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Key Words: Mineral Oil, MOSH, MOAH, *Pegasus* BT 4D, GCxGC-TOFMS

Introduction

Mineral Oil Hydrocarbons (MOH) have become a hot topic in several fields such as food, packaging, and cosmetics. Several large scandals have emerged over the past few years on MOH contamination; the most recent was in October 2019, when MOH contamination was found in infant formula. Therefore, the demands for a reliable and robust workflow for the identification and quantification of MOH is urgent. In fact, official control laboratories, commercial laboratories, private industries, and non-governmental organizations are all seeking harmonization of the methodologies and protocols employed for analysis of these contaminants.

MOH are unquestionably sourced from different types of petroleum fractions which are named according to specific use cases: paraffinum liquidum, paraffin, petrolatum, mineral oil, paraffin oil, and ozokerite, to name a few. The uptake for the consumer may come from different sources. For instance, they can be used as additives in food, laxatives or lubricants in medicine, emollient in consumer products, or printing inks and packaging materials for food contact materials.[1-3]

MOH are classified into two main sub-classes: MOSH (Mineral Oil Saturated Hydrocarbons) including linear, branched, and alkyl substituted cycloalkanes, and MOAH (Mineral Oil Aromatic Hydrocarbons) including mainly alkyl-substituted (poly) aromatic hydrocarbons such as alkyl benzenes and alkyl naphthalenes.

To date, the reference method for analysis of MOH contaminants is the hyphenated liquid-gas chromatography system coupled with flame ionization detection (LC-GC-FID), initially proposed by Biederman et al. in 2009.[4] Although this is largely used across many laboratories, the question about the reliability of the data produced employing this technique is still open.[5-7] In fact, the lack of a confirmatory method using a selective detector (i.e. mass spectrometry), validated protocols, and standardized interpretation and integration of the final chromatograms are constantly feeding the debate regarding the requirement of more accurate, robust, and reliable methods. In this respect, it was reported that the interpretation and integration of the chromatograms account for ~20% of the uncertainty in the determination of MOSH and MOAH.[8] This high percentage can be associated to many variables such as: the nature of the chromatograms, which are unresolved humps rather than single peaks, the need to quantify the hump in an extended carbon range (up to C50) resulting in its elution on top of the column's bleed, and/or the need to subtract the peaks riding on top of the hump that derive from interferences. These factors leave much space for interpretation.

In this application note we describe a novel workflow that supports the reduction of the uncertainty related to data integration and interpretation, resulting in reliable and robust results.

An LC-GCxGC-TOFMS/FID system has been used in combination with a new ChromaTOF® brand software version, designed and implemented for the MOSH/MOAH application (Figure 1).
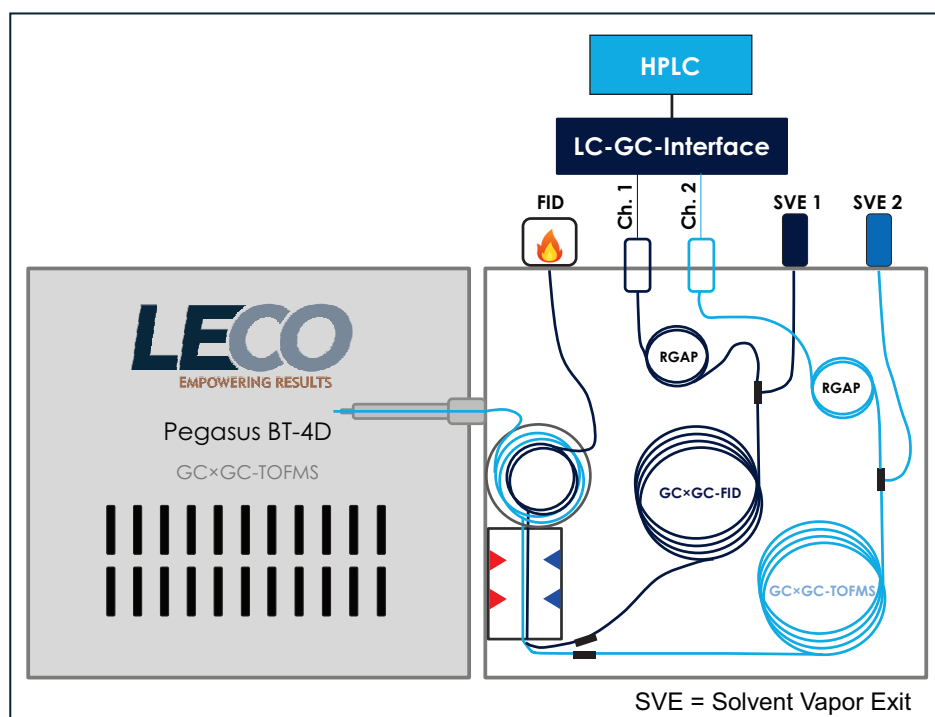

Figure 1. LC-GCxGC-TOFMS/FID scheme.

Experimental

All the relevant experimental parameters regarding the LC-GCxGC-TOFMS/FID platform are reported in Table 1. MOSH and MOAH internal standards (Restek #31070) and Retention Time standards (Restek #31076) were used for quantification and to define the carbon fractions according to published guidelines.[9,10]

A solution constituting of a paraffin oil (Supelco #18512) and an alkane mixture (C8-C40, Supelco) was used as reference sample to verify the system performance. Two animal feed samples provided by a collaborator were also analyzed using the platform in 1D and 2D mode.

Data processing was fully performed within the *ChromaTOF* environment, thanks to a special software version dedicated to MOSH/MOAH.

The MOSH and MOAH fractions of the same sample were simultaneously transferred, one to the FID and the other to the *Pegasus* BT 4D TOFMS. Therefore, a total of two analyses for each sample are required to acquire both GCxGC-TOFMS and GCxGC-FID signals from the same fraction.

Table 1. Instrument Parameters

LC	1260 Infinity II (Agilent)
Column	Allure Silica 250 mm x 2.1 mm i.d. x 5 µm (Restek #9160572)
Detector	Variable Wavelenght Detector at 230 nm
Conditions	Solvent A: Hexane; Solvent B: Dichloromethane. 0 min→100% A; 1.5-6 min →65% A @ 0.3 mL/min @ 6.10 min backflush → 100% B at 0.5 mL/min (9 min)
Interface	CHRONECT LC-GC (Axel Semrau)
Transfer conditions	MOSH = 2-3.5 min and MOAH = 4.4-5.9 min (450 µL each fraction)
Transfer gas pressures	90 kPa MOSH and 110 kPa MOAH
GC	LECO GCxGC QuadJet™ Thermal Modulator and FID
Columns	1D: Rxi-17SilMS, 15 m x 0.25 mm i.d. x 0.25 µm coating (Restek #14120) 2D: Rxi-1ms 2 m x 0.15 mm ID x 0.15 µm coating (Restek #43801)
Flows	2.5 mL/min @ TOFMS channel; 3.1 mL/min @ FID channel
Oven Program	60 °C (hold 8 min), ramp 8 °C/min to 350 °C (hold 5 min)
Secondary Oven	+5 °C (relative to the main oven temperature)
Modulator	+15 °C (relative to the secondary oven temperature)
Modulation Period	10 s
Transfer line	340 °C
MS	LECO *Pegasus* BT 4D
Ion Source Temp	250 °C
Mass Range	40-600
Acquisition Rate	200 spectra/s
FID Conditions	H_2: 30 mL/min; Air: 300 mL/min; Make-up gas (He): 25 mL/min Temperature: 360 °C

Results and Discussion

The scope of this application note was to verify the reliability, accuracy, and robustness of the data produced by the LC-GCxGC-TOFMS/FID system compared to the conventional LC-GC-FID data processed and integrated according to the recommendation given by the JRC.[9] Prior to any analysis, the system was checked for its performance by injecting the MOSH/MOAH retention time standard, confirming that the response factor of each component (i.e. C10 to C50) against the C20 was within the 0.8-1.2 range.

For the real-world samples, it appeared evident that the main limitation of the LC-GCxGC-TOFMS/FID system was the integration of the entire 3D hump generated by the MOSH/MOAH contaminations, in particular the removal of the riding peaks on top of it. In fact, most of the commercial GCxGC software options on the market have algorithms based on 2D single peak integrations rather than whole unresolved signals. Additionally, in the case of single peak riding on top of the hump, the difficulty was in integrating only the portion on top of the hump, without removing the part laying below. Despite previous attempts at MOSH/MOAH quantification using GCxGC-FID for confirmation, as was suggested by the EFSA in 2012, this specific issue of riding peaks has never been properly addressed.[11-14]

To overcome these drawbacks, LECO's own software engineers developed a novel algorithm capable of "trimming the riding peaks" on top of a hump. The algorithm works with both 1D (GC-FID) and 2D (GCxGC-FID) data and allows for an automatic subtraction of a blank signal to exclude any contribution from the column bleed. In 1D, the algorithm automatically removes the well-shaped narrow peaks on top of the hump, retaining the hump signal through the creation of a new line appropriately interpolating the base of the trimmed peaks (Figure 2).
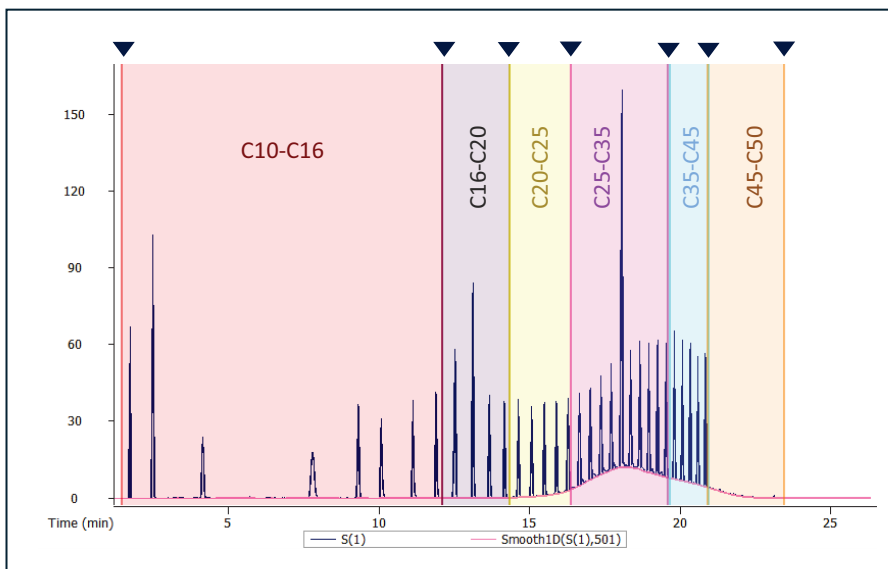
Figure 2. In blue, the trace of a spiked paraffin oil (Supelco #18512) with the alkanes mixture C8-C40 (Supelco). In pink, the smoothed trace created by ChromaTOF software, interpolating the base of the trimmed peaks.

In 2D, almost the same concept has been applied: *ChromaTOF* automatically reconstructs a 1D chromatogram for every 2D point and then removes the riding peaks with the same algorithm described above. Thus, a "clean" cloud is obtained, and the corresponding FID signal can be visualized either as a 1D or 2D chromatogram. In the first case, 1D reconstructed visualization, the user can perform a more traditional inspection of the data and evaluate the smoothing of the data. In the second case, 2D visualization, the user can take advantage of all the useful information still contained in the two-dimensional contour plot, and separately classify and quantify the MOAH subclasses (Figure 3).
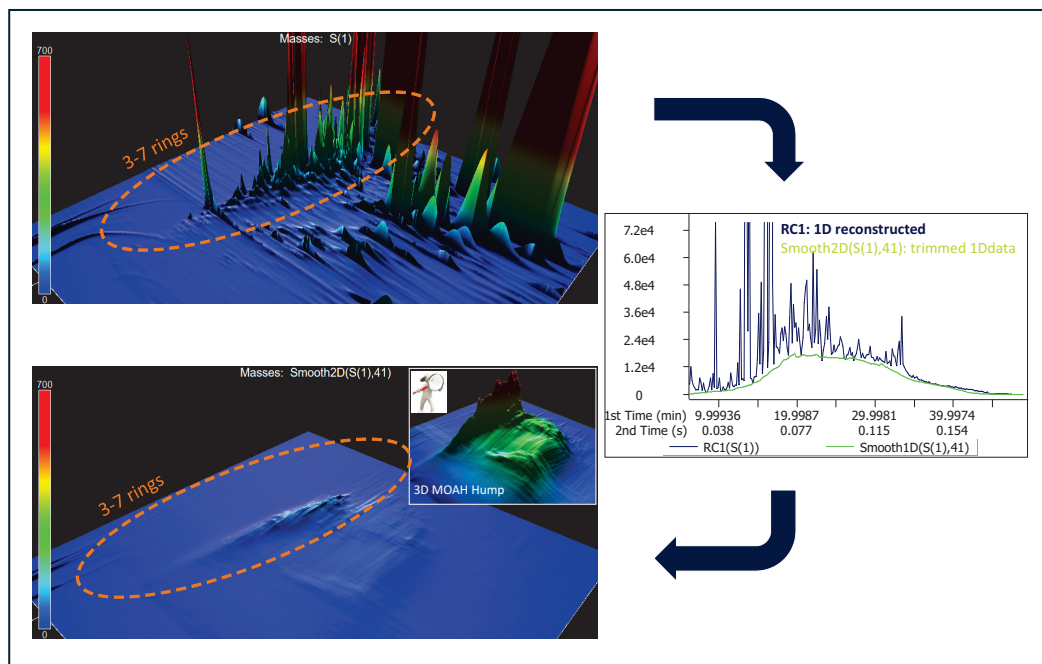

Figure 3. The top 3D plot shows a food sample contaminated by MOAH (3-7 rings aromatics). The same plot has been reprocessed by ChromaTOF to remove the peaks on top of the hump (bottom 3D plot). On the right side, a reconstructed 1D chromatogram used for in-depth evaluation of the trimming process.

The advantage of parallel detection with a TOFMS is the possibility to search for both targeted and non-targeted components. Having the mass spectra allows additional confirmation about the identity of the peaks that need to be subtracted as natural interferents (Figure 4 top). Additionally, the use of the feature "spectral filters" allows a faster confirmation of the chemical nature of the components detected. This feature provides extra support to challenging interpretations, resulting in the reduction of uncertainty (Figure 4 middle). Finally, the presence of a specific class of components (e.g. 3-7 rings aromatics) is also easily identified using the TOFMS trace, permitting a more in-depth evaluation of the contamination occurring in a specific sample (Figure 4, bottom).
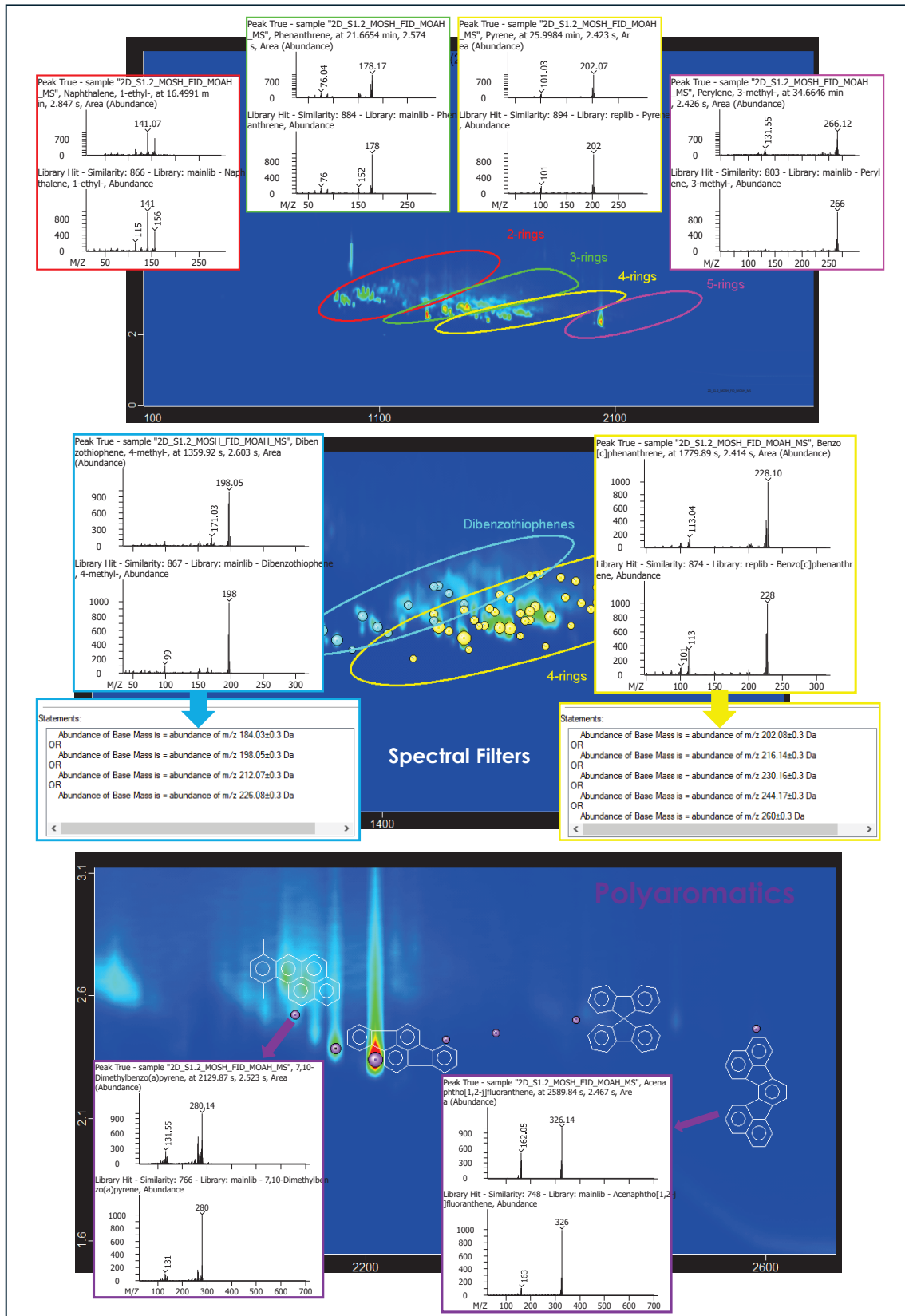

Figure 4. Top: GCxGC-TOFMS classification of 2-5 ring aromatics, based on their characteristic m/z fragments; Middle: Application of specific spectra filter to refine the classification; Bottom: Identification of polyaromatics (> 5 rings).

The *ChromaTOF* software version for MOSH/MOAH was tested analyzing the paraffin oil-alkane mixture in triplicate, both in 1D (LC-GC-FID) and 2D (LC-GCxGC-TOFMS/FID). The comparison to the expected value of 3.7% and 2.2% in 1D and 2D showed a general agreement between the two methods. In addition, two animal feed samples were analyzed to compare 1D and 2D results. The results, evaluated as the total MOSH and MOAH amount in the C10-50 range, were comparable as shown in Figure 5.
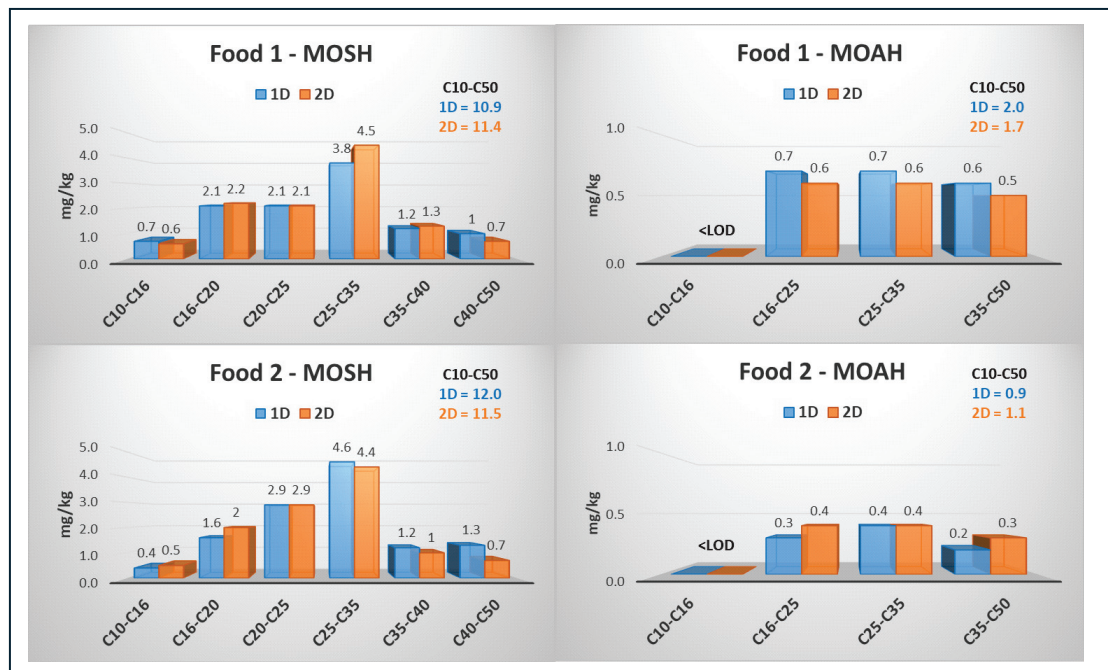

Figure 5. Comparison of quantitative data obtained by LC-GC-FID and LC-GCxGC-TOFMS/FID. The graphs show a general agreement between the two methods.

Conclusion

The application note demonstrates the successful coupling between the officially EU recommended methodology for analyzing MOSH and MOAH (i.e. LC-GC-FID) and LECO's GCxGC-TOFMS/FID technology.

This unified LC-GCxGC-TOFMS/FID method, in combination with the new *ChromaTOF* software version dedicated to MOSH/MOAH, reduces most of the uncertainty deriving from the use of non-selective separation and detection methodologies. The results reported in Figure 5 clearly demonstrate the reliability of the quantitative data produced by the novel platform. This work needs to be considered as state-of-the-art advancement, as more research needs to be performed to enlarge the knowledge regarding this wide class of contaminants and its common interferents now that their presence can be more reliably confirmed.

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