GERSTEL AppNote Nr. 228

Automated Analysis of Mineral Oil Hydrocarbons from Food using a novel LC-GC-FID Coupling Platform

Fred D. Foster[1], John R. Stuff[1], and Karsten B. Schwarz[2]

[1]GERSTEL, Inc. 701 Digital Drive, Suite J Linthicum, MD 21090, USA
[2]GERSTEL, GmbH & Co. KG, Eberhard-Gerstel-Platz 1, 45473 Mülheim a.d.R., Germany

Keywords

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Abstract

Mineral oil hydrocarbon (MOH) contaminants can be found in foods such as cereals, baked goods, fats and oils, coffee and many more. MOH can be introduced through process aids, additives, and machine- and lubricating oils used during food processing. Packaging such as jute bags used to store and transport foods, recycled cardboard, and printing inks are other major sources of MOH contamination. MOH are separated into Mineral Oil Saturated Hydrocarbons (MOSH) and Mineral Oil Aromatic Hydrocarbons (MOAH). Some MOAH are known carcinogens and MOSH are known to accumulate in human body tissue. Subsequently, food products should be analyzed and monitored for their presence.

Determination of MOSH and MOAH in food extracts involves the use of a coupled LC-GC-FID system. The analysis system used for the work presented here includes a GERSTEL Early Vapor Exit in which excess HPLC eluent is removed from the MOSH and MOAH fractions before they are transferred to their respective GC separation columns. Accurate and reproducible analysis results for MOSH and MOAH in various food extracts are achieved very efficiently using the GERSTEL MOSH/MOAH software to integrate the unresolved complex mixtures.

Introduction

Food producers, contract laboratories, and Food Safety Inspection agencies analyze food samples for the presence of Mineral Oil Saturated Hydrocarbons (MOSH) and Mineral Oil Aromatic Hydrocarbons (MOAH) using an online LC-GC-FID strategy first described by Biedermann and Grob [1] and in accordance with the regulatory method DIN EN 16995:2017-08 [2]. Briefly, hexane extracts of food samples, packaging material or other matrices are separated into MOSH and MOAH fractions using normal phase LC. These fractions are transferred online to separate GC inlets connected to analytical GC columns with uncoated guard columns used as retention gaps. The carrier gas stream removes excess elution solvent from the GC-FID system via an Early Vapor Exit (EVE) system. Volatile analytes are retained in the analytical system by "solvent trapping". Higher boiling analytes are distributed over the entire length of the guard columns and refocused at the beginning of the GC analytical columns using the retention gap technique. Following the eluent evaporation step, the vapor exit is closed and the MOSH and MOAH fractions are analyzed simultaneously by gas chromatography using two separate GC columns connected to flame ionization detectors (FIDs).

After collecting the data, the analyst is normally required to painstakingly integrate the peaks arising from naturally occurring hydrocarbons present in the food sample and then subtract their areas from that of the unresolved complex mixture to determine the amounts of MOSH and MOAH present in the food sample. This step can be extremely time consuming and can lead to differences in results when different analysts perform the manual integrations. Software automation of the integration of MOSH/MOAH data can not only provide higher throughput for the analysis but also better reproducibility by eliminating differences in data handling. The GERSTEL MOSH/MOAH Data Processing Software uses dedicated algorithms developed specifically for hump- and peak detection to ensure efficient data processing as well as accurate results and reporting. Manual adjustments and reintegration are easily performed at any time.

Experimental

Materials

The MOSH/MOAH standard was purchased from Restek® as a nine-component mixture that contains non-interfering internal standards as well as both MOSH and MOAH markers used to ensure that the LC fractions are cut correctly. The MOSH/MOAH retention time standard was purchased from Restek as well with ten alkanes that are used to accurately identify GC retention time limits for the integration. The GERSTEL Mineral Oil Reference Standard A was used to check system performance. All other reagents and solvents used were reagent grade.

Boxed dried food products (macaroni, quick oats, breadcrumbs, and raisins) were purchased from a local market.

Sample Preparation.

Mineral oil reference standard

- Aliquots of 2 g MOH reference standard A were weighed into separate glass 20 mL vials.
- A 10 mL volume of n-hexane was added to each vial using a glass graduated cylinder.
- A 10 µL aliquot of the MOSH/MOAH standard sample was added to each vial.
- The vials were vortexed for 30 s.
- Using glass pipettes, aliquots of the diluted samples were transferred into 2 mL autosampler vials, which were sealed and placed in the MPS for injection into the MOSH/MOAH LC-GC-FID system.

Boxed dried food products

- Separate 5 g samples of ground macaroni, quick oats, breadcrumbs, and minced raisins were weighed into 20 mL glass vials.
- A 10 mL volume of n-hexane was added to each vial using a glass graduated cylinder.
- A 10 µL aliquot of the MOSH/MOAH standard was added to each vial.
- The samples were vortexed for 30 seconds.
- The samples were agitated at 750 rpm for 18 hours at 55 °C.
- The samples were again vortexed for 30 seconds.
- The samples were centrifuged for 3 minutes at 1500 rpm (5 minutes at 3000 rpm for quick oats) to settle particulate matter.
- Aliquots of the hexane extracts were transferred into separate 2 mL glass autosampler vials using glass pipettes. The vials were sealed and transferred to the MultiPurpose Sampler for injection into the MOSH/MOAH LC-GC-FID system.

Recycled cardboard products

- Several 1 g samples of recycled cardboard box material (cut into 1 cm x 2 cm pieces) were weighed into separate 20 mL glass vials.
- A 10 mL volume of a (1:1) n-hexane-ethanol solution was added to each vial using a glass graduated cylinder.
- A 10 µL aliquot of the MOSH/MOAH standard was added to each vial.
- The samples were vortexed for 30 seconds.
- The samples were agitated at 750 rpm, for 2 hours at 30 °C.
- A 5 mL volume of LCMS grade water was added to each vial.
- The samples were again vortexed for 30 seconds.
- The samples were centrifuged for 3 minutes at 1500 rpm to settle particulate matter.
- Using glass pipettes, aliquots of the supernatant, hexane extracts were transferred to 2 mL autosampler vials for analysis in the MOSH/MOAH LC-GC-FID system.

GERSTEL AppNote Nr. 228

Instrumentation

All analyses were performed using a GERSTEL MultiPurpose Sampler (MPS) robotic configured with a GERSTEL LC-GC-FID system as shown in Figure 1. This system allowed the coupling of an Agilent 1260 Infinity II HPLC with a Restek Allure Silica column, (2.1 x 250 mm, 5 µm) and an Agilent 8890 GC with dual FID detectors. Sample injections were made using the GERSTEL MPS robotic into an injection valve with a 50 µL stainless steel sample loop. The system was operated using the MAESTRO software fully integrated into the ChemStation version of OpenLab CDS (C.01.10) from Agilent® Technologies.

Figure 1: GERSTEL MOSH/MOAH Sample Prep Solution LC-GC-FID system.

GERSTEL AppNote Nr. 228

LC Method Parameters

Mobile Phase: A – n-Hexane
B – Dichloromethane

LC Gradient:

Time (min)	Flow (mL/min)	Pressure (bar)	% B
0	0.3	200	1
0.1	0.3	200	1
1.5	0.3	200	35
5.9	0.3	200	35
6.0	0.3	200	100
15.0	0.5	200	100
15.3	0.5	200	1
25.0	0.5	200	1
25.2	0.3	200	1
30.0	0.3	200	1

Run time: 30 minutes.

Injection volume: 50.0 µL (loop over-fill technique)

UV wavelength: 230 nm

GC Method Parameters:

Pneumatics: H2, constant pressure
MOSH : 90 kPa (3.8 min); 30 kPa/min; 60 kPa (25.2 min)
MOAH : 90 kPa (5.8 min); 30 kPa/min; 60 kPa (23.2 min)

Retention Gap(s): Hydroguard-treated MXT guard column (Restek)

Column(s): 15 m MXT-1 (Restek)
di = 0.25 mm
df = 0.25 µm

Oven: 55 °C (10 min); 20 °C/min; 360 °C (4.75min)

FID: 380 °C

Results & Discussion

Figure 2 shows a representative overlay chromatogram of both FID traces (red trace = MOSH, green trace = MOAH) with the UV trace (blue trace) from an injection of a neat MOH standards mixture. In the UV trace, after 4.5 minutes the breakthrough of dichloromethane and therefore the start of the MOAH-fraction is observed as a steep increase in absorption to a plateau level. In this way, the UV trace is used to verify that the HPLC gradient conditions are being delivered properly. In addition, the elution of perylene was detected around 5 minutes, it is used to determine the end of the MOAH fraction elution from the HPLC column.

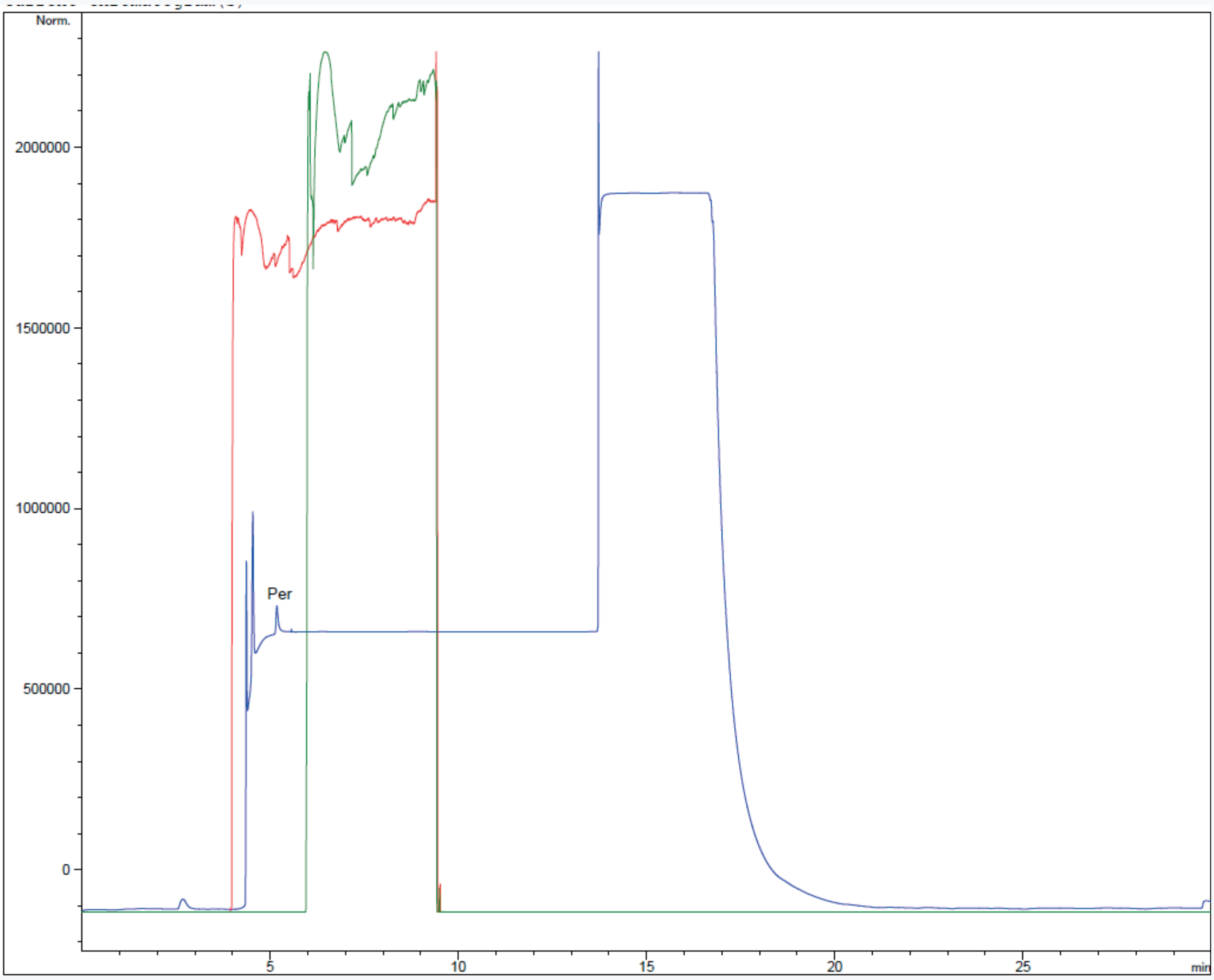

Figure 2: Representative LC-UV (blue trace) and FID chromatograms (red trace = MOSH, green trace = MOAH) from the MOSH/MOAH Sample Prep Solution system.

Figure 3 shows stacked FID chromatograms of MOSH and MOAH resulting from injection of a neat MOH standard mixture. In the top chromatogram, the MOSH fraction, the presence of n-decane (C10) or n-undecane (C11) are used to detect loss of volatile components during either transfer or re-concentration of sample extracts and to control the parameters for EVE-evaporation. Bicyclohexyl (CyCy) is the internal standard used for quantification of the MOSH fraction and n-tridecane is present to ensure absence of co-elution with the internal standard and as a marker for the identification in sample chromatograms. Cholestane (Cho) is used to indicate the end of the MOSH fraction and the remaining alkanes are present to enable reporting of results based on carbon chain length. In the bottom chromatogram, the MOAH fraction, the presence of pentylbenzene (5B) is used to detect loss of volatile components during evaporation (EVE). 1-Methylnaphthalene (1-MN) is the internal standard used for quantification of the MOAH fraction and 2-methylnaphthalene (2-MN) is used as a peak pair for easy identification of the internal standard. To verify the correct fractionation from the LC column, tri-tert-butylbenzene (TBB) is used to mark the beginning of the MOAH fraction and perylene (Per) marks the end of the MOAH fraction.

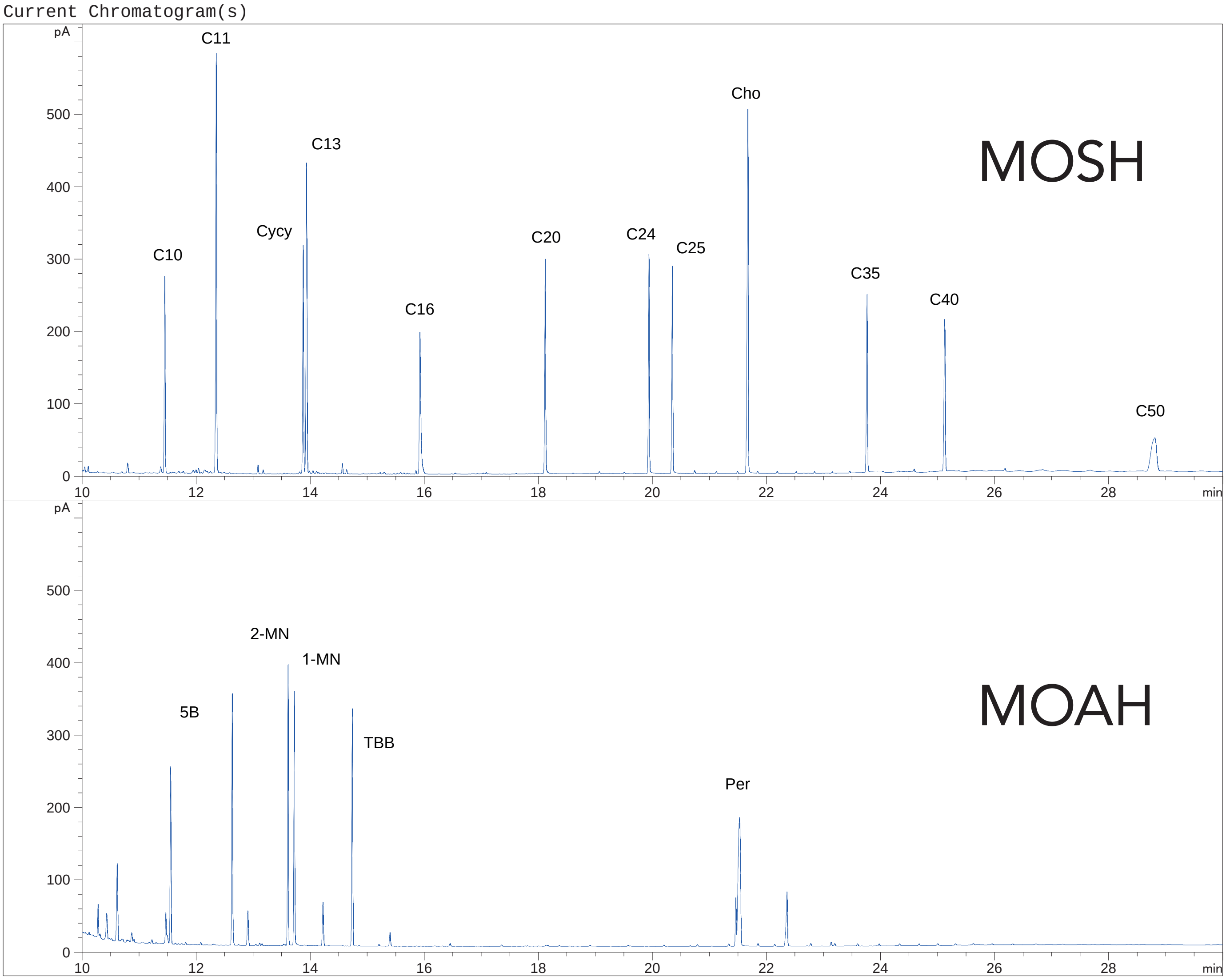

Figure 3: Stacked FID chromatograms of MOSH and MOAH fractions from a neat MOH standard mixture.

A MOH reference standard containing MOSH and MOAH at known concentrations in a triglyceride mixture is available from GERSTEL. Upon dilution in hexane and addition of the internal standard mixture, triplicate replicates of the MOH reference standard were injected into the MOSH/MOAH system. Figures 4 A-B show overlay chromatograms for MOSH (A) and MOAH (B) from the three replicates highlighting the reproducibility of the system.

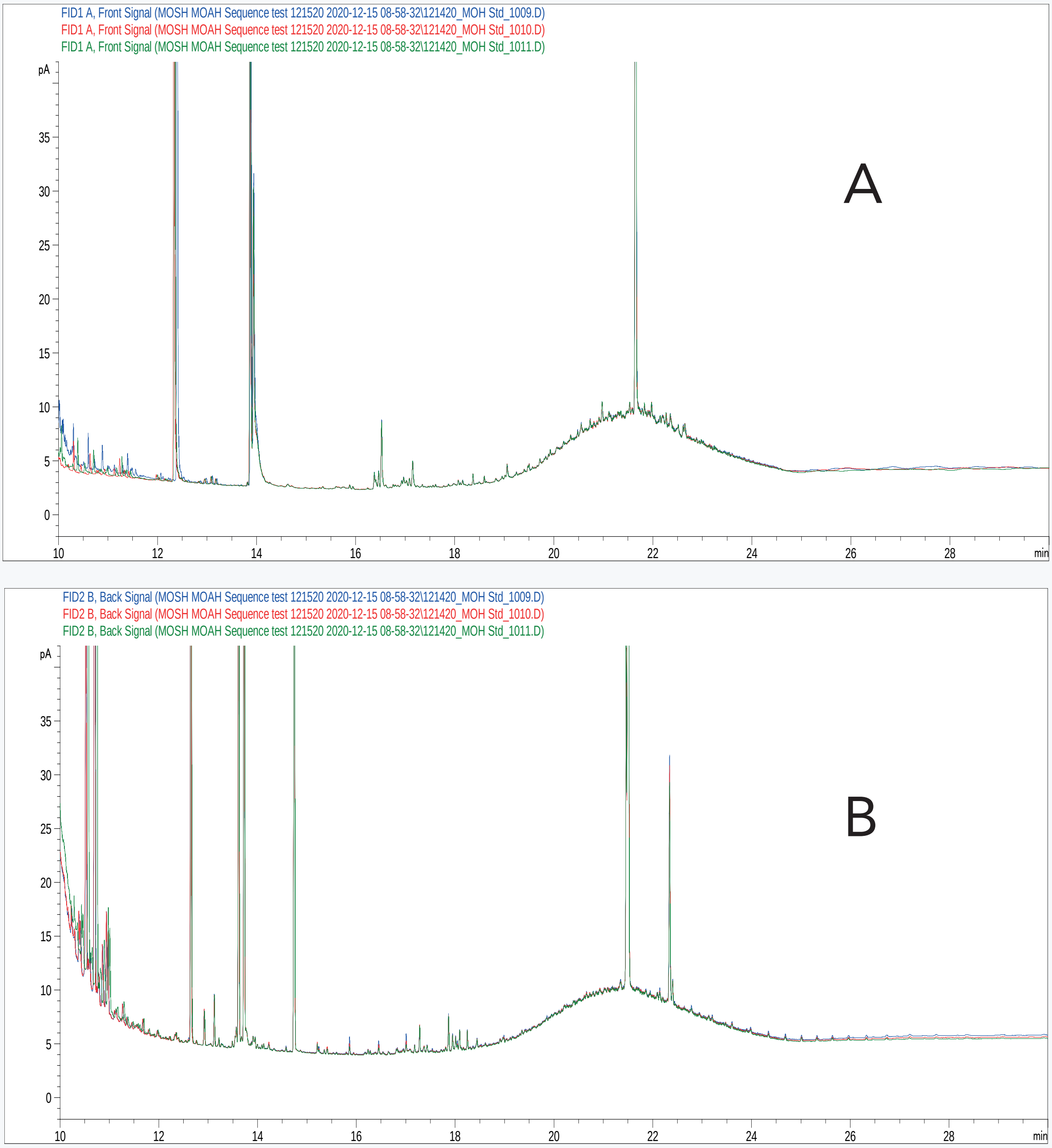

Figure 4 A-B: Overlaid chromatograms from MOH standard replicates for MOSH (A) and MOAH (B) demonstrating good reproducibility.

Example results from the GERSTEL MOSH/MOAH Data Analysis software are shown in Figures 5 A-B showing the integration of the unresolved complex mixtures for MOSH (A) and MOAH (B) from one of the replicates. The MOSH/MOAH Data Analysis software enables the efficient and reproducible integration of resulting MOSH/MOAH unresolved complex mixtures.

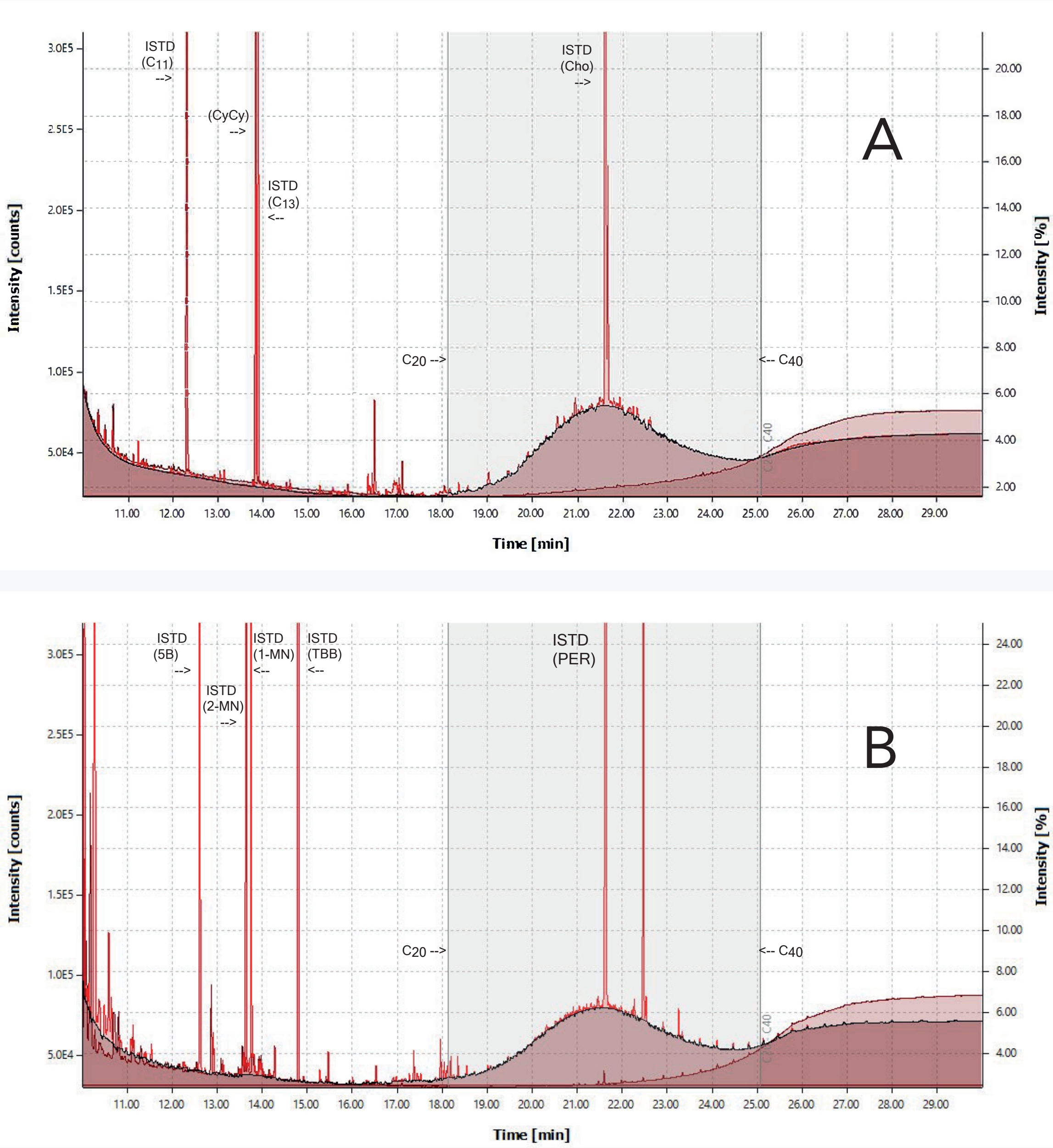

Figure 5 A-B: Representative integrated unresolved complex mixtures of MOSH (A) and MOAH (B) fractions using the GERSTEL MOSH/MOAH Data Analysis software. Blank run values, represented by the darker red areas, are subtracted from the areas of the unresolved complex mixtures when calculating the amount of MOSH and MOAH respectively.

Boxed food samples were extracted using the procedures described above. Triplicate hexane extracts from ground, dried macaroni, quick oats, breadcrumbs, and raisins were all submitted for analysis using the MOSH/MOAH system. Figures 6 A-D show the resulting overlay chromatograms for both the MOSH and MOAH fractions from the triplicate hexane replicates. The large unresolved complex mixture shown in the MOSH fraction of the breadcrumb samples particularly caught our attention.

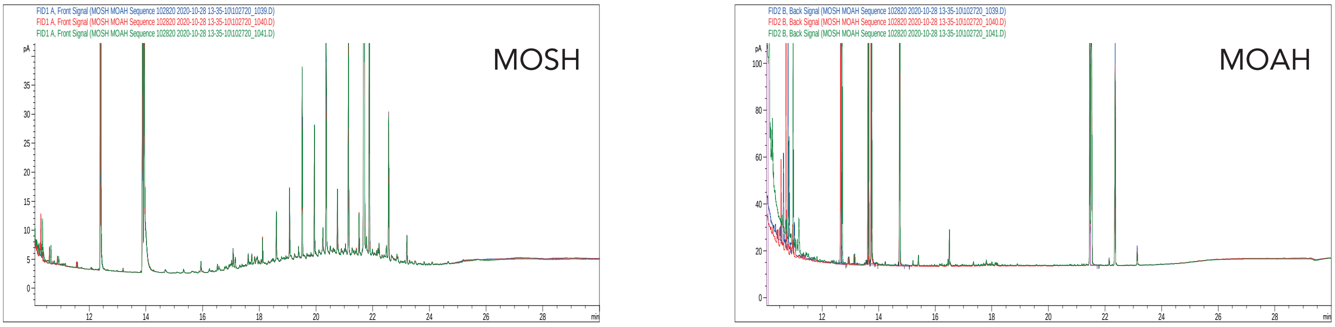

Figure 6A: Overlay MOSH chromatograms (left) and MOAH chromatograms (right) of three macaroni noodle extracts.

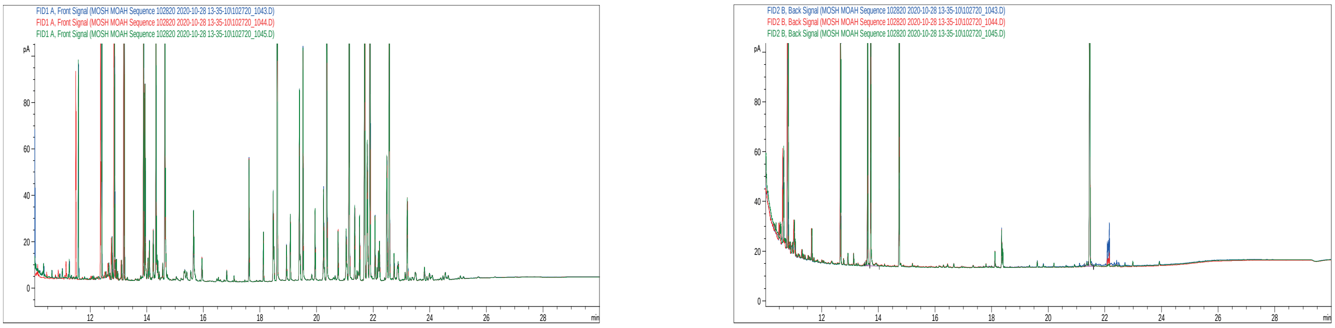

Figure 6B: Overlay MOSH chromatograms (left) and MOAH chromatograms (right) of three Quick Oats sample extracts.

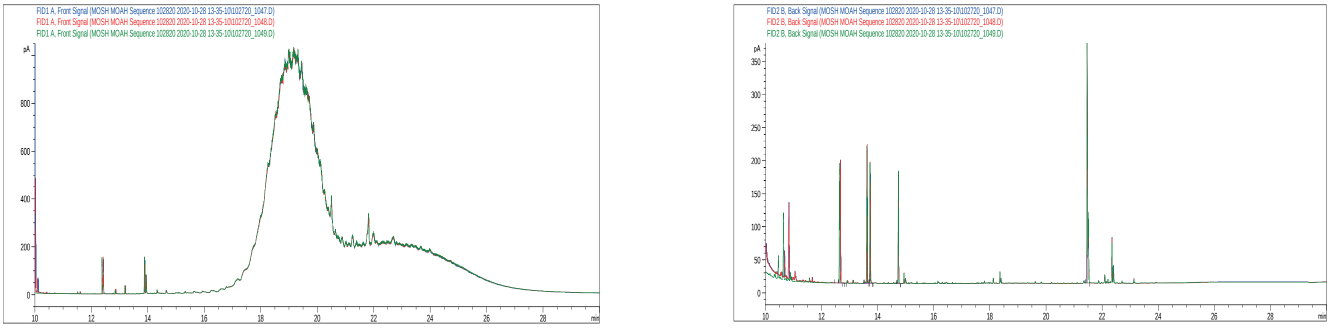

Figure 6C: Overlay MOSH chromatograms (left) and MOAH chromatograms (right) of three Breadcrumbs sample extracts.

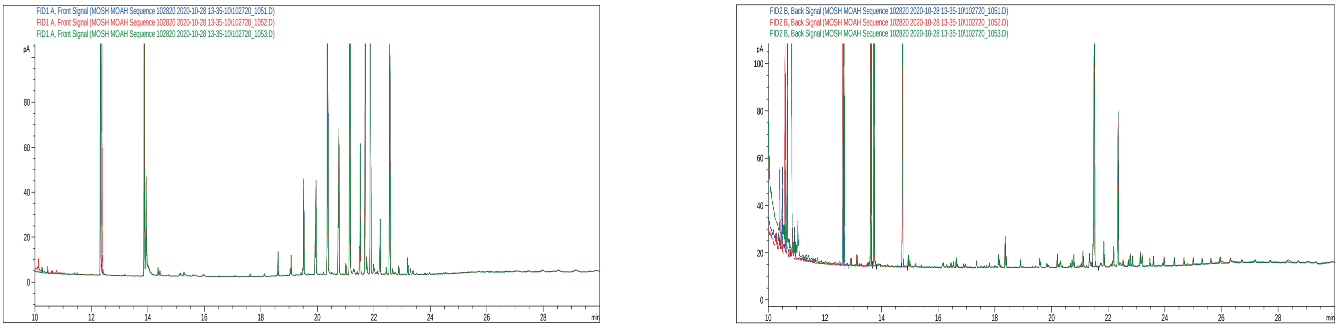

Figure 6C: Overlay MOSH chromatograms (left) and MOAH chromatograms (right) of three raisin sample extracts.

Figure 6 A-D: MOSH/MOAH chromatograms from extracted macaroni, oats, breadcrumbs, and raisin samples.

To verify that the unresolved complex mixture shown in the MOSH fraction of the breadcrumb samples was repeatable and not a result of sample contamination, three new breadcrumb samples from three new breadcrumb boxes were obtained, extracted as described above, and analyzed using the MOSH/MOAH system.

Figure 7 shows stacked chromatograms of the MOSH fraction from three different breadcrumb samples. The unresolved complex mixture from the breadcrumb samples was consistently detected in all breadcrumb samples analyzed.

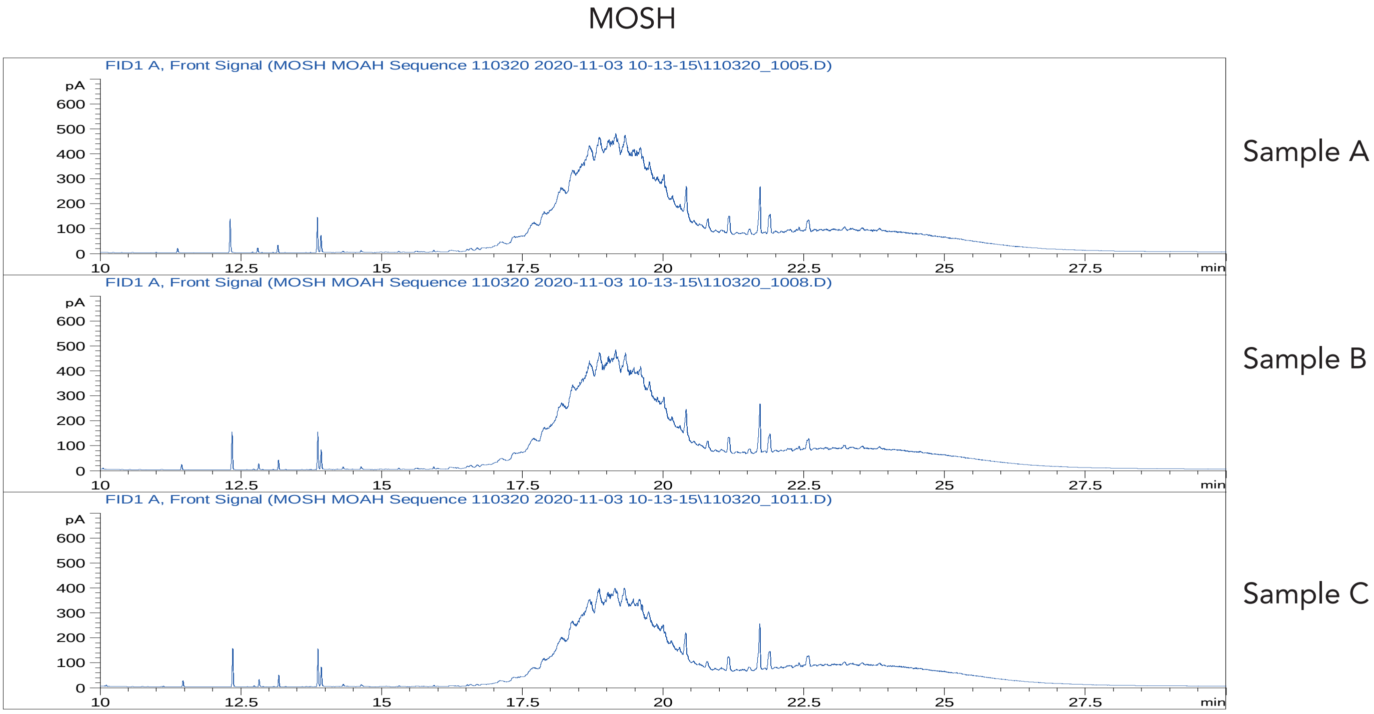

Figure 7: MOSH/MOAH results from breadcrumb samples from three separate boxes of the same brand.

Finally, the cardboard box of the breadcrumb sample was extracted using the procedure described above, the extract then further diluted 1:5 with hexane, followed by triplicate analysis of the hexane extract using the MOSH/MOAH system. The results were compared with the MOSH chromatograms from the breadcrumb extracts. Figures 8 A-B show the resulting overlay chromatograms of both the MOSH and MOAH fractions from triplicate injections of the hexane extract of the box that contained the breadcrumb sample. The resulting unresolved complex mixtures of the box extract replicates show similarities to those of the breadcrumb extracts, but also differences.

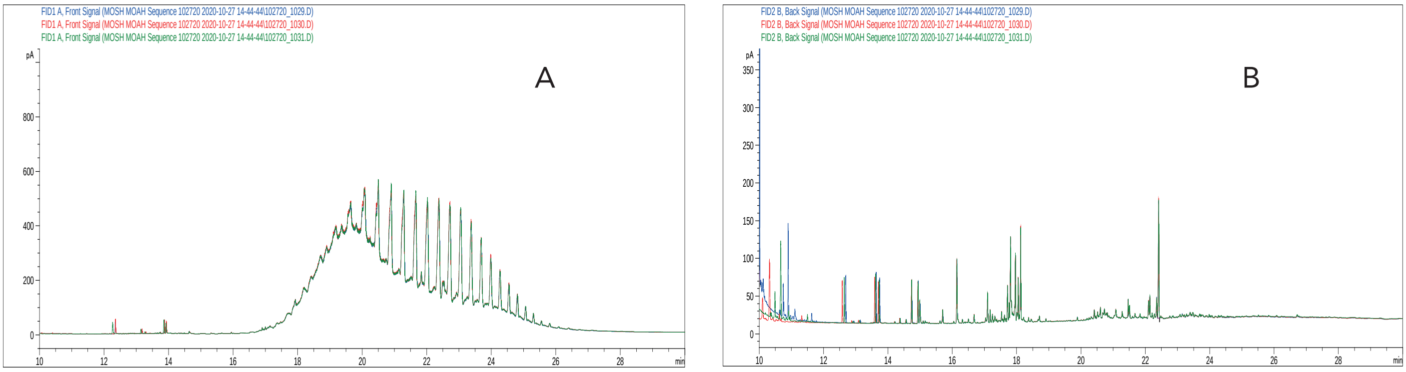

Figure 8 A-B: Overlaid chromatograms of the MOSH (A) and MOAH (B) fractions from triplicate hexane extracts of a breadcrumb sample box.

GERSTEL AppNote Nr. 228

Conclusions

As a result of this study, we were able to show:

- The GERSTEL LC-GC-FID system and the Early Vapor Exit (EVE) enable successful solvent trapping of the MOSH and MOAH fractions using just one integrated software and a single data acquisition method. For simplicity, the GC-FID traces and the LC-UV trace are all contained in a single data file.
- Mineral oil hydrocarbons fractions in hexane extracts of food samples and other matrices were successfully separated using normal phase LC, and each fraction subsequently transferred to and analyzed by the dual channel GC-FID.
- The GERSTEL MOSH/MOAH Data Analysis software enables the efficient and reproducible automated integration of the resulting MOSH/MOAH unresolved complex mixtures.

References

[1] M. Biedermann, K. Fiselier, and K. Grob, "Aric Hydrocarbons of Mineral Oil Origin in Foods: Method for Determining the Total Concentration and First Results", J. Agric. Food Chem. 2009, 57, 8711-8721.

[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; English version EN 16995: 2017, English translation of DIN EN 16995:2017-08.