numbered) alkanes in the range of C<sub>23</sub>-C<sub>33</sub> which can interfere and heavily disturb the analysis of the MOSH fraction. These interferences can be removed by flash chromatography on aluminium oxide columns (Alox-option).

## 3. Experimental Setup

The LC is directly connected to two high temperature GC columns with retention gaps which are installed in one GC oven. MOSH and MOAH fractions are separated on a silica gel column using a n-hexane /dichloromethane gradient. The interface between LC and GC is controlled by Axel Semrau LC-GC Chronect interface. After transferring the MOSH fraction on column 1 and MOAH on column 2 the temperature programme is started and both

fractions are separated simultaneously and detected by FID. This way, MOH can be separated into MOSH and MOAH, and their concentration can then be determined as a sum parameter. The separated fractions are directly transferred to the GC afterwards. The GC has two separated channels (two column sets and two FIDs) to determine MOSH and MOAH in parallel in a single run.

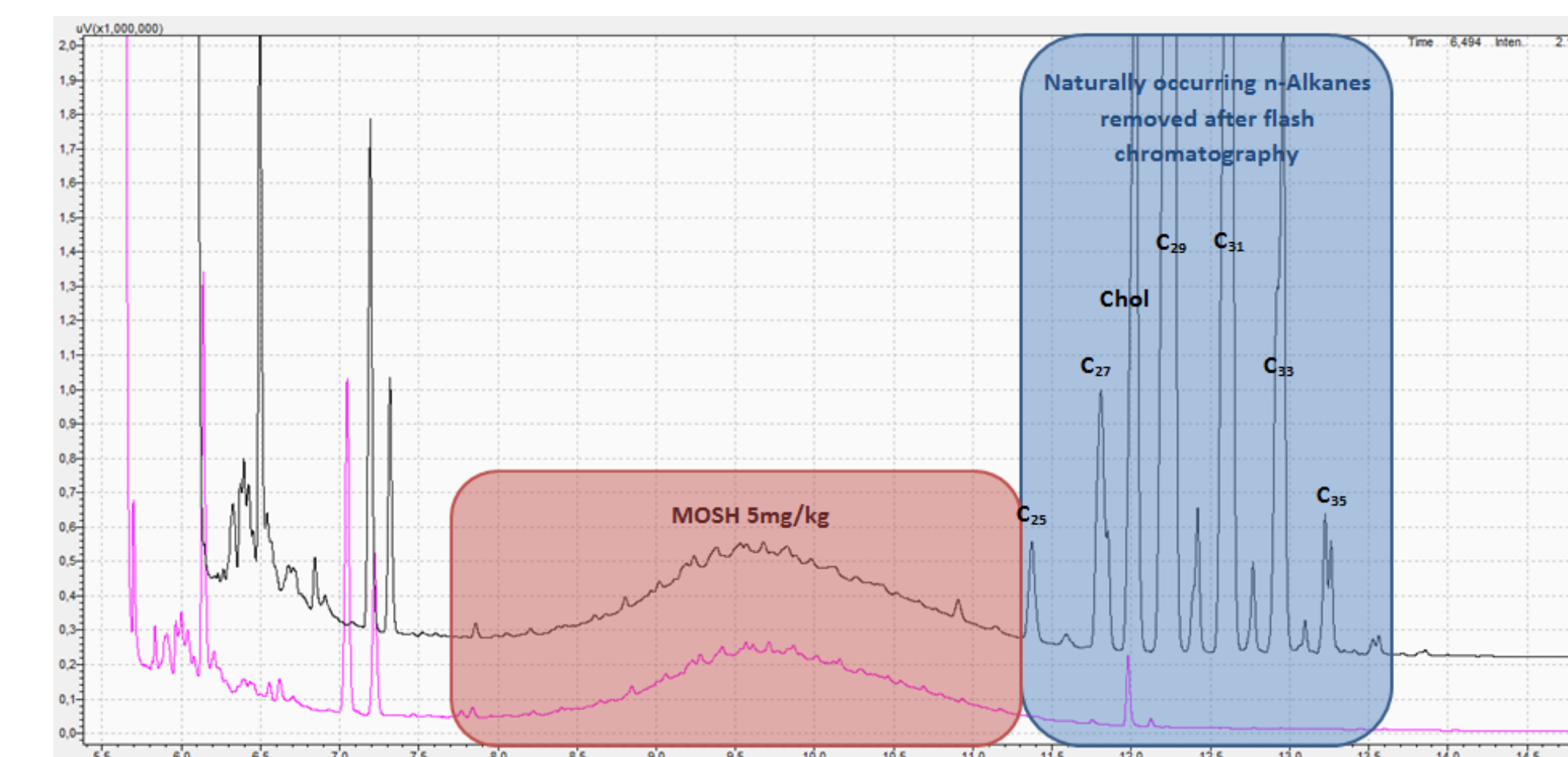


Figure 3: Chromatograms of a Rice sample before and after flash chromatography with Alox

## 4. Sample Preparation

Depending on the expected mineral oil concentration 1-10 g of homogenized and finely ground samples were used. The samples were extracted in Hexane, after the addition of an internal standard mixture (Restek MOSH/MOAH standard Cat.#:31070 containing 9 internal standards) at room temperature for 2 hours or overnight under occasionally shaking the flask.

The glass columns with inserted filters were filled with 10 g of aluminium oxide (90, basic, 0.063 mm - 0.2 mm, activated for 16h at 500 °C) and 3 g of silica gel (60, 60 µm-200 µm or 70mesh-230mesh respectively, activated for 16h at 40 °C). The columns were washed with 20 ml of n-Hexane prior to loading the sample. The extracts were centrifuged, if necessary, concentrated to a volume of about 1 ml under a stream of nitrogen. About 100 µL were taken and filled into an autosampler vial with micro insert for analysis of MOAH, the remaining samples was loaded onto the column. The MOSH fraction was eluted with 20 ml of n-Hexane, concentrated in an automated solvent concentrator and transferred into a 2 mL autosampler vial. This was placed in the autosampler rack of the LC-GC system. Aliquots of 50 µl were injected into the LC and 450 µL were transferred directly on the pre-columns for the MOSH fraction only.

## 5. Measurement

Figure 3 shows two chromatograms of a MOSH fraction of a rice sample spiked with 5 mg/kg of a mineral oil. Before flash chromatography with Alox n-Alkanes in the range of C<sub>25</sub>-C<sub>35</sub> are present. "False positive" integration would give a MOSH value of 10.8 mg/kg. After flash chromatography the n-alkanes were completely removed, and data evaluation is possible without any interferences. The result for the MOSH value is 4.31 mg/kg this time.

The MOAH value that was measured prior to flash chromatography gives a concentration of 0.64 mg/kg and results in a total MOH recovery of 4.95 mg/kg of the spiked 5 mg/kg.

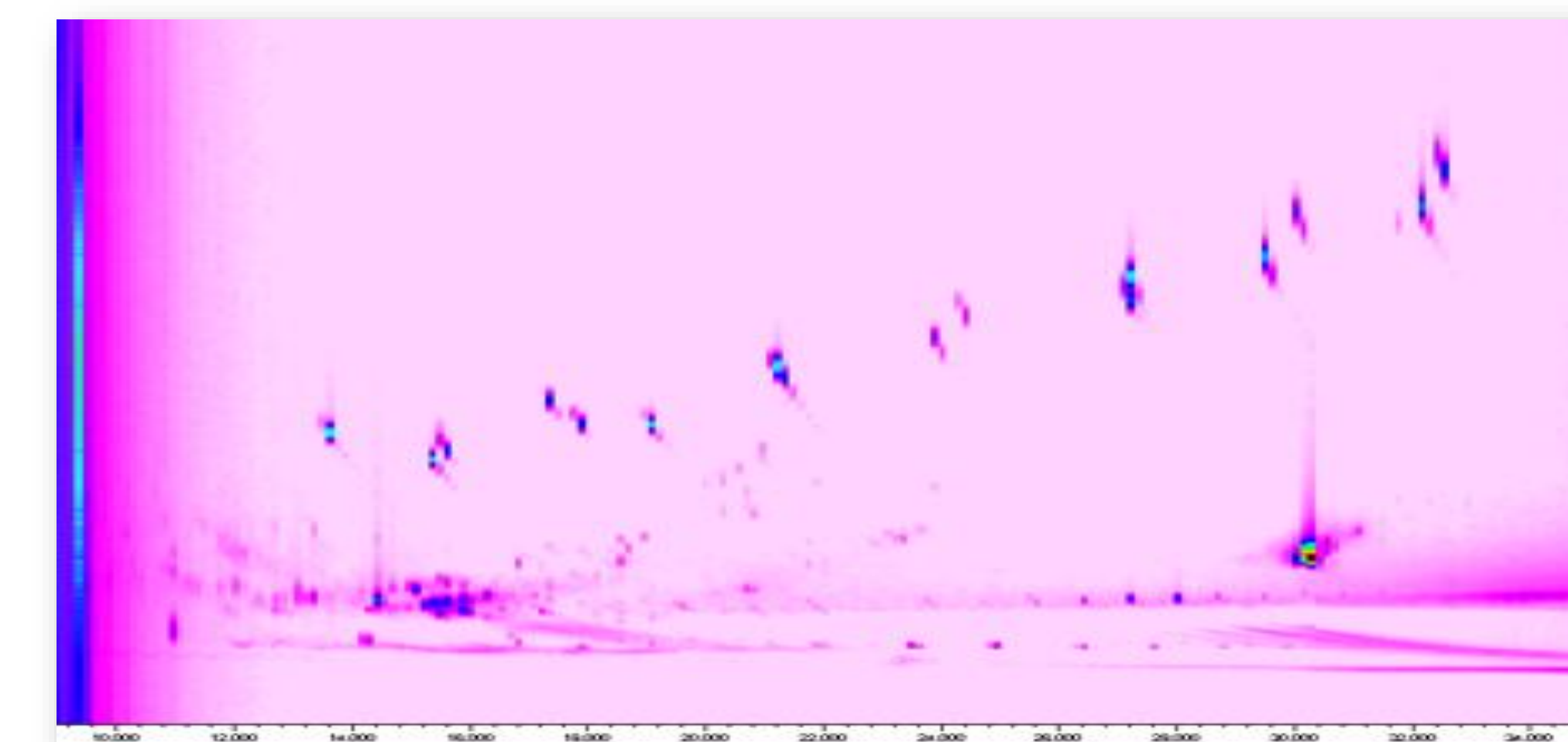


Figure 4: 2-D chromatogram of a standard PAH mix 14 (10 µl /ml, LGC, UK)

## 6. Comprehensive GCMS

In case of a detected MOAH concentration in food it is important to understand the origin of the contamination and the toxicity. Also, cross-contamination generated from MOSH, POSH, PAO and others should be excluded. That is why according to ISO 20122:2024 GCMS is required. More effective is GC x GCMS technique or comprehensive GCMS. Comprehensive GC-MS is a powerful technique that provides the two-dimensional chromatography data acquisition, resulting in a significantly improved resolution and sensitivity. Data processing is done via Chromsquare software (Chromaleont srl. Messina),

## 7. Conclusion

LC-GCxGCMS is a perfect solution for the analysis of MOSH and MOAH contaminants in food samples according to EU norm 16995:2022. The Shimadzu LC-GC online setup is designed for running fullautomatic analysis sequences and verification of complex samples in combination with comprehensive GCMS.

## 8. References

- [1] EU: COMMISSION RECOMMENDATION (EU) 2017/84 of 16 January 2017
- [2] EFSA Panel on food contact materials, enzymes, flavourings and processing aids (CEF). Scientific Opinion on Mineral Oil Hydrocarbons in Food. EFSA (EFSA Journal). 2012; <https://doi.org/10.2903/j.efsa.2012.2704>
- [3] EN 16995:2017 Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis
- [4] <http://www.bfr.bund.de/cm/343/bestimmung-von-kohlenwasserstoffen-aus-mineraloel-oder-kunststoffen.pdf>
- [5] Biedermann, M.; Grob, K. On-line coupled high performance liquid chromatography–gas chromatography for the analysis of contamination by mineral oil. Part 1: Method of analysis. Journal of Chromatography A 2011, 1255, 56-75, doi:10.1016/j.chroma.2012.05.095.