Determination of Mineral Oil Contamination in Foodstuff, Cosmetics and Packaging Materials

with the CHRONECT Workstation MOSH/MOAH

Application note 1801

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Introduction

Undesirable mineral oil residues are found in numerous products. These substances can be divided into two classes: Saturated (MOSH - mineral oil saturated hydrocarbons) and aromatic hydrocarbons (MOAH - mineral oil aromatic hydrocarbons). While the first substance class accumulates in the human body, the second compound class is suspected of containing carcinogenic substances. Substance classes such as POSH (polyolefin oligomeric saturated hydrocarbons) or PAO (poly-*alpha*-olefins) are chemically similar, but have different (synthetic) origins There are numerous contamination pathways by which these substances can get into food, for example:

- Recycled packaging materials produced from newspapers or magazines: The use of printing inks containing mineral oil is responsible for a gas phase migration into the food.
- Accidents during the transport or production of a food product
- Mineral oil-based lubricants within the production chain
- and many more

If a contamination on the basis of migration is assumed, especially hydrocarbons from the boiling range between C_{16} and C_{25} are observed, since they lie in a suitable volatility range. The health hazard of this contamination of food was already clearly formulated in December 2009 by the Federal Institute for Risk Co-Assessment (BfR). The BfR came to the conclusion in its assessment that the transition from mineral oils to foodstuffs should be urgently minimized. Measurements are recommended. The detection of these mineral oil contaminations in food has therefore been particularly investigated and optimized since then.

A further product field in which mineral-oil containing raw materials are used is the cosmetics sector. Highly refined mineral oils used in the cosmetics industry may also contain MOAH.

Device setup

For the determination of MOSH/MOAH contaminations from packaging, cosmetics or foodstuffs, sample purification and pre-separation by normal-phase HPLC has become the method of choice [1]. The samples are extracted with hexane, epoxidized if necessary and pre-purified with aluminum oxide depending on their origin and type [2].

The task of the HPLC step is to free the MOSH and MOAH fractions from interfering matrix components. By using an unmodified silica gel column, high amounts of fat and polar substances can be retained and effectively separated from MOSH and MOAH. This type of purification is also the basis of DIN EN 16995:2017-08 and the DGF standard method C-VI 22 (20), which fully complies with the system described here [3, 4]. The same applies to the method proposed by the European Commission for the determination of the content in infant food [5].

After pre-separation, both fractions are transferred to the GC dimension in large volumes without losses, separated by boiling point and detected by FID. The quantification is done as a sum parameter. A chromatographic run, i.e. the simultaneous determination of MOSH and MOAH from one sample, takes about 30 minutes.

The software Chrolibri was developed especially for MOSH/MOAH analysis and thus enables a simple evaluation.

The Hump Inspector software creates special mineral oil reference databases to determine the origin of the contamination.

Basic system components:

- CHRONECT LC-GC Interface
- Agilent Infinity II 1260 HPLC System
- Agilent 8890 GC with two FID
- CHRONECT Robotic RTC
- Used Software
  - CHRONOS to control the system
  - Clarity for data acquisition and evaluation
  - Chrolibri for evaluation of the unresolved peak humps
  - Hump Inspector to determine the source of contamination

Optional extension for epoxidation:

- FastWash Station
- Agitator
- Centrifuge

- Epoxidation procedure according to Nestola [6]

Optional extension for aluminum oxide purification:

- additional Agilent Infinity II 1260 HPLC pump
- HPLC valve
- Aluminum oxide HPLC column

Optional extension for MOSH depletion in cosmetics:

- HPLC valve
- Depletion HPLC column

Optional extension for fraction collection:

- Fract & Collect collection tool

Alternatively, the Nexera LC-40B XR and the Nexis GC-2030 from Shimadzu can be used. We reserve the right to make changes in the device configuration.

Sample preparation

All samples must be prepared before the actual measurement. Depending on the sample matrix, this can be simpler or more complex. Cosmetics or edible oils can be injected directly after dilution with *n*-hexane and addition of an ISTD. Compound foods or packaging, on the other hand, are usually extracted with *n*-hexane and ethanol.

For foods with a high emulsifier content or for general reduction of limits of determination, manual saponification with subsequent hexane extraction and concentration is possible.

Depending on the sample matrix, the extracts obtained can be automatically epoxidized by the measuring system and purified by aluminum oxide chromatography before they are transferred to normal phase HPLC.

Results

HPLC separation

Figure 1 shows an HPLC-UV chromatogram of an injection of a standard mixture. This consists of the substances C_{11}, C_{13}, cyclohexylcyclohexane (Cycy), cholestane (Cho) as well as pentylbenzene (5B), 1- and 2-methylnaphthalene (MN), tri-*tert*-butylbenzene (TBB) and perylene (Per).

The HPLC separation is performed by an *n*-hexane/dichloromethane gradient. The listed single standards are used for quantification and control for a successful MOSH/MOAH separation. Perylene is particularly well visible in the UV signal.

After 6 minutes the LC column is backwashed with dichloromethane. This procedure, called backflush, ensures a fast and effective removal of retained matrix components. After 9 minutes the column is conditioned with *n*-hexane in forward direction until it is ready for the next injection.

Figure 2 shows the GC-side MOSH and MOAH chromatograms obtained from the HPLC measurement. The complete absence of aromatic hydrocarbons in the MOSH fraction and of saturated hydrocarbons in the MOAH fraction can be taken as an indicator of successful MOSH/MOAH separation.

According to the current state of analytics, MOSH and MOAH are quantified between C_{10} and C_{50}. This roughly corresponds to a boiling range of 170-570 °C and thus also gives an indication of the nature of the possible MOSH/MOAH-containing mineral oils. A standard containing both components regularly ensures that the analytical system is non-discriminatory. Figure 3 shows this standard.

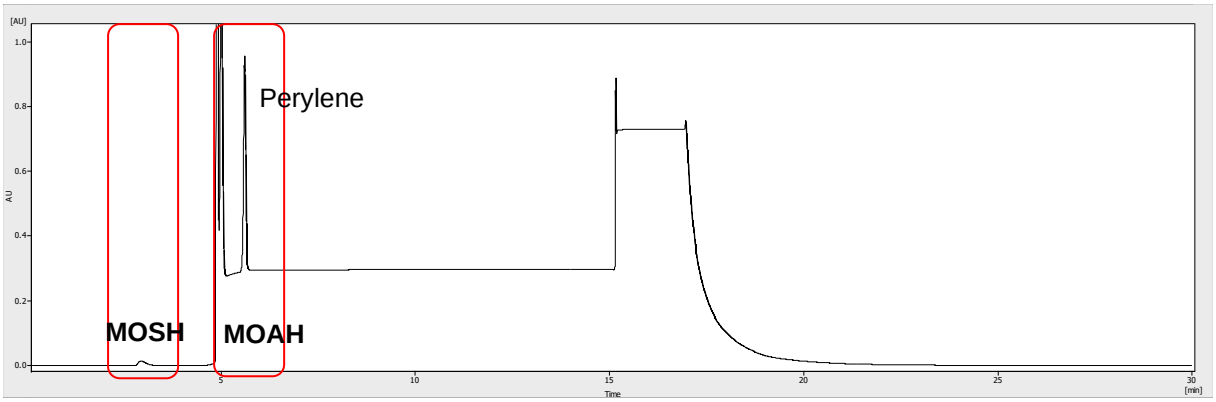

Figure 1: HPLC-UV chromatogram of a standard injection (wavelength: 230 nm). The marked fractions (450 µL each) are transferred to the gas chromatograph in large volumes without loss.

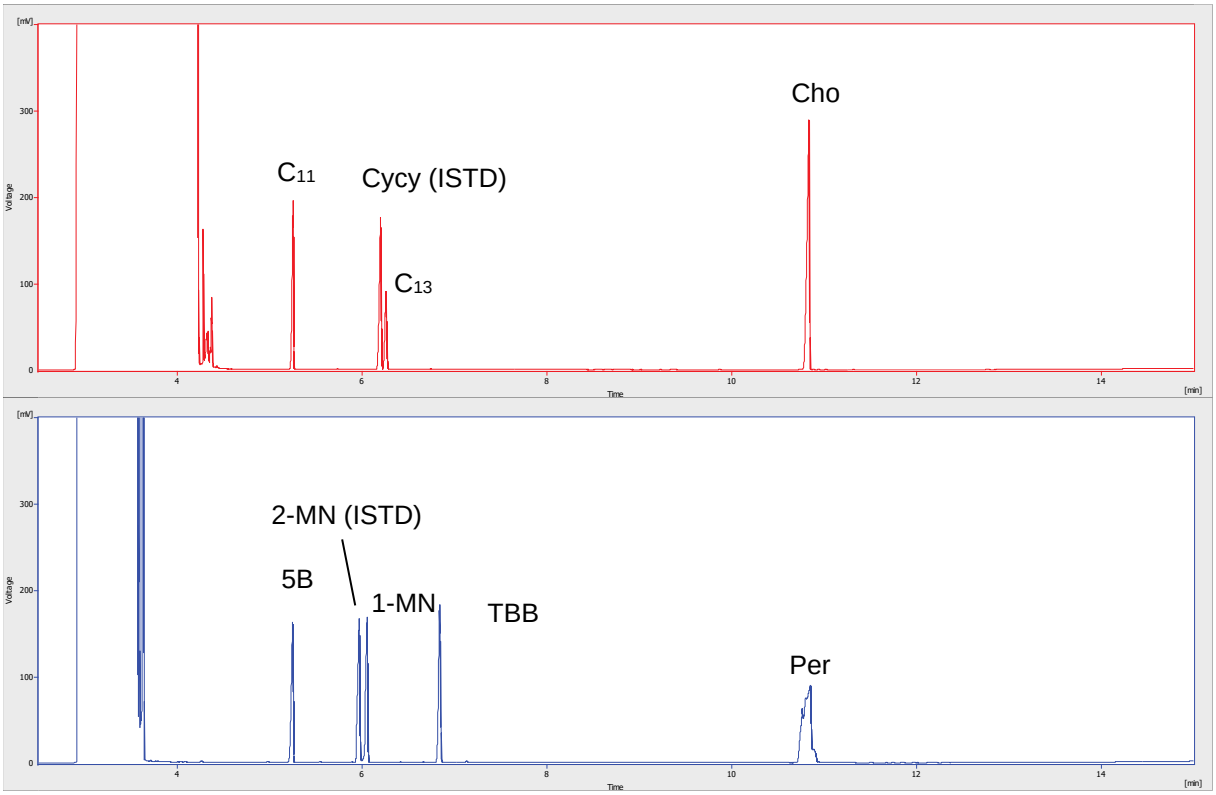

Figure 2: MOSH and MOAH GC-FID chromatograms of the injection from Figure 1. Both fractions were measured simultaneously. C_{11} and 5B are quantitatively recovered.

Following DIN EN 16995:2017-08, spiked sunflower oil was diluted in *n*-hexane and measured directly. Figure 3 shows a typical MOSH/MOAH chromatogram. Due to the high number of isomers, MOSH and MOAH do not form individual peaks but signal humps. The signal humps marked in Figure 4 are quantified as a sum directly with the internal standard. Peaks on top are normally subtracted and not included in the measurement.

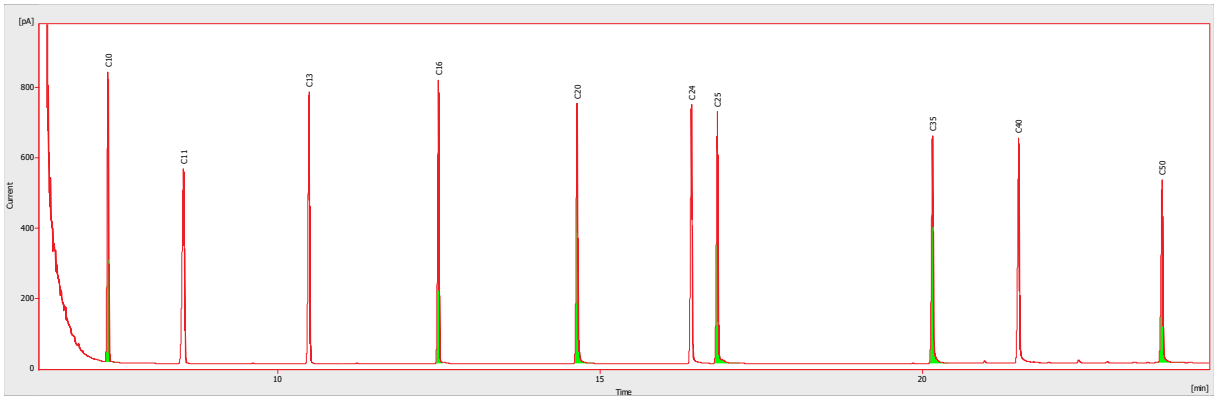

Figure 3: MOSH LC-GC-FID chromatogram of the injection of a standard with the components C_{10}, C_{11}, C_{13}, C_{16}, C_{20}, C_{24}, C_{25}, C_{35}, C_{40} and C_{50}. All alkanes have comparable peak heights and areas.

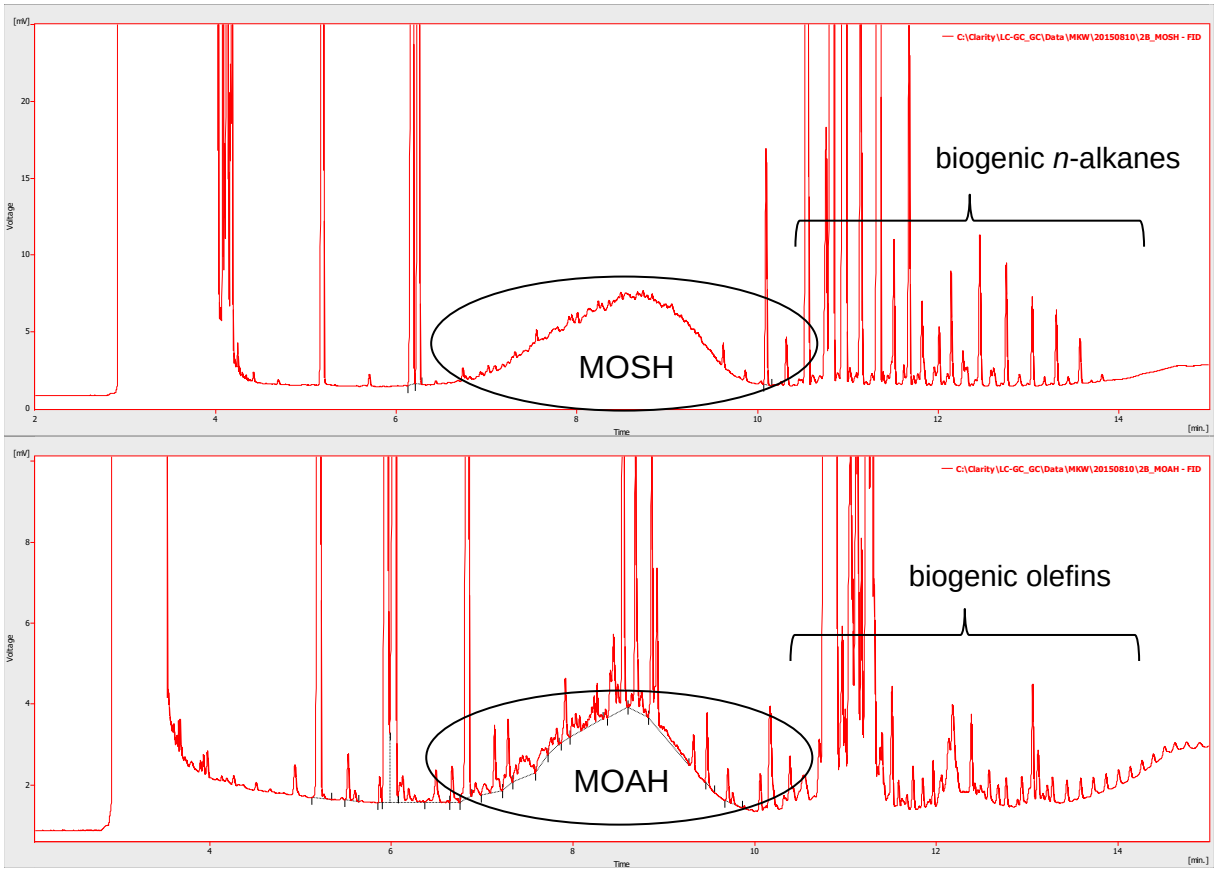

Figure 4: MOSH (top) and MOAH (bottom) GC-FID chromatograms of spiked sunflower oil. The contamination levels (integral of the marked areas) are 40 and 14 mg/kg, respectively.

As can also be seen in Figure 4, besides the obvious MOSH/MOAH humps, numerous single peaks or peak clusters can be seen. These are of natural origin and must be distinguished from MOSH/MOAH.

For this reason, the additional purification techniques mentioned above exist to remove these biogenic components from the sample. On the one hand, the MOSH fraction contains natural *n*-alkanes with a mostly uneven number of carbon atoms, which are superimposed on MOSH.

On the other hand, there are olefinic hydrocarbons, such as β-carotenes, squalene or refining products of sitosterol, which mainly affect the quantification of MOAH.

Figure 5 shows sunflower oil, which was automatically purified by aluminum oxide and epoxidation before the actual measurement.

The MOSH fraction is completely free of biogenic *n*-alkanes. Thus, even lower MOSH amounts can be quantified. The same applies to the MOAH fraction, which is largely free of olefins. Here, it must be taken into account that depending on the sample matrix no quantitative epoxidation might be possible. Both purification techniques are mandatory for a successful and reliable detection of MOSH and MOAH.

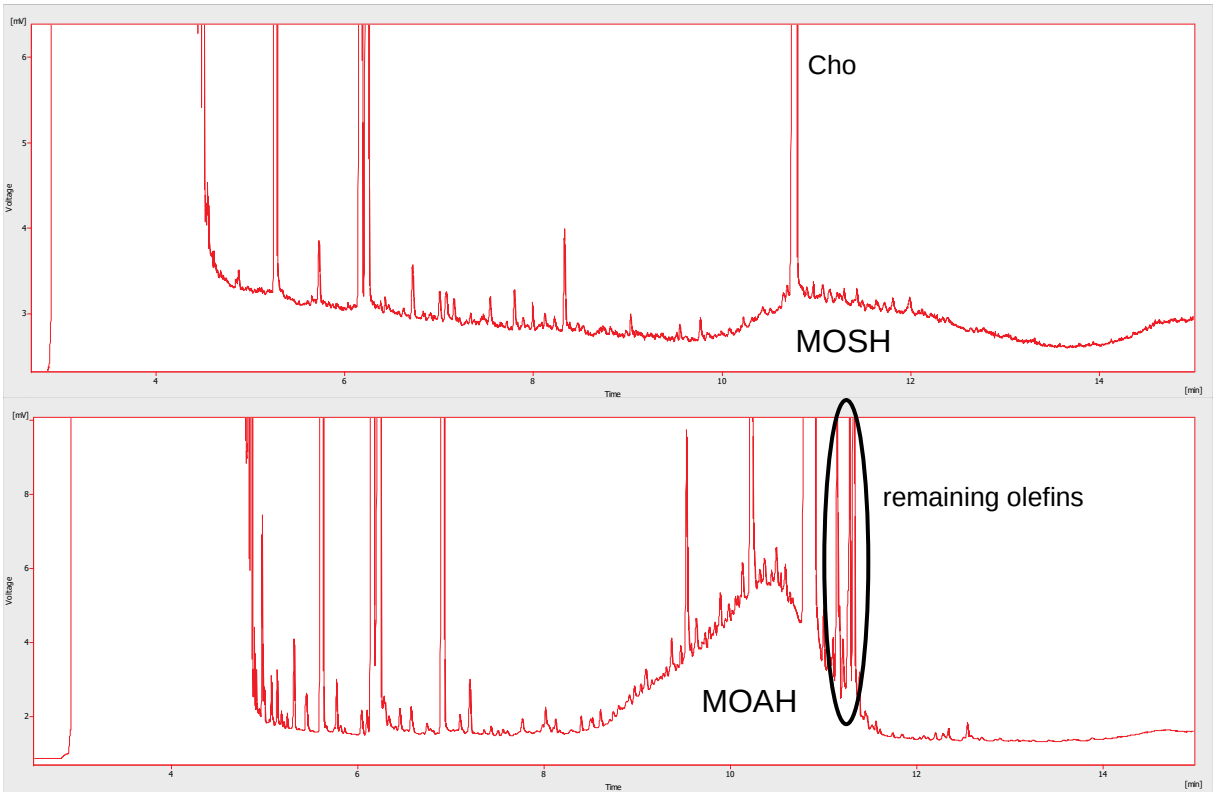

Figure 5: MOSH and MOAH GC-FID chromatograms of spiked sunflower oil after aluminum and epoxidation purification. The contaminations are 3 and 25 mg/kg respectively (MOAH was spiked for better visualization). Depending on the matrix, the epoxidation conditions have to be adjusted to obtain best possible results.

Validation techniques

Foodstuff

In cases where a 100 % statement regarding the presence of MOSH or MOAH cannot be made, additional validation techniques are necessary. The most common technique is two-dimensional gas chromatography with mass spectrometric detection (GCxGC-MS) [7]. With the Fract & Collect option, the measuring system offers the possibility of collecting purified MOSH and MOAH fractions and transferring them to other analytical techniques - if desired, even online as in the case of LC-GCxGC-MS coupling.
Figure 6 shows the MOAH fraction of an LC-GC-FID fraction and the same sample measured by LC-GCxGC-MS. In this case, the sample was transferred to the subsequent analysis by means of Fract & Collect.

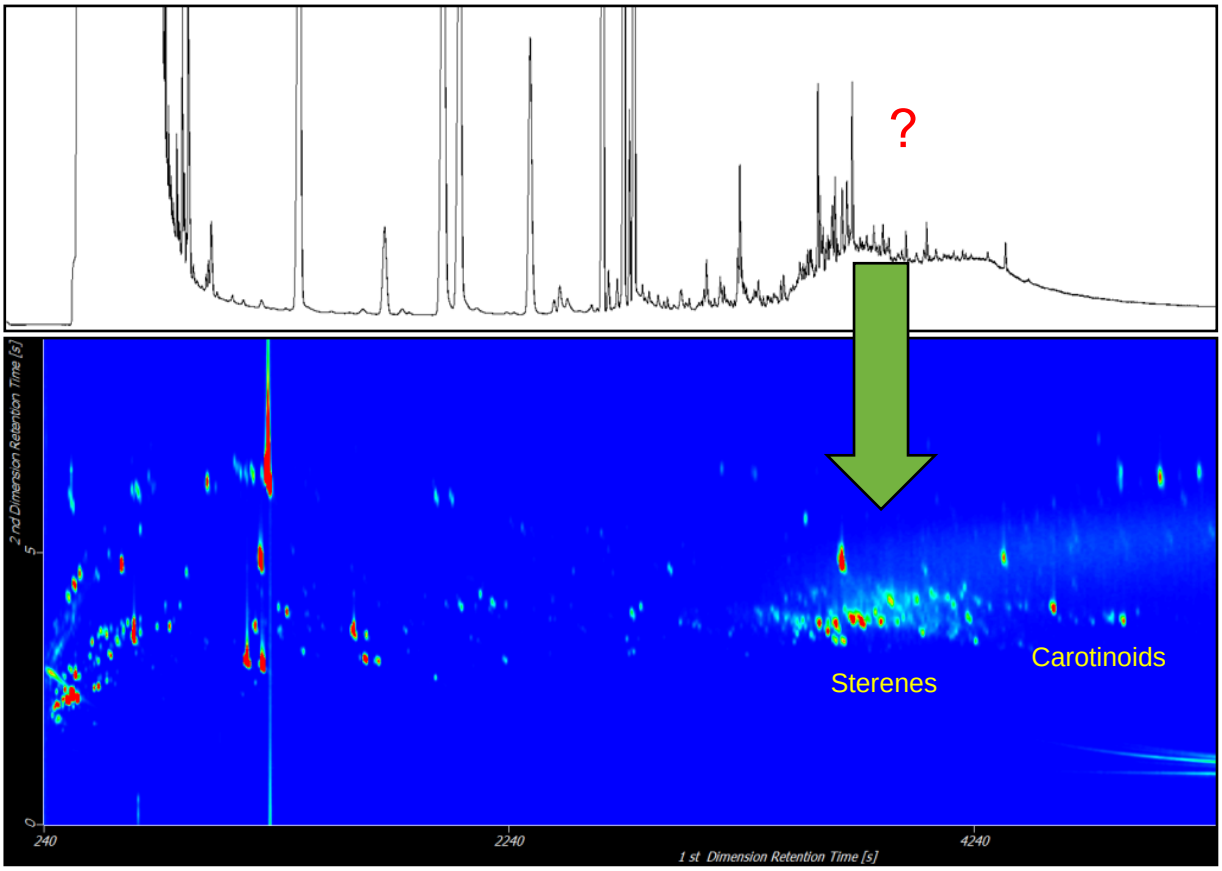

Figure 6: MOAH fraction: LC-GC-FID (top) and LC-GCxGC-MS chromatograms (bottom) of palm oil after epoxidation. The mass spectra clearly show the presence of biogenic substances. Thus, the observed hump in the FID chromatogram is at least not entirely attributable to the presence of MOAH.

Cosmetics

In the case of cosmetic samples based on mineral oil, which often contain only traces of MOAH, a further challenge in MOSH/MOAH analysis becomes apparent. Due to the natural presence of MOSH, it cannot be excluded with certainty that MOSH is falsely detected as MOAH. This cannot be guaranteed by FID or MS detector. The reason for this problem is a mass overload of the normal phase HPLC used, which leads to the fact that the two substance classes can co-elute.

One solution is the targeted removal (depletion) of MOSH from such samples. The idea behind this is to reduce the high amount of MOSH without losing MOAH so that MOAH becomes quantifiable.

This is achieved either by the Fract & Collect option with subsequent re-injection of the sample, or by an additional HPLC normal phase separation dimension that is connected upstream of the main separation column and can thus remove large amounts of MOSH.

Summary

The coupling of normal phase HPLC and GC-FID allows a reliable quantification of mineral oil residues in food, cosmetics and packaging. The use of highly porous silica gel columns allows the direct injection of fat-containing samples, thus avoiding manual fat separation. Additional purification techniques, such as aluminum oxide chromatography and epoxidation, further reduce the limits of quantitation and increase the reliability of quantification.

The direct coupling to other analytical techniques, such as GCxGC or NMR, further increases the reliability of quantification. The determination limits of 10 mg/kg specified in DIN EN 16995:2017-08 can be safely achieved with this system. With additional enrichment techniques, as described in the DGF standard method C-VI 22 (20), named by the BfR and other authors, even lower limits below 1 mg/kg can be achieved [2, 4, 8-10].

Literature

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The CHRONECT Workstation MOSH/MOAH is a development by Axel Semrau.

Subject to technical changes

Axel Semrau GmbH & Co. KG
Stefansbecke 42
45549 Sprockhövel
Germany
Tel.: +49 2339 / 12090
www.axelsemrau.com
info@axelsemrau.de