Application note | 002208

Food safety

Automated LC-GC system for MOSH and MOAH analysis in food, according to EN 16995:2017

Authors

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Keywords

MOSH, MOAH, mineral oil, hydrocarbons, food contaminants, LC-GC, TRACE 1610 GC-FID, Vanquish HPLC

Goal

To streamline the analysis of mineral oil saturated and aromatic hydrocarbons as food contaminants by automating sample preparation and on-line injection into a hyphenated LC-GC system, therefore increasing sample-throughput, as well as improving data accuracy and precision.

Introduction

MOSH and MOAH are acronyms used in the food industry to refer to different types of contaminants that can be present in food and beverages. MOSH stands for mineral oil saturated hydrocarbons and includes paraffinic and naphthenic saturated hydrocarbons. MOAH stands for mineral oil aromatic hydrocarbons and includes alkylated cyclic aromatic hydrocarbons. Both may occur in food packaging material, as well as in contaminated water or soil. As such, they can migrate into food and drinks and therefore pose a risk to human health. To preserve the safety and quality of products for consumers, it is important for the food industry to take steps to identify and minimize the presence of MOSH and MOAH in food and beverages.

The presence of MOSH and MOAH in food and beverages is regulated in the European Union by the European Food Safety Authority (EFSA), which provides guidance on the

acceptable levels of these contaminants in food, setting specific maximum levels for MOSH and MOAH, based on the potential risk to human health. In the United States, the determination of MOSH and MOAH in food and beverages is regulated by the Food and Drug Administration (FDA), which sets guidelines for the acceptable levels of these contaminants in food.

Recently, the Standing Committee on Plants, Animals, Foods and Feed (SCoPAFF) of the European Commission published a draft joint statement effectively defining upper limits for MOAH in food at 2 mg/kg for edible oils and fats.

The choice of the analytical method for the analysis of MOSH/MOAH depends on the type of sample being analyzed, the specific contaminants being studied, and the level of sensitivity and precision required. It is important to use validated and well-established analytical methods to ensure accurate and reliable results.

Liquid chromatography-gas chromatography (LC-GC) is an analytical technique that combines the separation power of liquid chromatography with the separation and detection capability of gas chromatography to analyze complex mixtures, including food and beverages, for MOSH and MOAH.[1] In LC-GC, the extracted sample is first cleaned up using LC, which separates saturated and aromatic hydrocarbons fractions according to their polarity, as well as fractionating the matrix. The hydrocarbon fractions are then transferred to a GC column where they are further separated and analyzed. This technique offers several advantages, including high separation power, high sensitivity and selectivity permitting the detection and quantification of even trace levels of MOSH and MOAH in complex matrices, and reducing the risk of interferences from other compounds in the sample.

In the past, this analysis implied time-consuming manual operations for sample extraction and clean up, as well as multiple injections per sample to complete the determination of both MOSH and MOAH. Modern technology allows streamlining the analysis of MOSH/MOAH by automating sample preparation and on-line injection into the hyphenated LC-GC system, therefore increasing sample throughput, as well as improving data accuracy and precision. This application note illustrates the applicability of automated workflows for MOSH/MOAH analysis in food matrices, presenting different hardware configurations available to meet different analytical needs.

Experimental

The MOSH and MOAH analyzer used for this note was designed and validated by SampleQ, Interscience,[2] a Thermo Fisher Scientific channel partner for automated systems, offering a complete solution for MOSH and MOAH analysis.

Instrumental set up – Basic system LC-GC/FID

The basic LC-GC system required to perform MOSH/MOAH analysis includes a dual channel Thermo Scientific™ TRACE™ 1610 GC-FID system set up for large volume injections of sample fractions delivered from a Thermo Scientific™ Vanquish™ HPLC, equipped with a silica gel column. The LC is connected to the GC through the Thermo Scientific™ 1600 Auxiliary Oven, which hosts the injection valve, the transfer valve, the backflush valve, and the LC column, to ensure the sample path is heated to avoid any risk of clogging. This compartment also features a continuous check of LC leakage, with an active stop of the pump in case a leak is detected. A schematic of the plumbing is reported in Figure 1. This configuration is expandable with additional items to increase analytical capabilities, as shown in the following sections.

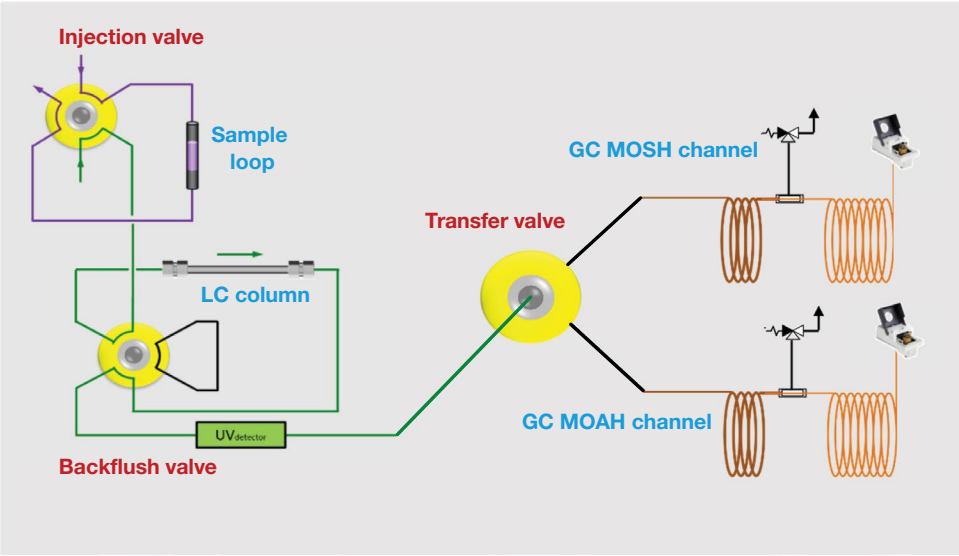


Figure 1. Schematic of the LC-GC hardware setup

The combined LC-GC system is served by a Thermo Scientific™ TriPlus™ RSH SMART robotic autosampler, to perform the injection of the extracted sample into the LC injection valve. The entire sample path from injection to detection is represented in Figure 2.

After injection, the LC column allows the MOSH fraction (saturated hydrocarbons) to elute with pure hexane as mobile phase and be transferred to the GC, while the MOAH fraction (aromatic hydrocarbons) is retained. Then, the mobile phase is changed to a mixture of hexane/dichloromethane (65%/35%) to allow the MOAH fraction to elute and be transferred to the GC. The dual channel GC configuration allows for automated analysis of MOSH and MOAH fractions with a single injection of the sample, thanks to the transfer valve, which automatically diverts each fraction into the proper GC channel. The GC column set up includes for each channel a precolumn connected to the analytical column through a solvent vapor exit (SVE) valve, required to perform the transfer of a large fraction of eluent into the capillary columns (large volume injection). The SVE valves allow the discharge of the majority of the solvent before transferring the MOSH or MOAH components into the capillary column for further separation and FID detection for reliable quantitation (Figure 3).

The detailed LC-GC system configuration is reported in Appendix 1. The entire system is fully controlled by the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), including method set up, data acquisition, processing, and reporting.

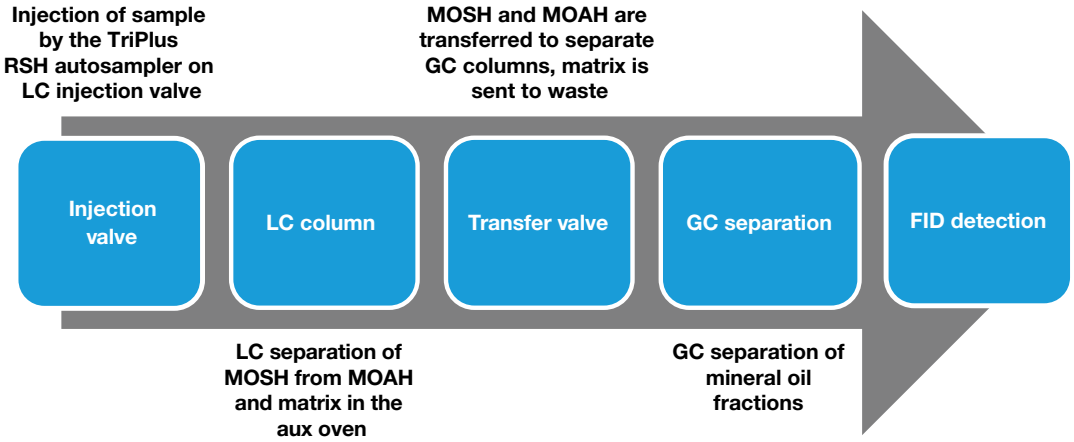


Figure 2. Schematic of the sample path

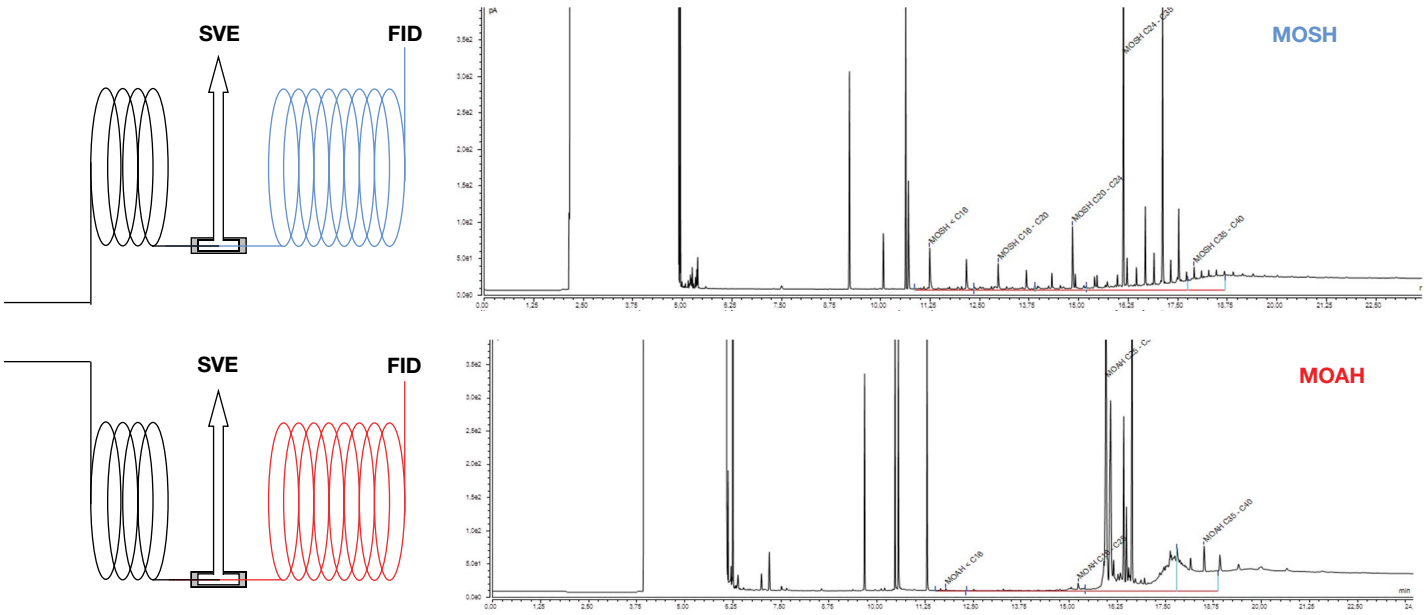


Figure 3. GC columns set up for large volume injection and separation of the MOSH and MOAH fractions. MOSH and MOAH are simultaneously analyzed on the dual-channel GC. An example of the FID chromatograms of each fraction are reported.

Standards and solvents

- MOSH/MOAH standard (Restek, P/N 89031070), including nine components (150-600 µg/mL): *n*-undecane (C11), *n*-tridecane (C13), bicyclohexyl (CyCy), cholestane (Cho), 1-methylnaphtalene (1-MN), 2-methylnaphthalene (2-MN), *n*-pentylbenzene (5B), perylene (Per), 1,3,5-tri-*ter*-butylbenzene (TTB)
- MOSH/MOAH retention time marker (Restek, P/N 89031076). This 10-component mix contains mineral oil saturated hydrocarbon (MOSH) markers that are used to accurately identify GC retention time limits for integration.
- Pure mineral oil
- *n*-Hexane, Picograde™ (Scientest, P/N SO-1244-B025)
- Dichloromethane, Picograde™ (Scientest, P/N SO-1185-B025)

Standards preparation

- Internal standard mixture:
  Dilute the MOSH/MOAH standard 625 times in hexane (i.e., 40 µL in 25 mL). ρ = 0.48 µg/mL
- Retention time marker:
  Dilute the retention time marker 100 times in hexane (i.e., 100 µL in 10 mL). ρ = 1 µg/mL
- Mineral oil stock solution
  Dissolve 50 mg mineral oil in 10 mL hexane. ρ = 5,000 µg/mL
- Mineral oil standard:
  Dilute the mineral oil stock solution 100 times in hexane (i.e., 100 µL in 10 mL). ρ = 50 µg/mL

Samples preparation

- Extra virgin olive oil (EVOO) spiked at 20 ppm and 50 ppm total mineral oil (MOSH + MOAH)
- Olive oil (OO) spiked at 20 ppm and 50 ppm total mineral oil (MOSH + MOAH)

Reagents for automated sample preparation

- 3-chloroperbenzoic acid (mCPBA), <77%. Prepare a solution 100 g/L in ethanol absolute >99%.
- Sodium thiosulfate pentahydrate, >99.5%. Prepare a solution 100 g/L in ultra-pure water.

System qualification criteria

Internal standard check

The analytical performance of the entire system was verified by injection of a standard mixture containing nine components. Four components were visible on the MOSH channel, including bicyclohexyl (CyCy) used as internal standard. The other five components were visible on the MOAH channel; here, 2-methylnaphtalene (2-MN) is used as internal standard. The recoveries of the seven components were calculated against the internal standards. The following criteria, more stringent than what required by official regulation, apply to this standard for system qualification:

- Recovery of three components on MOSH channel between 90 and 110%
- Recovery of 5B, TTB, and 1-MN on MOAH channel between 90 and 110%
- Recovery of perylene on MOAH channel between 80 and 100%
- Resolution 2-MN/1-MN (MOAH) > 2
- Resolution CyCy/C13 (MOSH) > 2

The recoveries and resolutions were automatically calculated in the Chromeleon CDS report designer (Figure 4).

Peak Name	Retention min	Area pA*min	Resolution to CyCy	Rel.Area (CyCY) %		Recovery %
C11	8.155	8.402	35.33	101.02		101.02%
CyCY	9.853	8.317	0.00	100.00		100.00%
C13	10.020	4.363	5.74	52.45		104.91%
Cholestane	14.385	16.905	154.20	203.25		101.63%

Peak Name	Retention Tim min	Area pA*min	Resolution to 2-mn	Rel.Area (2-MN) %		Recovery %
Pentylbenzene	9.258	8.727	28.11	97.95		97.95%
2-MN	9.927	8.909	0.00	100.00		100.00%
1-MN	10.002	8.644	3.17	97.03		97.03%
1,3,5-TTB	10.753	8.787	35.58	98.62		98.62%
Perylene	14.418	16.185	38.30	181.67		90.83%

Figure 4. Results of the internal standard check on MOSH channel (upper table) and MOAH channel (lower table). Resolution between 2-MN and 1-MN is 3.17; resolution between CyCy and C13 is 5.74.

Retention time marker check

The system performance was also verified by injecting the MOSH/MOAH retention time marker. This contains the relevant markers for the beginning and end of all fractions. The ratios between every component and C20 were calculated and must be ≥0.80, as shown in Figure 5.

Repeatability

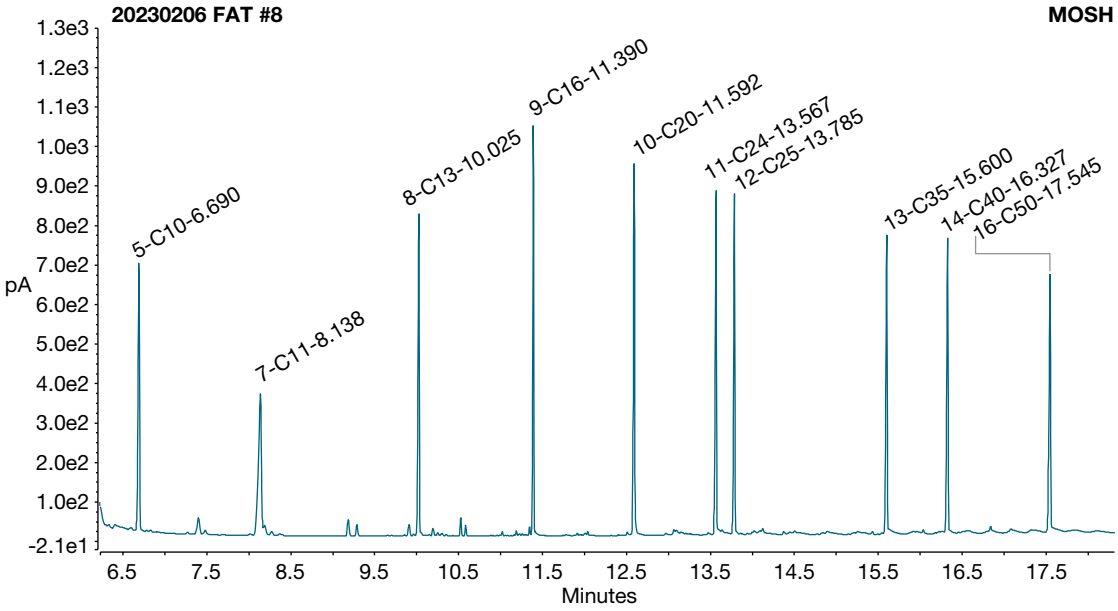
The mineral oil standard was injected 10 consecutive times with a blank injection in the middle. The amount of MOSH and MOAH was calculated for each injection as well as the total amount of mineral oil. An example of the mineral oil standard is given in Figures 6A, 6B, 6C. The repeatability results are reported in Table 1, showing an RSD% < 1% for both MOSH and MOAH fractions.

Automated sample preparation

More stringent regulations on MOSH/MOAH content in food and more attention from the food producers to minimize possible contamination pushed the need for a highly sensitive analytical technique, capable of reliably detecting levels of <1 mg/kg. Sample preparation is key to reduce possible interferences from biogenic hydrocarbons, which would co-elute with the MOSH and MOAH fractions during LC separation. Additional steps in the sample clean-up are necessary. The TriPlus RSH SMART autosampler can be configured with dedicated tools, offering the additional capability to automate the sample preparation procedures, including saponification and epoxidation, before executing on-line injection into the LC-GC system. The full TriPlus RSH SMART autosampler configuration is illustrated in Figure 7.

Saponification

In the case of food matrices with high fat content, the silica LC column is capable of separating triglycerides from the hydrocarbon fractions, but with a low capacity for fats. A preliminary saponification step by heating samples with potassium hydroxide in water is useful for a prior removal of fatty acids, improving the clean-up in the LC column. This allows for larger sample volume injection and reduced interferences in both MOSH and MOAH chromatograms, permitting a lower limit of quantitation. This step is recommended for samples with a fat content >50% and when lower LOQ are desired.



Component	RT (min)	Ratio to C20
C10	6.690	0.98
C11	8.138	0.91
C13	10.025	0.96
C16	11.390	0.95
C20	12.592	1.00
C24	13.567	0.98
C25	13.785	0.95
C35	15.600	0.92
C40	16.327	0.93
C50	17.545	0.88

Figure 5. Chromatogram and results of the retention time marker standard mixture on the MOSH channel. The same markers are used to check the MOAH channel.

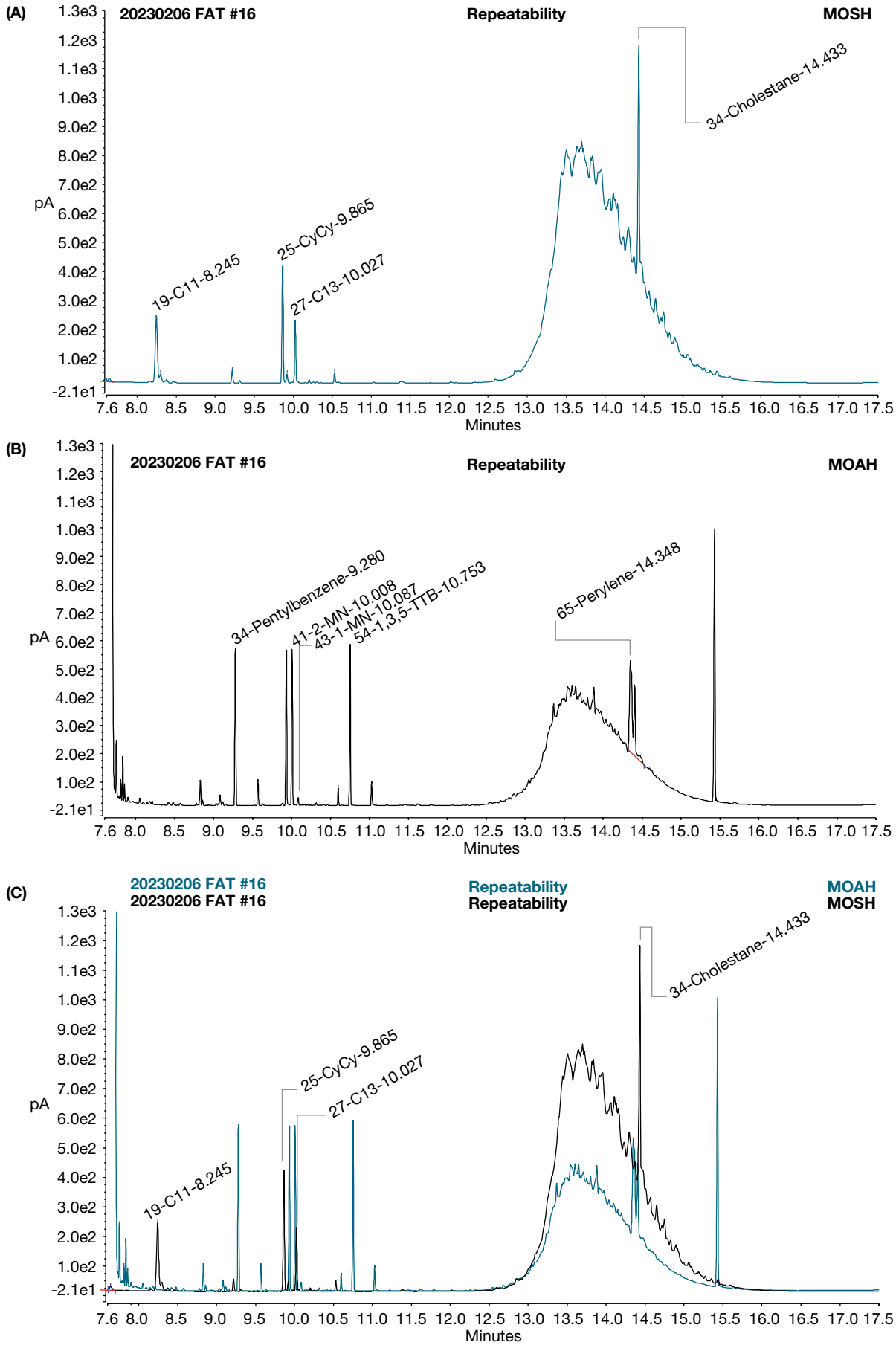


Figure 6. Example of chromatograms for mineral oil standard: (A) MOSH channel, (B) MOAH channel), and (C) overlay of the MOSH (black trace) and MOAH (blue trace) chromatograms

Table 1. Repeatability results for mineral oil standard (n=10)

Injection	Response MOSH	Response MOAH	Total MO
1	126.889	44.291	171.180
2	129.151	43.877	173.028
3	128.916	44.476	173.392
4	129.345	43.853	173.198
5	130.103	43.702	173.805
6	128.689	44.023	172.712
7	128.694	44.157	172.851
8	129.162	43.829	172.991
9	129.794	44.011	173.806
10	130.705	44.997	175.702
RSD	**0.79%**	**0.87%**	**0.65%**

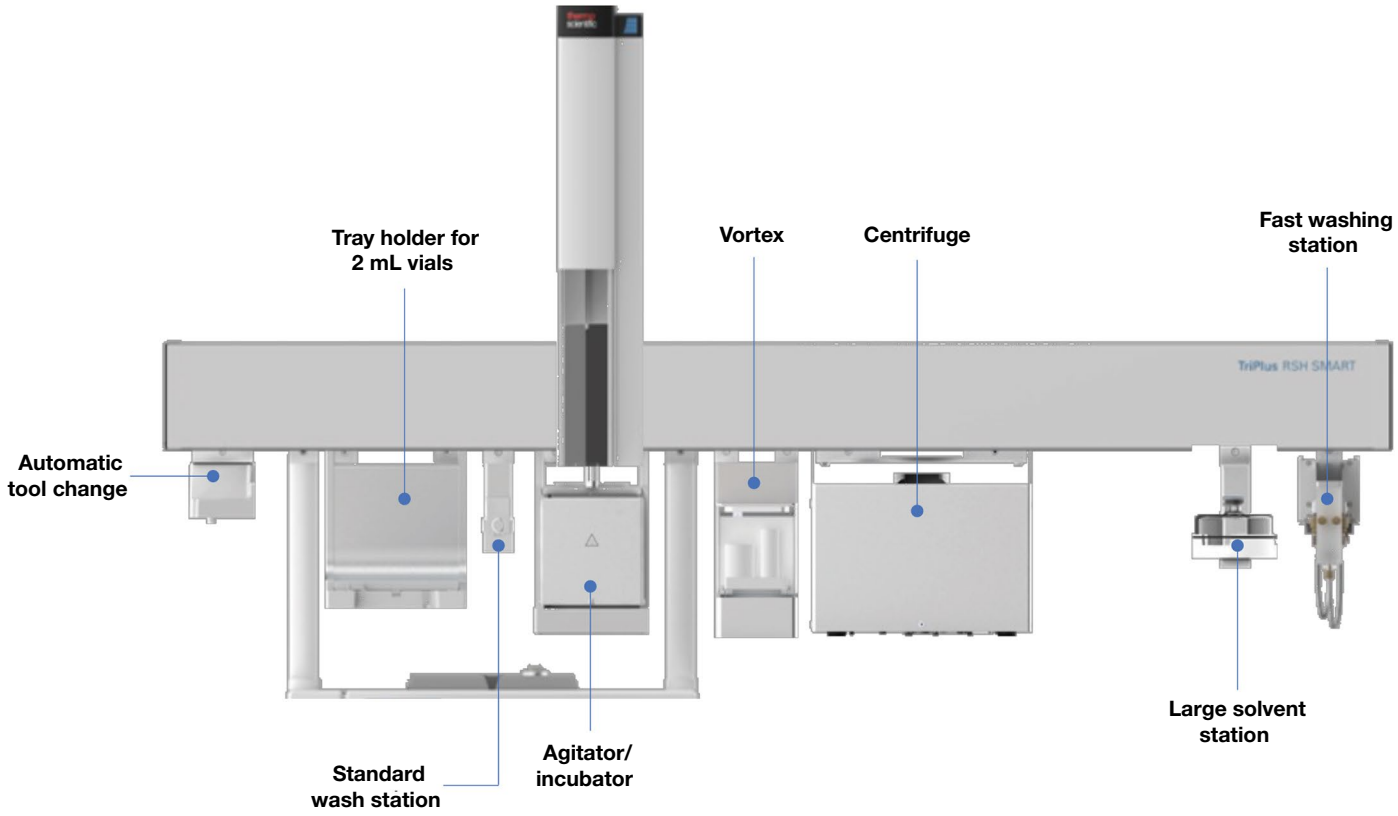


Figure 7. TriPlus RSH SMART Extended configured for automated sample preparation. More details are reported in the Appendix.

Epoxidation

Epoxidation allows removal of biogenic unsaturated hydrocarbons such as terpenes, carotenes, and squalene. This step is critical to remove poly-unsaturated hydrocarbons, which would interfere with the MOAH fraction. It can be combined with the saponification step and is recommended for almost all edible oils and fats, fruit, and vegetables. An example of a crude palm oil sample analyzed with and without epoxidation is reported in Figure 8, showing the comparison of the MOAH channel chromatograms.

The epoxidation reaction is executed with an organic peroxide,[3] which converts non-aromatic double bonds into the more polar epoxide ring, enabling their LC-based separation from the MOSH and MOAH fractions. The reaction conditions need to be controlled to avoid incomplete epoxidation or a too aggressive reaction potentially attacking the MOAH fraction. In this case, the decrease of the perylene peak (used as an IS) is symptomatic. Figure 9 reports a comparison of olive oil (OO) and extra virgin olive oil (EVOO) samples analyzed with epoxidation, checking the absence of the typical squalene peak present in the MOAH chromatogram for non-epoxidized olive oil samples. The intense peak visible at 14.5 min in the EVOO sample is unreacted squalene, indicating an incomplete epoxidation reaction and making a correct MOAH quantification impossible. The OO sample in turn does not show a peak corresponding to squalene, with a normally shaped peak not impacting the results. The perylene peak remains present in good recoveries in both samples, ensuring no MOAH was destroyed during epoxidation.

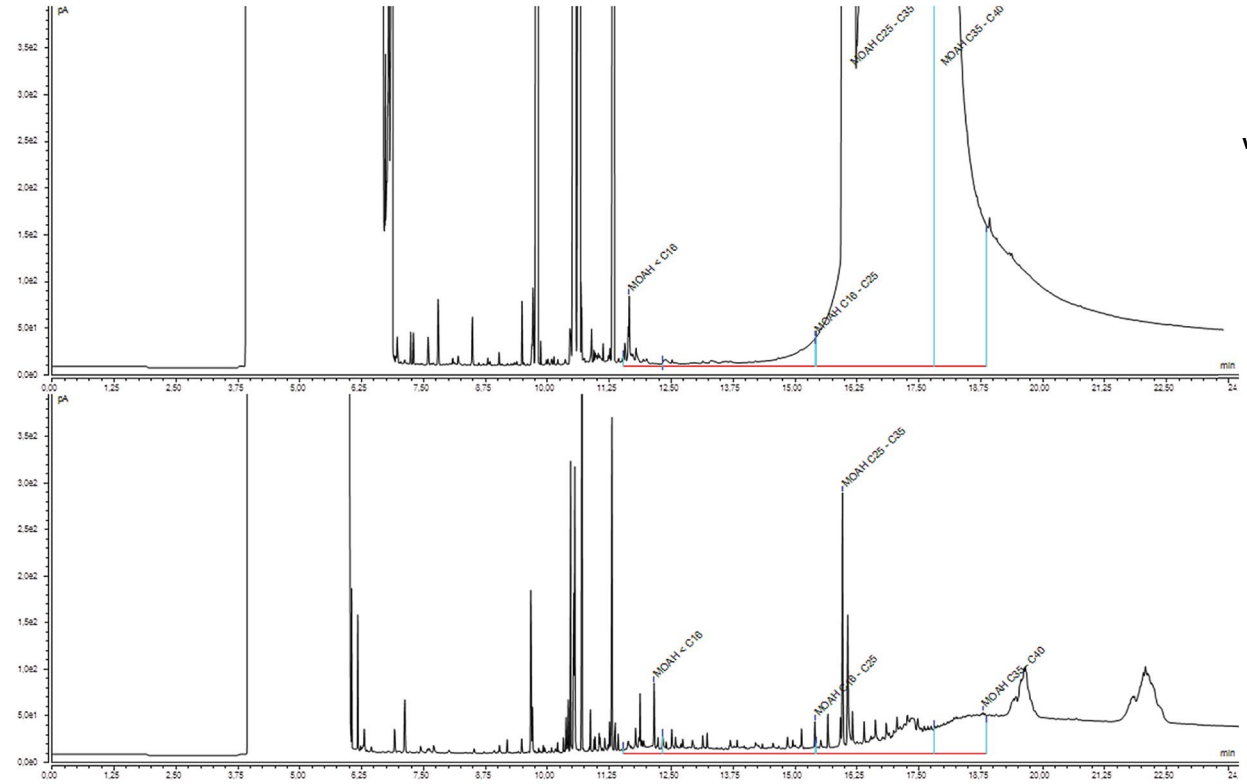


Figure 8. Example of MOAH chromatograms for crude palm oil sample with and without epoxidation

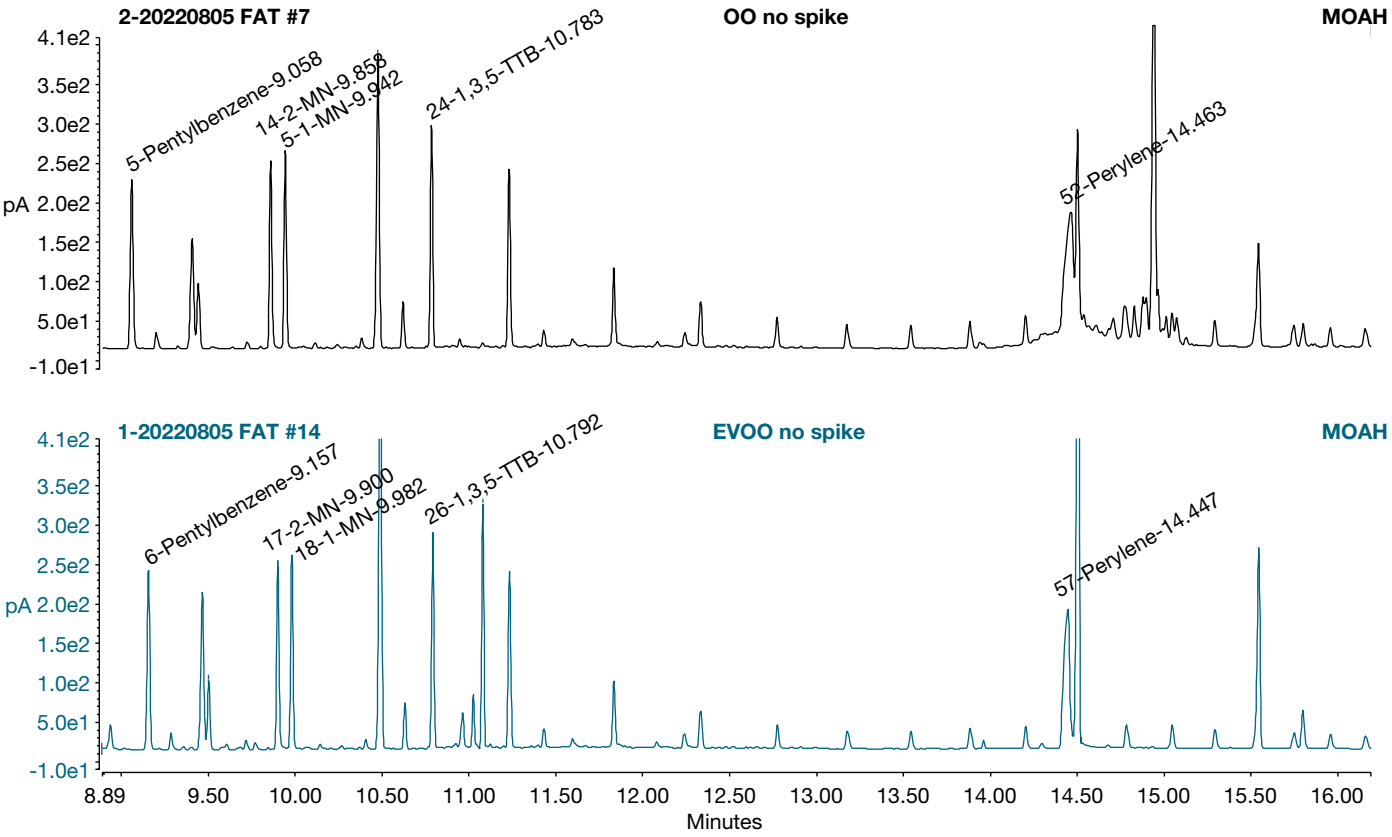


Figure 9. MOAH chromatograms of epoxidized OO (black trace) and EVOO (blue trace) samples

The automated sample preparation workflow executed by the TriPlus RSH SMART autosampler for the epoxidation reaction follows the flowchart summarized in Figure 10. The script is provided by SampleQ.

The reliability of the workflow, including the epoxidation, was evaluated by preparing and analyzing olive oil samples in duplicate and determining the resulting RSD% between replicate measurements, as reported in Table 2.

Table 2. Repeatability of the spiked olive oil samples

Samples	RSD MOSH (C10-C50) %	RSD MOAH (C10-C50) %	RSD total oil (C10-C50) %
EVOO spike low	1.30	4.02	2.02
EVOO spike high	2.67	0.18	1.92
OO spike low	1.10	1.94	1.37
OO spike high	0.29	1.41	0.64

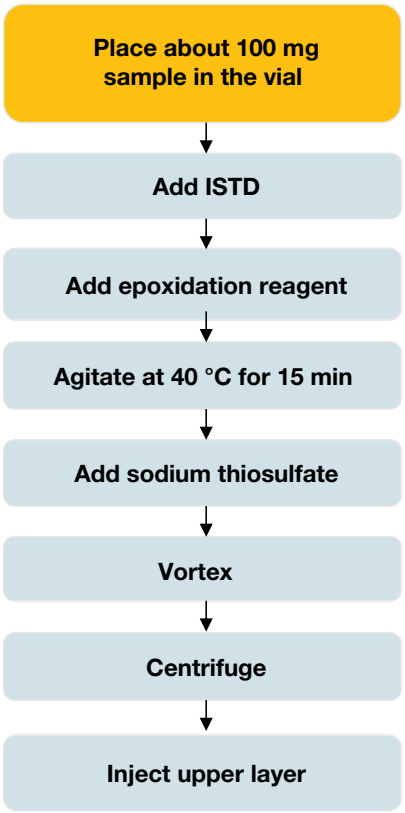


Figure 10. TriPlus RSH SMART automated sample preparation workflow for epoxidation

Configuration optional building blocks

The basic LC-GC/FID configuration can be expanded with additional modules to meet specific analytical requirements.

Aluminum oxide (Alox) clean-up module

Alox clean-up is useful for removal of natural *n*-alkanes present in food, which would interfere with the MOSH fraction. This is achieved in a secondary LC column packed with deactivated aluminum oxide. This optional module requires an additional LC pump and an HPLC column compartment with two extra valves. It is recommended for fruit, vegetables, and many common oils and fat and is required with almost all oils/fats in combination with saponification. The MOAH chromatogram is not impacted, while MOSH results are more accurate in case of presence of *n*-alkanes (Figure 11). However, it is not recommended in the absence of *n*-alkanes.

Fraction collector

The addition of the fraction collector implies another channel to the transfer valve in the auxiliary oven. It allows the collection of MOSH and/or MOAH fractions for off-line analysis on a separate GC or GC-MS instruments. If required, the fraction collection is fully automated in the instrument method.

GCxGC and MS

To get more information about the nature of the detected mineral oil, higher selectivity and identification capabilities can be added to the gas chromatographic system. In this case an additional GC or GC-MS can be added equipped with the GCxGC flow modulator.

GCxGC increases the separation power by adding a second dimension to the GC separation. It generates a longer analysis time but a greater insight of the composition of the mineral oil fractions, especially when coupled to a mass spectrometer, facilitating the identification of false positives.

Data acquisition and control software

Chromeleon CDS

The entire system is fully controlled by Chromeleon CDS, including the automated sample preparation workflow executed by the TriPlus RSH SMART autosampler. Customizable e-panels offer easy control of each module—robotic autosampler, GC, GC-MS, aux oven, LC system—giving a direct control of the most critical components (Figure 12).

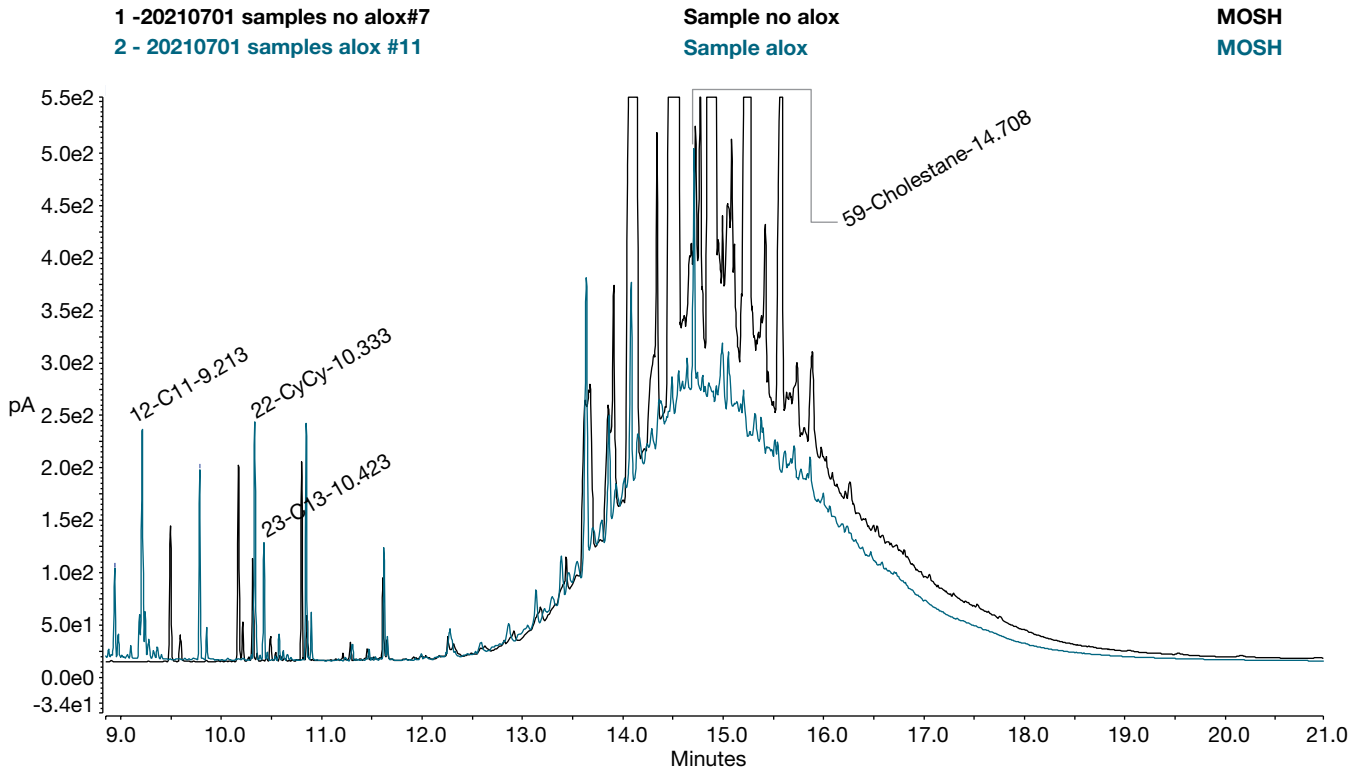


Figure 11. MOAH chromatogram before (black trace) and after Alox clean-up (blue trace)

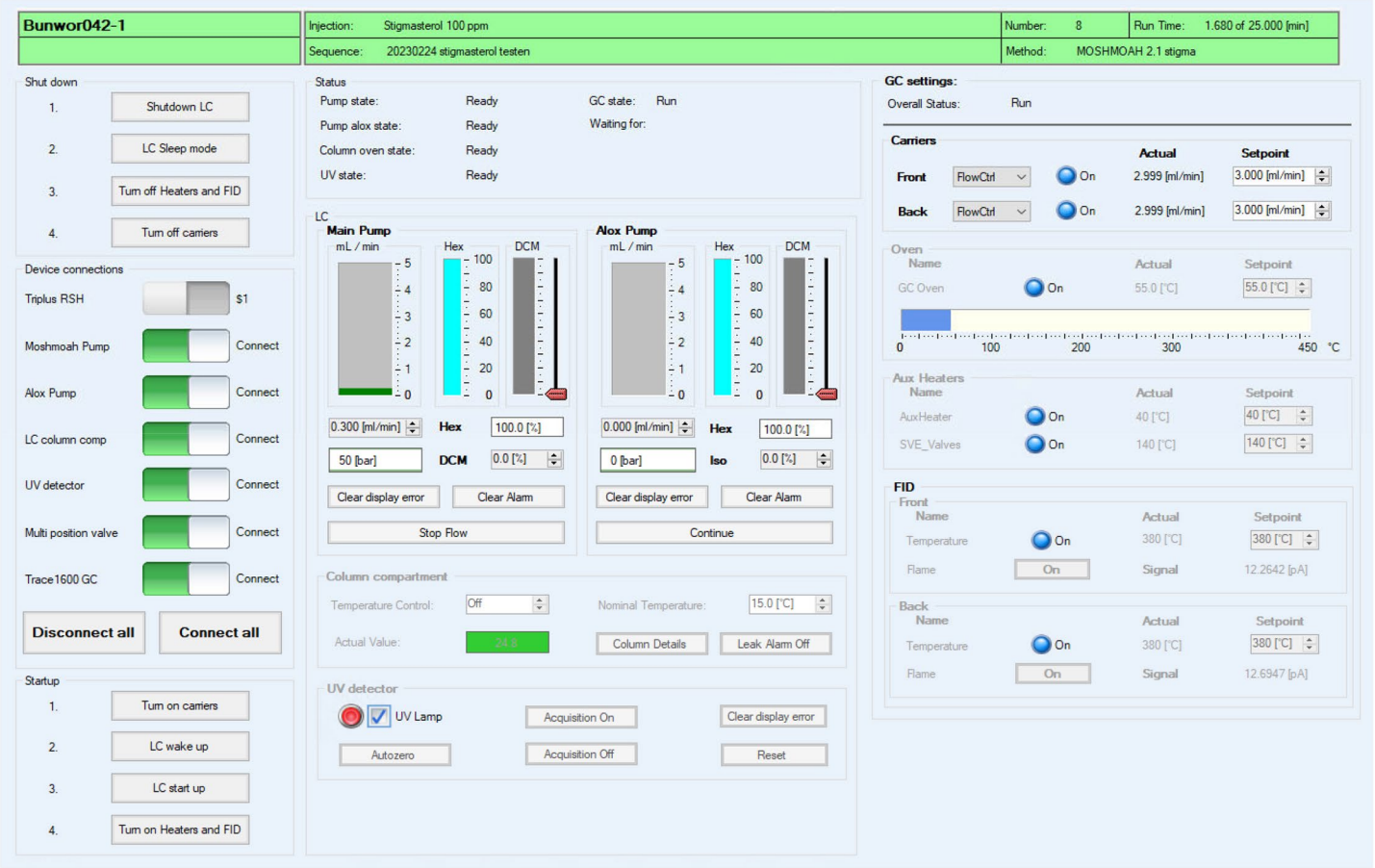


Figure 12. Chromeleon CDS e-panel

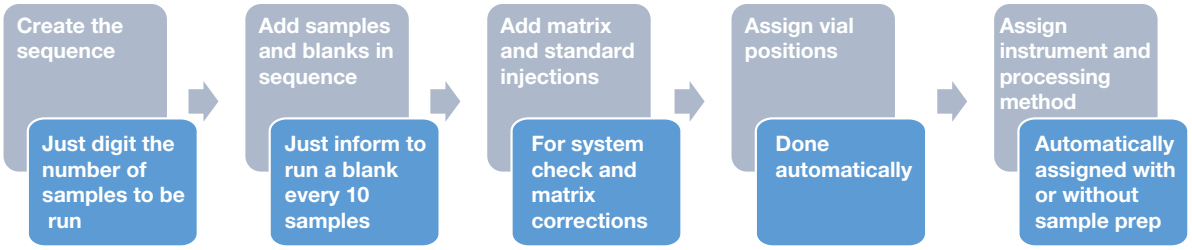


Figure 13. Steps required in a manual sample sequence set-up (grey box), which are fully automated in the e-Workflow (blue box)

Chromeleon CDS e-Workflow

The sample sequence creation is typically a time-consuming process with different steps that may lead to mistakes if done manually (Figure 13). The use of the Chromeleon CDS e-Workflows™ greatly facilitates this process with automated sequence set up. The sample sequence can be created in just three clicks, thanks to automated assignment of vial positions, injection types, and instrument and processing methods, saving time and preventing possible errors.

Reporting

Reporting of the results is fully automated with no need for an external calculator or spreadsheet-based data processing file. The report template is provided and includes MOSH and MOAH fractions quantitation (mg/kg), as well as internal standard recovery, as shown in Figure 14.

The reports are fully customizable to meet specific requirements and can be exported in .pdf, .csv, .txt, or any format that suits the established LIMS system.

Results

Peak name	Retention Time Start	End	Area Uncorrected	Area UCM	Area Matrix corrected	ISTD factor	Amount mg/kg
MOSH C10-C16	7.090	11.532	24.887	2.646	2.646	0.124	0.329
MOSH C16-C20	11.532	12.743	1.277	0.497	0.497	0.124	0.062
MOSH C20-C25	12.743	13.943	9.806	9.774	9.774	0.124	1.214
MOSH C25-C35	13.943	15.773	76.650	60.158	60.158	0.124	7.474
Sum MOSH C16-C35	**12.743**	**15.773**	**86.456**	**69.931**	**69.931**	**0.124**	**8.750**
MOSH C35-C40	15.773	16.500	4.707	4.707	4.707	0.124	0.585
MOSH C40-C50	16.5	17.638	3.894	3.894	3.894	0.124	0.484
Sum MOSH C10-C50	7.09	17.638	121.221	81.675	81.675	0.124	10.147

Peak Name	Retention Time Start	End	Area Uncorrected	Area UCM	Area Matrix corrected	ISTD factor	Amount mg/kg
MOAH C10-C16	8.500	11.532	41.892	5.552	5.552	0.120	0.666
MOAH C16-C20	11.532	12.743	0.803	0.514	0.514	0.120	0.062
MOAH C20-C25	12.743	13.943	0.360	0.209	0.209	0.120	0.025
MOAH C25-C35	13.943	15.773	21.004	3.649	3.649	0.120	0.438
Sum MOAH C16-C35	**11.532**	**15.773**	**22.167**	**4.372**	**4.372**	**0.120**	**0.525**
MOAH C35-C50	15.773	17.683	1.513	1.253	1.253	0.120	0.150
MOAH >C50	16.500	25.000	17.150	17.150	17.150	0.120	2.058
Sum MOAH C10-C50	8.5	17.683	87.740	15.550	15.550	0.720	1.341

Peak Name	Retention min	Area pA*min		Rel.Area (CyCY) %	Amount ppm	Recovery %
C11	7.917	8.425		104.67	1.05	104.67%
CyCY	9.893	8.049		100.00	1.00	100.00%
C13	10.058	4.178		51.90	0.52	103.80%
Cholestane	14.508	16.492		204.89	2.05	102.44%

Peak Name	Retention Tim min	Area pA*min		Rel.Area (2-MN) %	Amount ppm	Recovery %
Pentylbenzene	9.173	8.635		103.63	1.04	103.63%
2-MN	9.940	8.332		100.00	1.00	100.00%
1-MN	10.022	8.686		104.24	1.04	104.24%
1,3,5-TTB	10.815	8.652		103.84	1.04	103.84%
Perylene	14.512	16.869		202.44	2.02	101.22%

Figure 14. Example of automated MOSH/MOAH reports in Chromeleon CDS

Conclusion

Overall, hyphenated LC-GC is a valuable analytical tool for the analysis of MOSH and MOAH in food and beverages, offering high sensitivity, selectivity, and separation power to detect low levels of these critical contaminants in complex matrices. The use of a validated method and appropriate quality control procedures is necessary to ensure the accuracy and reliability of the results.

The MOSH/MOAH analyzer is modular and expandable. Starting from a basic configuration, additional optional modules are available to meet different analytical requirements, ensuring the best approach for QA/QC labs as well as research laboratories.

Automated sample preparation with a robotic autosampler before LC-GC analysis facilitates otherwise time-consuming procedures necessary to enhance sample clean-up and meet the required LOQ imposed by the official regulations, while ensuring accurate and highly precise results.

The use of a single software for instrument control, data acquisition, data processing, and reporting greatly facilitates operations, making a very complex analysis much easier. Customizable control panels and reporting are available to meet specific needs. Long and articulated sample sequences can also be set up very quickly using the Chromeleon e-Workflow, saving time and removing possible input errors.

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Appendix 1

Table A1. MOSH/MOAH analyzer - Basic LC-GC configuration with automated saponification and epoxidation workflow

Autosampler	**TriPlus RSH SMART Extended equipped with the following tools**
	• Heater/Agitator
	• Centrifuge
	• Vortex
	• Fast washing station
	• Dilutor
	• Trayholder for 2 mL vials
	• Liquid syringe tool 100 µL
	• Liquid syringe tool 1 mL
	• Large solvent station
	• Automatic tool change
HPLC	**Vanquish HPLC – Normal phase**
	• Degasser SRD-3200
	• LC Pump HPG-3200 SD
	• UV detector VWD-3100
Heated valves box	**TRACE 1600 Auxiliary Oven**
	• LC 6-port injection valve with 100 µL sample loop
	• 6-port backflush valve
	• 10-port transfer valve
	• Restek™ Allure™ silica gel LC column
Gas chromatograph	**TRACE 1610 GC**
	• Injector: 2x Thermo Scientific™ iConnect™ Solvent Vapor Exit modules
	• Detector: 2x Thermo Scientific™ iConnect™ FID modules
	• 2x Column set: Deactivated 10 m, 0.53 mm i.d. metal precolumn connected to MXT-1 15 m, 0.25 mm i.d., 0.1 µm
	• Hydrogen sensor

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