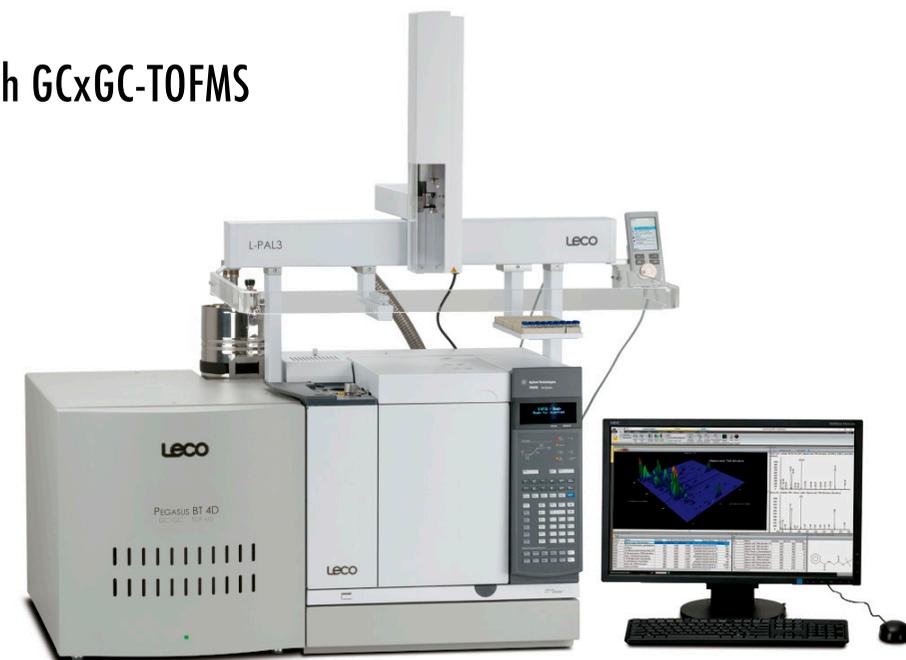


# Instrument: Pegasus® BT 4D

## Determining MOSH/MOAH with GCxGC-TOFMS

**F**ood contamination with mineral oil hydrocarbons (MOH) has been a growing concern since 2012, when the European Food Safety Authority (EFSA) flagged them as a potential health concern<sup>[1]</sup>. MOH are typically 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 the alkyl benzenes, alkyl naphthalenes, etc.

With respect to the analytical methods, two choices have been proposed so far for the quantification of these substances: 1) an off-line method consisting of a solid phase extraction-SPE, followed by a GC-FID analysis, and 2) the most popular, online LC-GC-FID method. However, depending on the matrix, both methods sometimes result in inaccuracies and provide challenges from a qualitative/quantitative point of



view, due to the lack of an associated confirmatory method (i.e. GC-MS, GCxGC-MS).

In this respect, LECO's Pegasus® BT 4D GCxGC-TOFMS system can help to unveil the complexity of contaminated food samples, providing superior chromatographic resolution and identification capabilities. In fact, as also suggested by the EFSA opinion, the GCxGC-MS tech-

nology needs to be used as a confirmatory tool in case of uncertain results.

As an example of the extraordinary resolution power, it is possible to differentiate the MOSH from the POSH (Polyolefin Oligomeric Saturated Hydrocarbons), and/or MOAH from the biogenic substances, such as the terpenoids. Both the POSH and the terpenoids are, in fact, referred to as interferents and can often be quantified as MOSH or MOAH in error, when using the conventional methodologies (i.e. LC-GC-FID), thus leading to erroneous results.

In addition to the increased separation power and identification confidence, LECO ChromaTOF® software's "Classifications" feature can be routinely applied to quickly gather information from the samples about the presence of a specific chemical class such as hopanes or steranes, or the recently emphasized 3-7 rings from MOAH<sup>[2]</sup>.

This note describes a GCxGC-TOFMS workflow to separate and identify the MOSH/MOAH fractions from the biogenic substances normally occurring in a spice sample, namely cumin.

### Experimental

A cumin extract was obtained and the spice was pre-separated into individual MOSH and MOAH fractions, each (via sequential analytical runs) were concentrated (to ~100 µL), and injected into the

**Table 1**

GCxGC	LECO GCxGC QuadJet™ Thermal Modulator
Injection	2 µL, Splitless mode (330 °C)
Columns	<sup>1</sup> D: Rxi-17 SilMS, 12 m x 0.25 mm i.d. x 0.25 µm coating (Restek) <sup>2</sup> D: Rxi-1-HT, 1.05 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Carrier Gas	He; 1 ml/min constant flow
Oven Program	40 °C (hold 1 min), ramp 5 °C/min to 360 °C, hold 10 min
Secondary Oven	+7 °C
Modulation (P <sub>M</sub> )	5 s
Transfer Line	340 °C
MS	LECO Pegasus BT 4D
Ion Source Temp	280 °C
Mass Range	40-700
Acquisition Rate	200 spectra/s

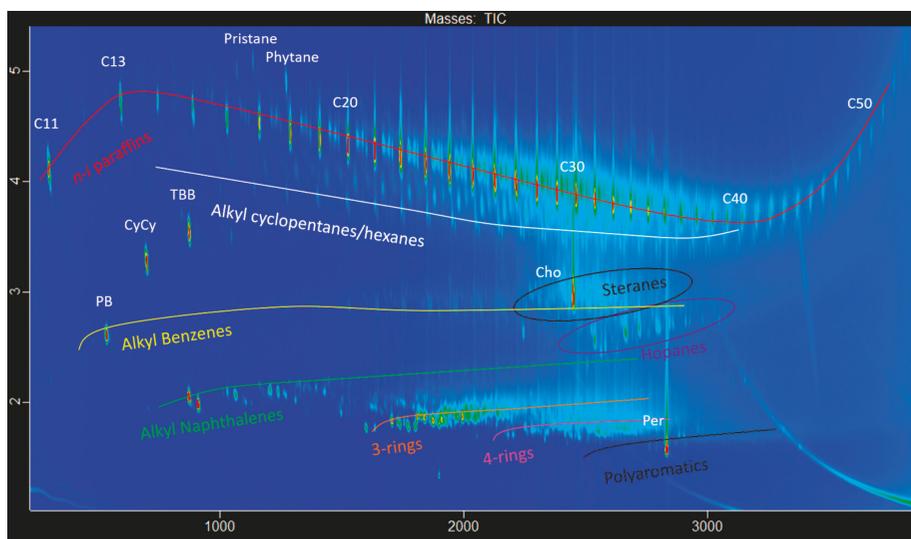
Pegasus BT 4D GCxGC-TOFMS system using the direct LC-GC fractionation and coupling approach. Prior to the spice analysis, using the same procedure, a standard sample, named VGO-IS, constituted by a petroleum fraction spiked with the MOSH/MOAH Internal Standard (Restek Corporation, #31070) was injected too as a reference. The GCxGC-TOFMS experimental parameters are reported in Table 1.

## Results and Discussion

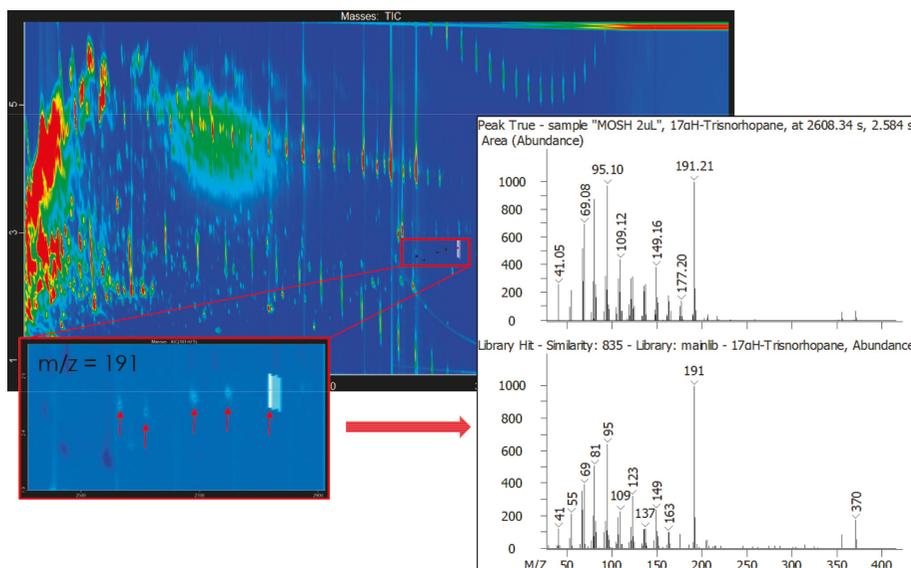
The analysis of the VGO-IS reference sample (obtained by mixing a Vacuum Gas Oil; VGO- carbon range >C50-) with the MOSH/MOAH Internal standards, enabled creation of a reference contour plot including all the most relevant chemical classes to be investigated in the MOSH/MOAH field. Figure 1 shows the GCxGC contour plot obtained from the injection of this sample.

As evident from Figure 1, the extraordinary power of the GCxGC technology allows the separation of many classes of components resulting in a very structured contour plot. A reverse column setup was chosen because of its specific capability to resolve chemical classes otherwise problematic to separate using the conventional "normal" setup (e.g. n-i paraffins from the POSH). The reverse setup also allows clear separation of hopanes ( $m/z$  191), considered as proof of mineral oil contamination. These markers are often present in trace amounts and are very difficult to separate and/or detect with the LC-GC-FID methodology. Nevertheless, thanks to the combination of GCxGC and TOFMS technology, they are clearly resolved and show a very characteristic pattern of elution. The same can be observed for the steranes, which elute slightly above the hopanes and can be easily targeted using the  $m/z$  217.

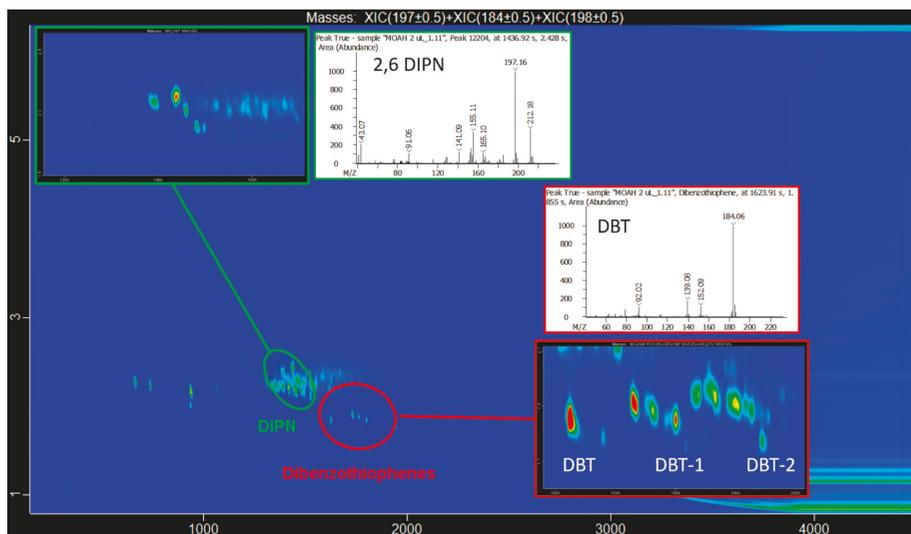
Finally, an accurate evaluation of the separation capabilities within the MOAH fraction was performed as well, to insure an adequate separation of the aromatic ring structures within the fraction. This was necessary in agreement with the most recent recommendation from the EU in 2019, which highlights the importance from a toxicological point of view of the 3-7 ring polycyclic aromatic compounds (3-7 PAC). Following the analysis of the reference sample, the MOSH and MOAH fractions from the cumin extract were also analysed using the same Pegasus BT 4D GCxGC-TOFMS system setup and data analysis approach. The main



**Figure 1. GCxGC-TOFMS contour plot from the VGO sample + the MOSH/MOAH internal standards (VGO-IS).**



**Figure 2. Hopanes detection in the cumin MOSH fraction ( $m/z$  191.21).**



**Figure 3. Detection and Classification of the DIPN and Dibenzothiophene (DBT) in the MOAH fraction from cumin.**

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scope for the analysis was to screen for the presence of the MOSH and MOAH contaminations and the presence of interferents, which might have affected the LC-GC-FID quantification results.

Figure 2 shows the presence of hopanes in the MOSH fraction of the cum-in, confirming the presence of mineral oil contamination, whereas Figure 3 shows the presence of specific MOAH markers such as the Diisopropyl-naphthalenes (DIPN) and the Dibenzothiophenes (DBT) originating most probably, from the cardboard ink of the packaging and the jute bags used for the transportation of the spice after the harvest, respectively.

Together with these markers of contamination, the presence of the aromatic compounds was also confirmed. As an example, Figure 4 shows the classification of mono-, di-, and tri-aromatic hydrocarbons within the MOAH fraction.

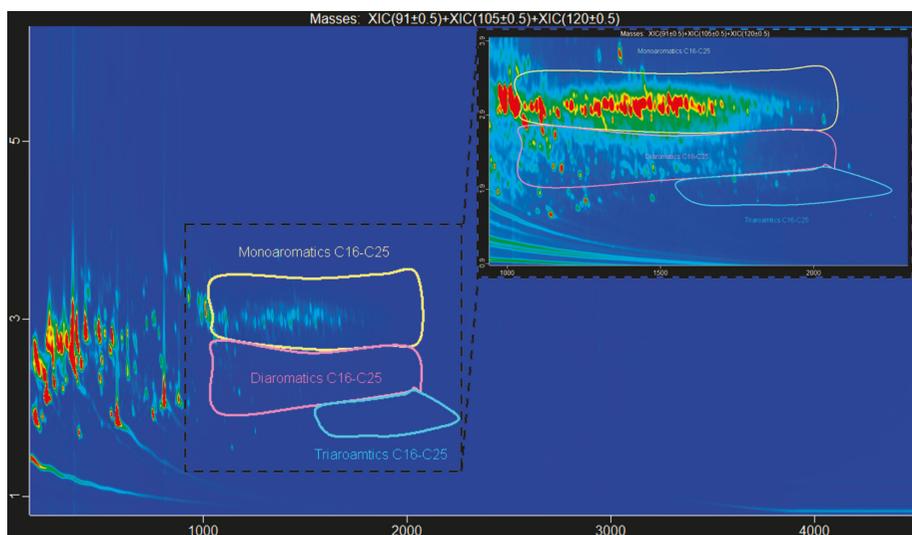
All these chemical families have been automatically detected by applying LECO's ChromaTOF "Classifications" feature, previously established using theVGO-IS as a reference standard.

Further, an additional group of substances was detected as well, namely some biogenic sesquiterpenes normally present in the cumin. Figure 5 shows both the position of this group of substances in the contour plot, partially overlapping with the monoaromatic fraction, and the identification of one of the components encountered ( $\gamma$ -amorphene, 850/1000), as an example.

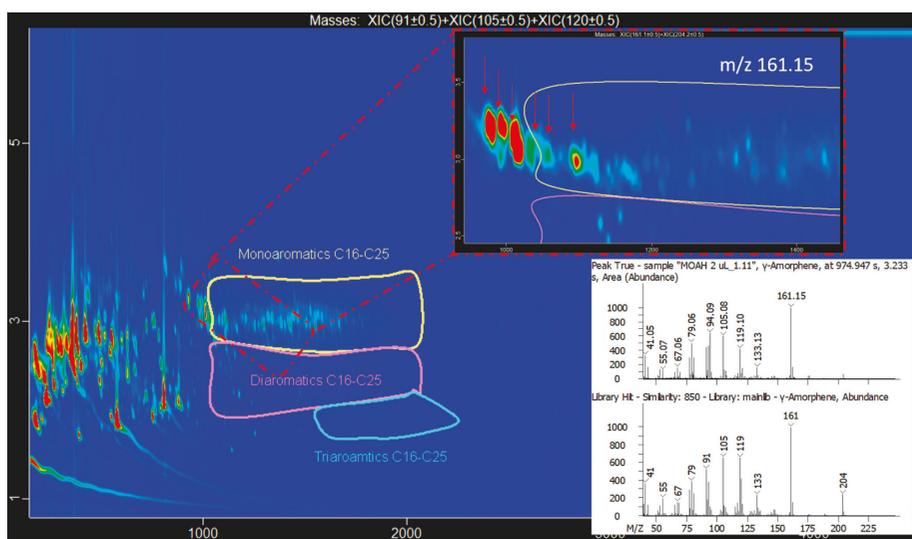
Such information is just an example of how the GCxGC technology, in combination with the power of LECO's TOFMS, can improve users' confidence when quantifying and qualifying MOSH/MOAH samples.

## Conclusions

LECO's GCxGC-TOFMS technology is demonstrated to be a seamless choice to confirm



**Figure 4. Detection and Classification of mono-, di- and, tri- rings aromatics hydrocarbons within the cumin MOAH fraction.**



**Figure 5. The red circled zone comprises some naturally occurring sesquiterpenes, such as the positively identified  $\gamma$ -amorphene.**

the presence of MOSH/MOAH contaminants in food samples. In fact, as advised by the EFSA opinion, a confirmatory GCxGC-TOFMS analysis has been performed on the cumin extract. This established the presence of a MOSH/MOAH contamination, but also the presence of natural biogenic substances, not belonging to these fractions and possibly biasing the quantitative results obtained with the conventional methods.

Current activities include using a wide range of challenging matrices, using FID for quantification, and also based on the approach described here, they are being carried out and validated in conjunction with further development and finalisation

of a fit for purpose software tool for an optimal and complete MOSH/MOAH analysis workflow.

## References

- [1] *Scientific Opinion on Mineral Oil Hydrocarbons in Food (EFSA)*. EFSA Journal 2012;10(6):2704. 10.2903/j.efsa.2012.2704
- [2] *EFSA Technical Report. Rapid risk assessment on the possible risk for public health due to the contamination of infant formula and follow on formula by mineral oil aromatic hydrocarbons (MOAH)*. <https://doi.org/10.2903/sp.efsa.2019.EN-1741>