Application Note

Instrument: Pegasus® BT 4D

Frankincense and Myrrh: Visual Characterization and Comparison of Essential Oils with GCxGC Structured Chromatograms

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Key Words: Essential Oil Analysis, GCxGC, MS, TOFMS, Structured Chromatograms, Characterization, Comparison

Introduction

Frankincense and Myrrh are historically interesting materials that continue to be researched today for better understanding of their composition and their potential uses. They are both resins that come from the Boswellia (Frankincense) or Commiphora (Myrrh) trees, and both are commonly used as perfumes and incense. In this work, we investigate the associated essential oils, which are extracts from these tree materials. Gas chromatography (GC) is a common analytical tool for the analysis of essential oils because their major components tend to be volatile and semi-volatile analytes. GC separates the individual chemical components, and when coupled with mass spectrometry (MS), it also provides identification information and signals that can be readily quantified. This separation, identification, and quantification of individual chemical components provides important characterization information about these samples. Even more information can be learned by extending the separation to a second dimension with GCxGC. GCxGC adds a complementary second column in series with the primary column, effectively separating the entire sample by both mechanisms. This provides an increase in the overall chromatographic resolution and often separates analytes that coelute in a single dimension separation. Another benefit of GCxGC is that it inherently produces structured chromatograms where chemically similar analytes tend to elute in ordered bands through the GCxGC separation space. This provides important identification context to support MS library and retention index matching and also allows for rapid visual characterization of the compound classes and polarity of the components of a sample of interest. In this application note, we highlight these benefits and compare Frankincense and Myrrh from their structured chromatograms.

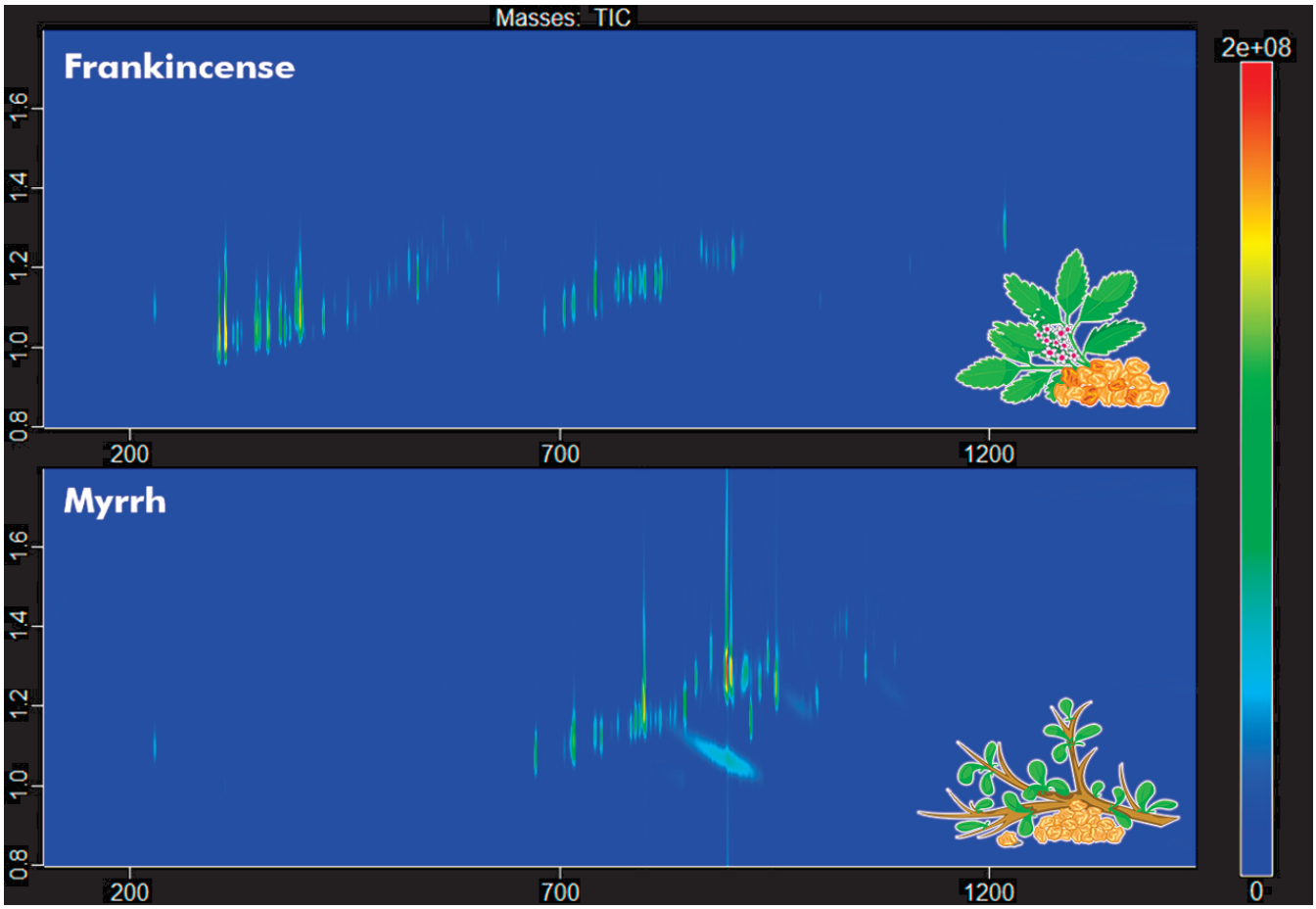

Figure 1. GCxGC Chromatograms for Frankincense and Myrrh essential oils.

Experimental

The essential oils were diluted to 1% in acetone and analyzed with GCxGC-TOFMS, as described in Table 1. Data for an alkane standard (C6 through C24) were also collected with the same separation conditions for Retention Index determinations.

Table 1. Instrument (*Pegasus* BT) Conditions

Auto Sampler	**LECO L-PAL 3 Autosampler**
Injection	1 uL, split 100:1
Gas Chromatograph	**LECO GCxGC QuadJet™ Thermal Modulator**
Inlet	250 °C
Carrier Gas	He @ 1.4 mL/min, corrected constant flow
Columns	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
	Rxi-17SilMS, 0.45 m x 0.25 mm x 0.25 µm coating (Restek)
Temperature Program	40 °C ramp 10 °C/min to 280 °C
	Secondary oven: +25 °C relative to primary oven
Modulation	1 s with temperature maintained +15 °C relative to 2nd oven
Transfer Line	300 °C
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250 °C
Mass Range	33-500 m/z
Acquisition Rate	200 spectra/s

Results and Discussion

The Frankincense and Myrrh essential oil chromatograms are shown in Figure 1. Many differences between these samples are clearly apparent in visual review of the chromatograms. This is expected, as the aroma description and the anticipated chemical composition of these materials also differs significantly. There are some specific target analytes that were of interest based on their expected presence in these essential oils. Frankincense is described as containing terpenes (for example, α-pinene, β-pinene, and limonene), terpenoids (terpinene-4-ol), and esters (octyl acetate). Myrrh is described as having a heavy contribution of furanosesquiterpenoids (for example, furanoeudesma-1,3-diene, lindestrene, and dihydropyrocurzerenone). These target analyte classes, indicated on the chromatogram in Figure 2, were located within the data by reviewing the automated peak finding results that were generated by ChromaTOF® brand software. Identifications were tentative, but were determined with spectral matching compared to NIST library databases and also by first dimension retention index verification. The observed spectral information compared to the NIST library spectra for these target analytes are shown in Figure 3 and the peak metrics for the identifications (spectral similarity score, observed RI, and library RI) are listed in Table 2.

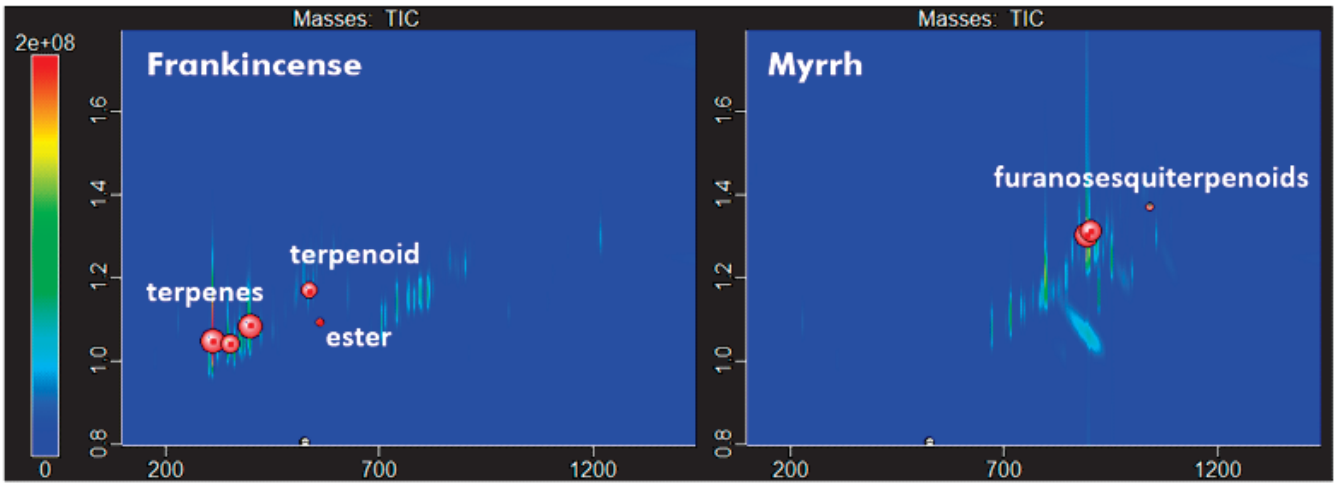

Figure 2. Chromatographic location of target analytes of interest in the Frankincense and Myrrh essential oils. Red peak markers indicate the peak location with size of the bubble proportional to peak area.

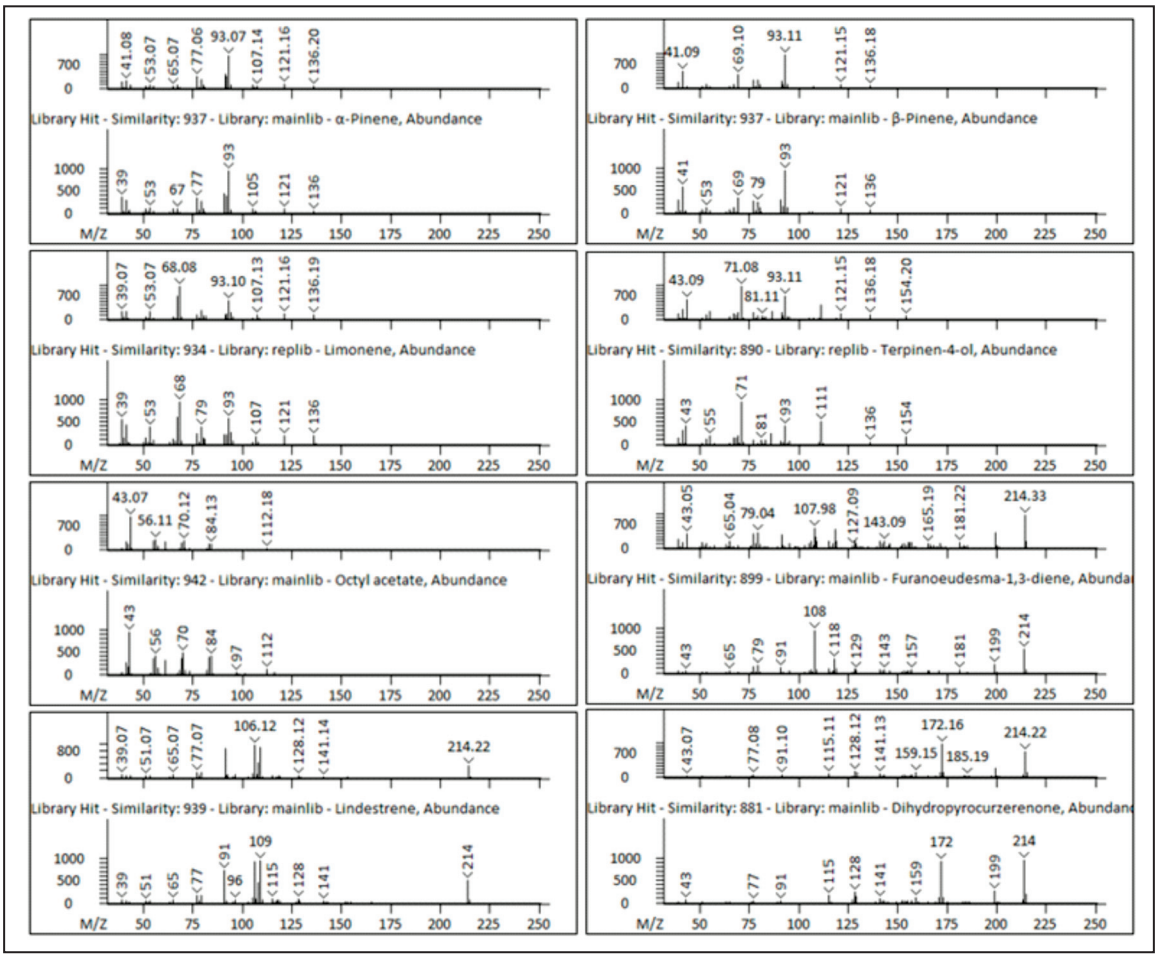

Figure 3. Observed spectra (top) and the NIST Library match (bottom) for the target analytes indicated on Figure 2 and tabulated in Table 2.

Table 2. Target Analytes of Interest

Name	R.T. (s)	CAS	Formula	Similarity	RI	Lib. RI
α-Pinene	309.99, 1.052	80-56-8	$C_{10}H_{16}$	937	936.5	937
β-Pinene	350.98, 1.044	127-91-3	$C_{10}H_{16}$	937	981.7	979
Limonene	396.98, 1.088	138-86-3	$C_{10}H_{16}$	934	1032	1030
Terpinen-4-ol	533.97, 1.172	562-74-3	$C_{10}H_{18}O$	890	1183.4	1177
Octyl acetate	557.97, 1.097	112-14-1	$C_{10}H_{20}O_2$	942	1210.9	1210
Furanoeudesma-1,3-diene	891.95, 1.306	115526-32-4	$C_{15}H_{18}O$	899	1644.3	1629
Lindestrene	898.95, 1.315	2221-88-7	$C_{15}H_{18}O$	939	1654.6	1652
Dihydropyrocurzerenone	1037.94, 1.374	59462-26-9	$C_{15}H_{18}O$	881	1872.6	1861

These particular analytes differ between the samples, and the overall characterization and comparison of other differences between these samples can be further explored by taking advantage of this structured nature of the GCxGC data. A GCxGC separation that pairs a non-polar column with a polar column creates data where the elution order in the first dimension is primarily related to volatility, and elution order in the second dimension is primarily related to polarity or chemical structure. The least polar analytes elute with the earliest second dimension retention time while the most polar analytes elute with the latest second dimension retention times. This property leads to structured bands of analytes with the same functional group through the GCxGC separation space. It can be noted in Figure 2 that the three terpenes in Frankincense elute together and the three furanosesquiterpenoidsalso elute near each other. These elution bands that relate to chemical structure provide helpful context for improving analyte identifications, provide rapid information on analyte polarity, and are also helpful for general characterization of the samples, as shown in Figures 4-10.

The structured bands for terpenes are shown in Figure 4. Terpenes are a common constituent of many plant-based materials. They can have important odor contributions and are hypothesized to participate in some therapeutic properties. These terpenes elute in three separate bands in the GCxGC space, as shown in Figure 4. The first band is the monoterpenes, the second is the sesquiterpenes, and the third is the diterpenes. These bands encompass the target terpenes shown in Figure 2 and are helpful for getting a sense of how the terpene profiles compare between Frankincense and Myrrh. For example, Frankincense has a higher monoterpene content while the majority of Myrrh's terpenes are sesquiterpenes.

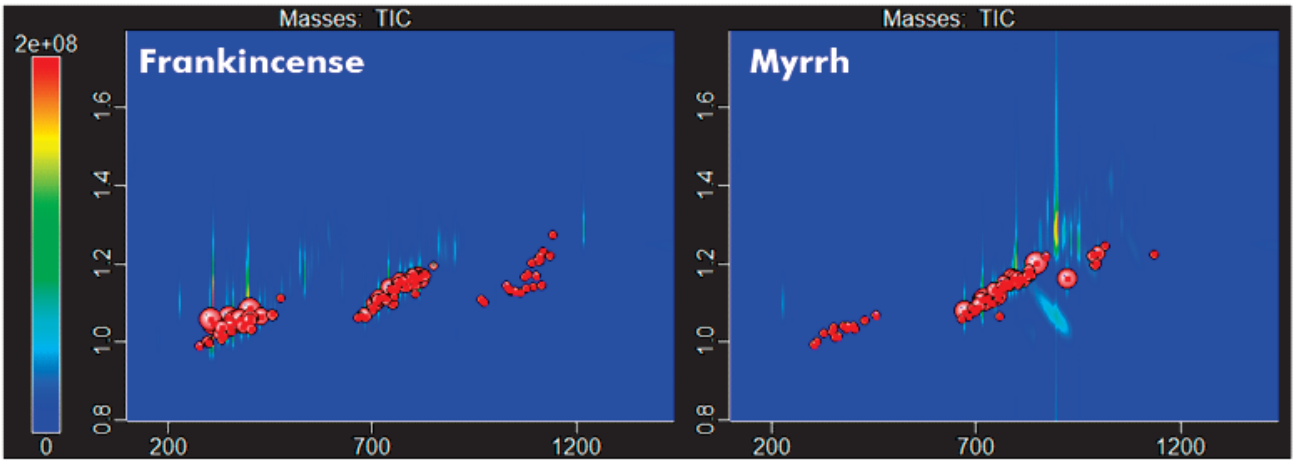

Figure 4. Terpene peaks are indicated on the chromatograms.

The terpenoids that are oxygenated and slightly more polar than the terpenes elute in bands with slightly longer second dimension retention times as compared to the terpenes, as indicated in Figure 5. Frankincense has more monoterpenoids, while Myrrh has more sesquiterpenoids. Furanosesquiterpenoids are a specific terpenoid that are characteristic of Myrrh as was shown in Table 2 and Figure 2. There are other furanosesquiterpenoids that elute in the band of compounds with these targets, as shown in Figure 6. These elute slightly later in the second dimension than the terpenoids, above the sesquiterpenes, and are observed in the Myrrh but not the Frankincense.

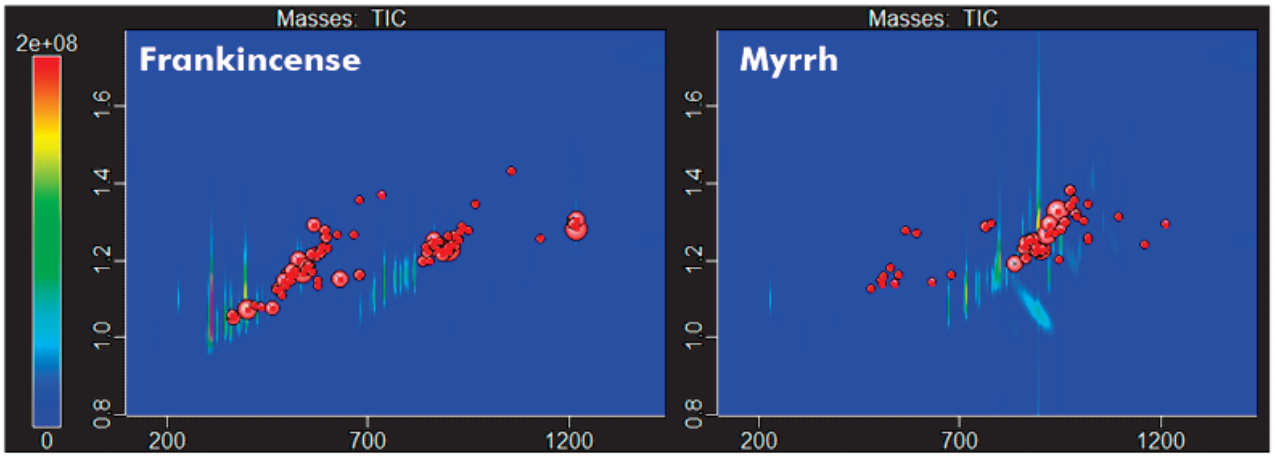

Figure 5. Terpenoid peaks are indicated on the chromatograms.

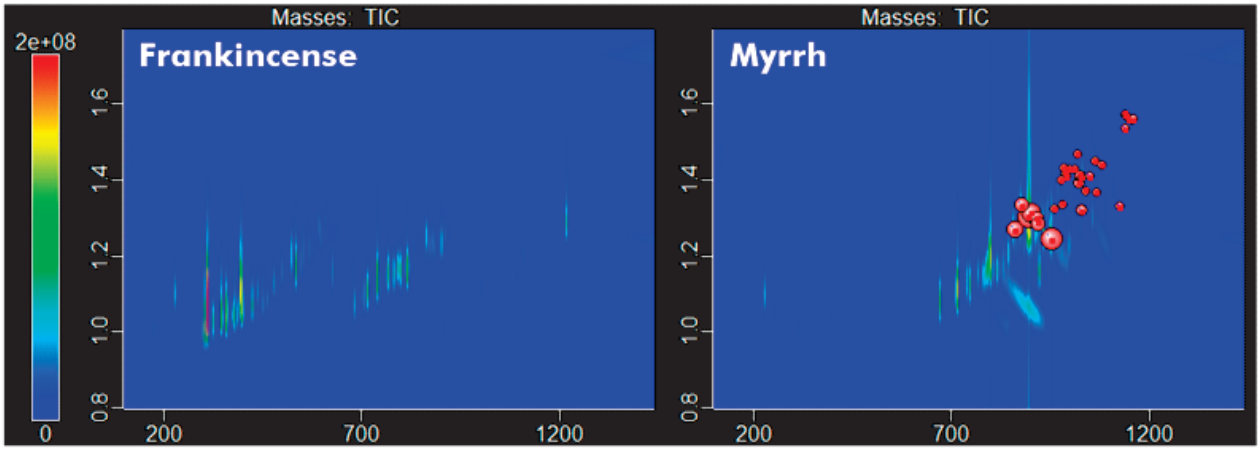

Figure 6. Furanosequiterpenoid peaks are indicated on the chromatograms.

Esters are another important class of compounds that are important aroma contributors. We observed octyl acetate as one of the target analytes, shown in Figure 2 and described in Figure 3 and Table 2. Other ester compounds are observed in the Frankincense essential oil, as indicated in Figure 7. The esters are present at higher levels in the Frankincense compared to the Myrrh.

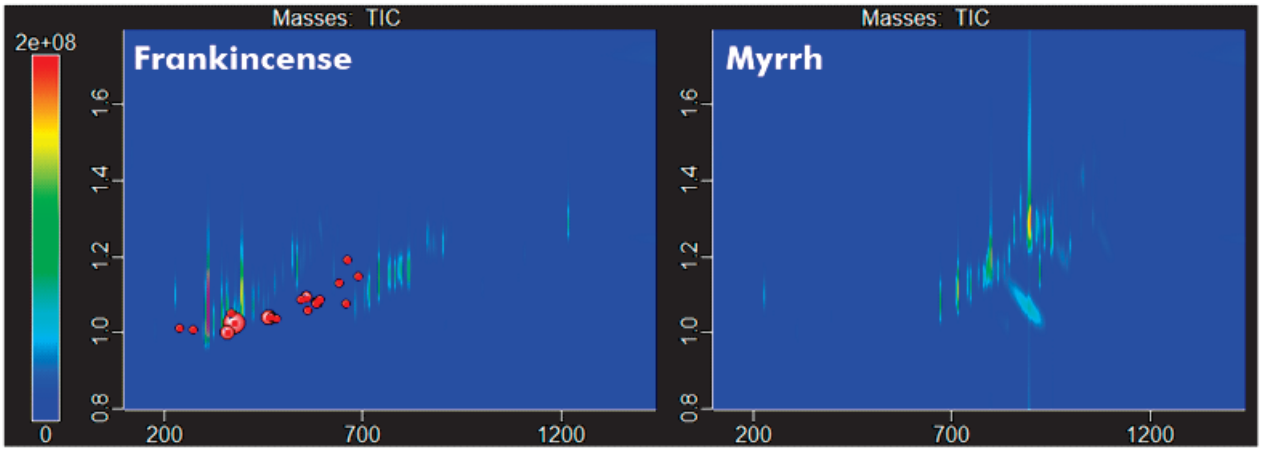

Figure 7. Ester peaks are indicated on the chromatograms.

These chemical compound class bands can be observed for other analyte types as well. Alkanes are indicated in Figure 8, aromatic compounds are indicated in Figure 9, and oxygenated aromatics are shown in Figure 10. In all cases, these bands are helpful for understanding the differences between the essential oils and provide a rapid visual indication of the polarity of the chemical components.

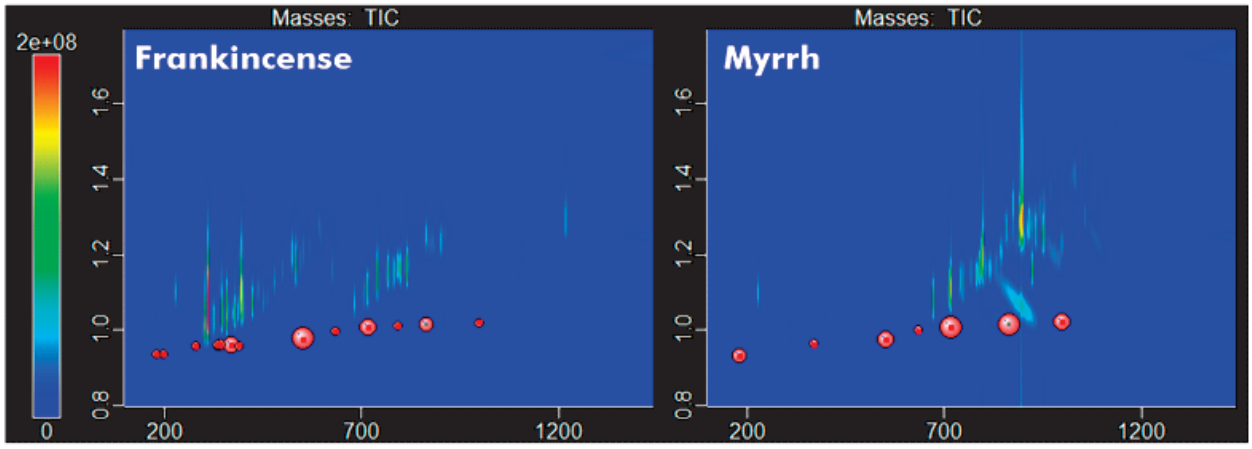

Figure 8. Alkane peaks are indicated on the chromatograms.

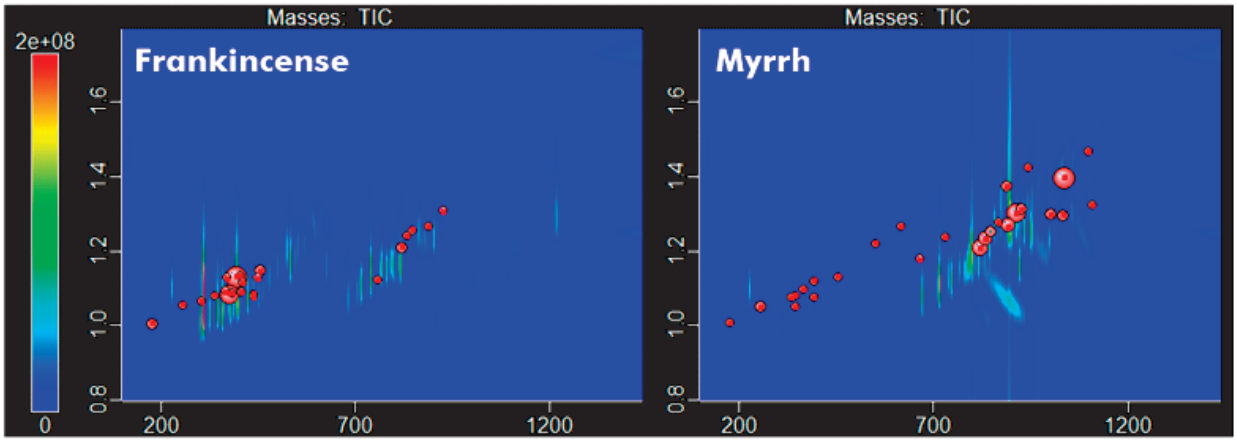

Figure 9. Aromatic peaks are indicated on the chromatograms.

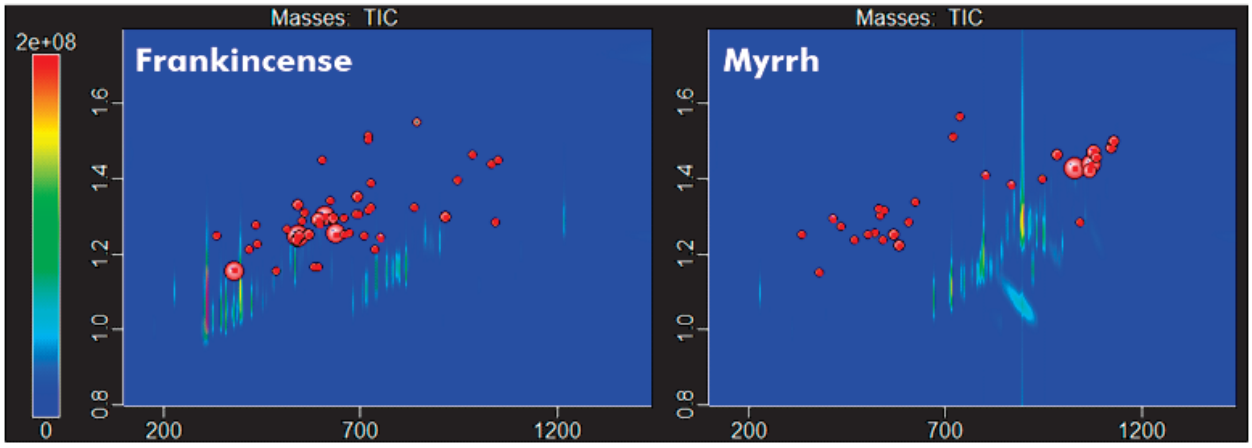

Figure 10. Oxygenated aromatic peaks are indicated on the chromatograms.

Conclusion

In this work, we have demonstrated the application of GCxGC-MS for the characterization of Frankincense and Myrrh essential oils. GCxGC provided structured chromatograms that were helpful for general characterization and comparison between the samples. Compound classes elute in structured bands through the GCxGC separation space, facilitating rapid visual comparisons.

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