Quality Assurance and Consumer Protection

MOSH/MOAH analysis - efficiently automated

Instrumental analysis is a critical pillar of consumer protection, especially for food safety. To ensure a reasonable cost benefit balance, high laboratory productivity and good quality of results, leading contract laboratories increasingly strive to automate their processes. An example is the determination of mineral oil residues in food, food packaging, and cosmetics.

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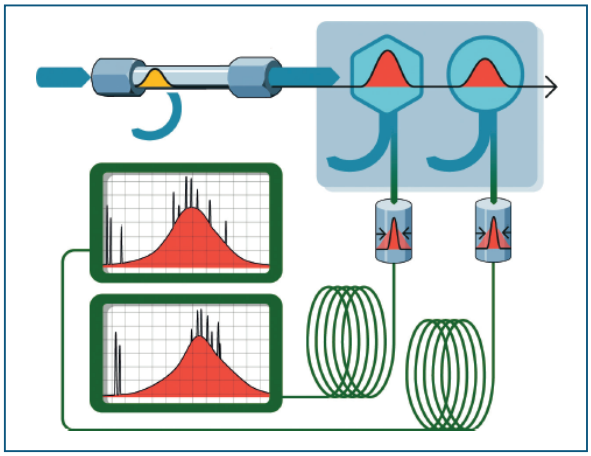
Industrial production, processing and transportation invariably put food at risk of contamination with mineral oil hydrocarbons, which are subdivided into two chemically distinct fractions: MOSH (Mineral Oil Saturated Hydrocarbons) and MOAH (Mineral Oil Aromatic Hydrocarbons). According to the European Food Safety Authority (EFSA), the MOAH fraction contains highly potent carcinogenic compounds, and both fractions accumulate in human tissue. From a toxicological standpoint and in the best interest of the consumer, food, and food contact materials, which mainly means food packaging, should be monitored for the presence of MOSH and MOAH[1]. The same applies to cosmetics, which are of course in direct contact with our skin.



Principle of MOSH/MOAH determination by LC-GC-FID. Normal phase LC separation of MOSH and MOAH and subsequent parallel GC-FID analysis.

GERSTEL MPS robotic-HPLC-GC-FID solution for automated epoxidation and MOSH/MOAH determination.

Sizing up the application details

In MOSH/MOAH analysis, the main points are to extract mineral oil hydrocarbons from the sample and subsequently to determine them using a suitable analysis method. MOSH/MOAH analysis involves several extract cleanup steps to separate, concentrate, and transfer the MOSH and MOAH fractions from the sample matrix into a solution that can be analyzed in a sensitive and robust manner, yielding accurate results. Interfering matrix compounds such as triglycerides are removed automatically on the HPLC column during separation of the fractions.

The hydrocarbons contained in the MOSH and MOAH fractions are determined separately, but simultaneously, in a dual channel gas chromatography system using flame ionization detection (GC-FID).

Depending on the sample type and matrix, additional sample preparation steps may be required to eliminate naturally occurring hydrocarbons from the extract. Some samples must be epoxidized prior to the LC-based fractionation to remove unsaturated polyenes that would otherwise interfere with the MOAH determination. Depending on the matrix, the MOSH fraction must be subjected to a further cleanup step using an aluminum oxide (AlOx) column to remove n-alkanes of plant origin.

To sum it up, determining MOSH and MOAH residues in food or cosmetics is a highly labor intensive task when performing the sample preparation manually. Contract laboratories and routine laboratories that are measured not only by the quality of their results, but also by their productivity and by how fast results are delivered, are well advised to automate the process.

Focus on the customer benefit

With the aim of maximizing efficiency, productivity, accuracy, and precision of the MOSH/MOAH analysis, GERSTEL has developed an integrated system that automates sample preparation, including extract cleanup, and LC-GC-FID determination of both fractions.

The hardware consists of an Agilent 1260 Infinity II HPLC system and a dual channel GC-FID system (Agilent 8890 GC) coupled by an LC-GC interface (GERSTEL) and combined with a MultiPurpose Sampler (GERSTEL MPS robotic). The system is highly modular and easily adapted to changes in requirements. Systems based on the MPS Single Head autosampler fulfill all requirements for efficient automated determination of the MOSH and MOAH fractions in prepared extracts optionally including AlOx cleanup. Dual Head systems additionally enable cleanup and sample preparation steps such as saponification and epoxidation using performic acid or *m*-Chloroperoxybenzoic acid (mCPBA), including high energy agitation, centrifugation, evaporative concentration, cool storage of reagents and samples, wash stations for syringes, and more. Both Single- and Dual Head MPS systems enable maximum efficiency in performing all sample preparation and introduction steps. Optionally, a dedicated module for AlOx cleanup of the MOSH fraction can be added.

LC-GC coupling

When developing the LC-GC coupling, we focused on ruggedness and ease of maintenance of the entire system to maximize system up-time, sample throughput, and laboratory productivity. Our concept includes a special GC column mounting system that enables unfettered access to columns and connectors for fast and easy replacement and the column connector technology used ensures long-lasting gas

tight connections even after multiple instances of disconnecting and reconnecting columns. The user has full control of all system parameters and pre-columns are easily rinsed or replaced as needed. The solvent vapor venting system (GERSTEL Early Vapor Exit, EVE) uses a novel approach: Valves are unheated for simplicity and for easy replacement of capillary connectors to the EVE. An integrated purge system removes residual solvent from the valve and connected tubing for long-lasting rugged operation.

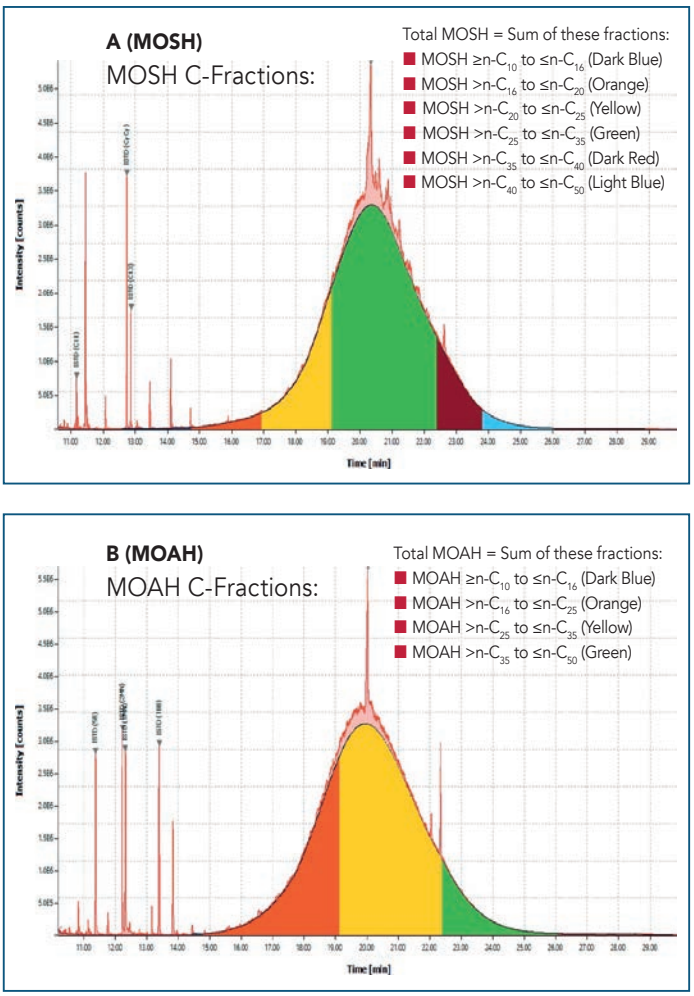


Figure 2: Unresolved complex mixtures (UCM) of MOSH (A) and MOAH (B) fractions extracted from an edible oil sample and integrated using the GERSTEL MOSH/MOAH Data Analysis software. The colored peak (signal) areas represent individual c-fractions. The total amounts are calculated as the sum of all c-fractions between n-C10 and n-C50. The values are included in the final report. System control of the GERSTEL MPS robotic-HPLC-GC-FID solution for MOSH/MOAH analysis is performed through the MAESTRO software; data handling is simplified using a dedicated software.

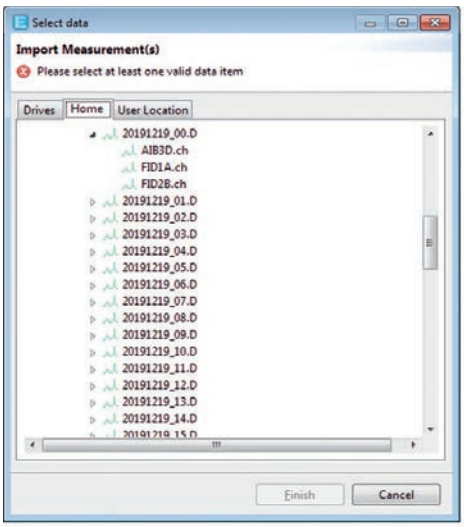
Analysis Details

A quick summary of the analysis: Sample cleanup and fractionation is performed using normal phase liquid chromatography (NP-LC), based on polarity differences. The MOSH fraction elutes before the MOAH fraction using a solvent gradient consisting of *n*-hexane (C_6H_{14}) and dichloromethane (CH_2Cl_2). Matrix residues are backflushed from the column using a flow of 100 % dichloromethane before the column is reconditioned and prepared for the following sample with a forward flow of *n*-hexane. A UV detector set to 230 nm is used to verify separation and transfer to the GC of the two fractions. The UV detector cannot be used for quantification of the fractions since many of the compounds contained, and the internal standards [3], are insufficiently UV-active and do not deliver equimolar responses. Following HPLC separation, the large volume fractions (450 µL each) are transferred directly to the dual channel GC system with two GC columns in the oven and two FID detectors for simultaneous determination of the MOSH and MOAH fractions. Initially, the fractions are focused and prepared for GC analysis by evaporating the solvent and venting the fumes through the specially designed EVE system. Following GC separation, analytes are detected by FID, a detector type that delivers well known mass responses for the range of analytes contained in the MOSH and MOAH fractions. Quantitation is performed as the sum of all C10-C50 compounds with individually added sections in accordance with the JRC Guidance [3]. The internal standard mixture used for MOSH/MOAH analysis includes nine different compounds. These are used for quantification and as markers and controls for both the HPLC fractionation and the GC separation performance. A separate „retention time standard" with 10 compounds from C10 to C50 is analyzed regularly to determine the prescribed size ranges.

Benefits of Automation

The description above illustrates the complexity of the MOSH/MOAH sample preparation. Automation reduces the risk of errors while improving efficiency, reliability, and reproducibility on a 24/7 basis. Further, the ease of operation and the process miniaturization result in improved sustainability while freeing up analyst time to focus on more pressing tasks such as planning, data evaluation and reporting. All parts of the MOSH/MOAH analysis system are under unified control of the MAESTRO software integrated with

the Agilent OpenLab CDS. Operation is user friendly and intuitive. Data handling has been simplified by a dedicated software package for automated MOSH/MOAH data handling. "Saddle peaks" resulting from naturally occurring compounds are clearly visible on top of the MOSH and MOAH humps consisting of thousands of mineral oil related compounds. The size of each hump is decisive, the peaks on top do not count, they are automatically disregarded or subtracted by the software. The humps are automatically integrated with a blank chromatogram used as the lower baseline. The results are reported for each size range as well as for the whole C10 - C50 range. Manual correction of integration parameters is possible at every step, if required. The software enables the user to change integration parameters manually as needed after inspecting the results followed by efficient automated batch reintegration of an entire series of analysis results.



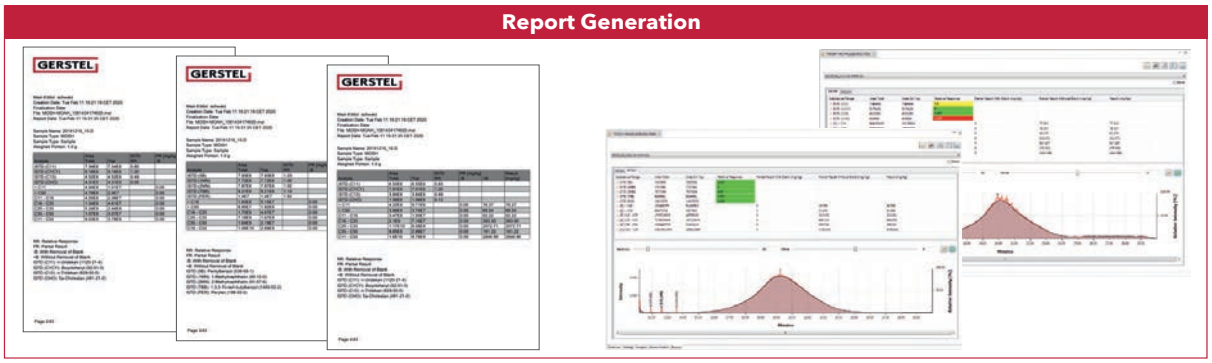


Figure 3: Report Generation: Results are presented in one of several standard report formats that are easily customized to meet individual needs of the laboratory. Data can be exported in a variety of formats for further processing.

Final Words

The fully automated GERSTEL MOSH/MOAH solution meets the requirements of international standard methods. The fractionation is rugged and reliable, good recovery of the n-alkanes up to C50 as percentage of the n-C20 is achieved, and no significant carry over is seen. Further, the system is reliable for routine operation, meeting all requirements for occupational safety, data integrity, intuitive operation, and ease of maintenance. Obtained analysis results correspond well with reference analyses performed by independent laboratories, which have successfully taken part in round robin tests.

References:

[1] European Food Safety Authority (EFSA), Scientific Opinion on Mineral Oil Hydrocarbons in Food, EFSA Journal 2012;10(6):2704, http://bit.ly/2TnvTWb

[2] DIN EN 16995 Foodstuffs - Vegetable oils and foodstuff on basis of vegetable oils - Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis EN 16995:2017-08

[3] JRC Technical Reports: Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials (in the frame of Commission Recommendation EU 2017/84), http://dx.doi.org/10.2760/208879

Automated MOSH/MOAH Sample Prep

Three sample preparation steps are generally used for MOSH/MOAH analysis:

1) Aluminum oxide cleanup
2) Epoxidation
3) Saponification

Each of these methods handles a specific challenge in MOSH/MOAH analysis and can be used independently. It is possible to use only epoxidation or only aluminum oxide cleanup on one sample type, but it is also possible to use all of them consecutively on the same sample.

Cleanup with activated Aluminum oxide (AlOx):
Using activated aluminum oxide allows the removal of n-alkanes of plant origin from the MOSH fraction that might otherwise interfere with integration of the hump when evaluating the data. The n-alkanes are retained on the AlOx column and separated from the target compounds. In traditional manual sample preparation, single-use glass columns are used. The aluminum oxide has to be activated at 400 °C for 40 h and is discarded after every sample. Unfortunately, MOAH compounds are retained on the column as well and the AlOx columnn must be discarded after each manual cleanup step. As a consequence, when using manual sample preparation, two complete analyses are required for each sample: One for MOSH with AlOx preparation and one for MOAH without AlOx.

The GERSTEL solution is online AlOx cleanup after separation of the MOSH and MOAH fractions, preventing loss of the MOAH analytes and eliminating the need for a separate MOAH determination: Aluminum oxide is packed in a column, similar to an LC column, that lasts a few hundred samples (depending on the matrix) and is connected to the LC system via a second LC pump. While the MOSH fraction is passed through the AlOx column, the MOAH fraction is "parked" in an injection loop. Following cleanup, both fractions can be injected into the GC simultaneously, producing MOSH and MOAH results in a single instrument run. Additionally, this procedure saves immense amounts of solvent and single-use raw materials compared to the manual procedure.

Epoxidation: Epoxidation is used to derivatize olefins to epoxides. Olefins are mainly of plant origin and may interfere with the MOAH analysis. Epoxidation increases their polarity and thereby their retention on the HPLC column causing them to elute after the MOAH fraction. Epoxidation is performed by the MPS using a GERSTEL quickMIX, a centrifuge, and a decapper. The decapper is needed to eliminate siloxane contamination of the sample, resulting from multiple septum penetrations during sample preparation. The process is performed on-line in the system prior to sample injection. Epoxidation can be combined with saponification on a stand-alone MPS WorkStation used for off-line sample preparation.

Saponification: Samples with high fat content may need to go through saponification to remove excess fat before introduction to the analysis system and prevent triglyceride breakthrough in the LC column. The reaction is fully automated using the MPS with a GERSTEL quickMIX, GERSTEL mVAP multi-position evaporation station, Agitator, and Centrifuge to name the main options used. The system is modular and easily changed or upgraded with additional modules as needed when requirements change.

Saponification is performed in batches of up to six samples for maximum efficiency on a stand-alone MPS WorkStation and can be combined with epoxidation for a total throughput of 36 samples per day without limiting the sample capacity of the MOSH/MOAH analysis system.