Application Note

Instrument: Pegasus® BT 4D

Characterization of Citronella Essential Oil

LECO Corporation; Saint Joseph, Michigan USA

Key Words: Essential Oil Analysis, Retention Index, GC, GCxGC, MS, TOFMS, Deconvolution, Peak Capacity Increase, Structured Chromatograms

Introduction

Essential oils are routinely analyzed with Gas Chromatography (GC) to separate and quantify the individual components in the complex mixtures. GC is often paired with Mass Spectrometry (MS), which allows for identification of the individual analyte components that have been separated from each other. This separation, identification, and quantification of individual chemical components with GC-MS, provides important information that can be helpful for characterization, authentication, process optimization, and for a variety of other quality control objectives. GC-MS meets many analysis needs, but adding another dimension of separation via a complementary second dimension column with GCxGC can provide even more information about your sample and improves your ability to achieve these experimental goals. GCxGC maintains the primary separation and adds the secondary separation by coupling a complementary second column in series with the first. The sample is effectively separated by both mechanisms simultaneously. This increases the peak capacity and analytes that coelute in one dimension may separate in the other. With GC or GCxGC, tentative identifications are determined from matching the observed full m/z range spectral information to NIST library databases and by confirming first dimension retention index information with NIST databases. GCxGC often improves identifications, though, by providing additional context for structural information with second dimension elution order and by providing cleaner spectra by reducing coelutions. In this work, we analyze and characterize a citronella essential oil with both GC-MS and GCxGC-MS. We demonstrate the benefits of full m/z range data, deconvolution, increased peak capacity, and the structured nature of the chromatograms. GCxGC helps you see what you've been missing and learn even more about your sample of interest.

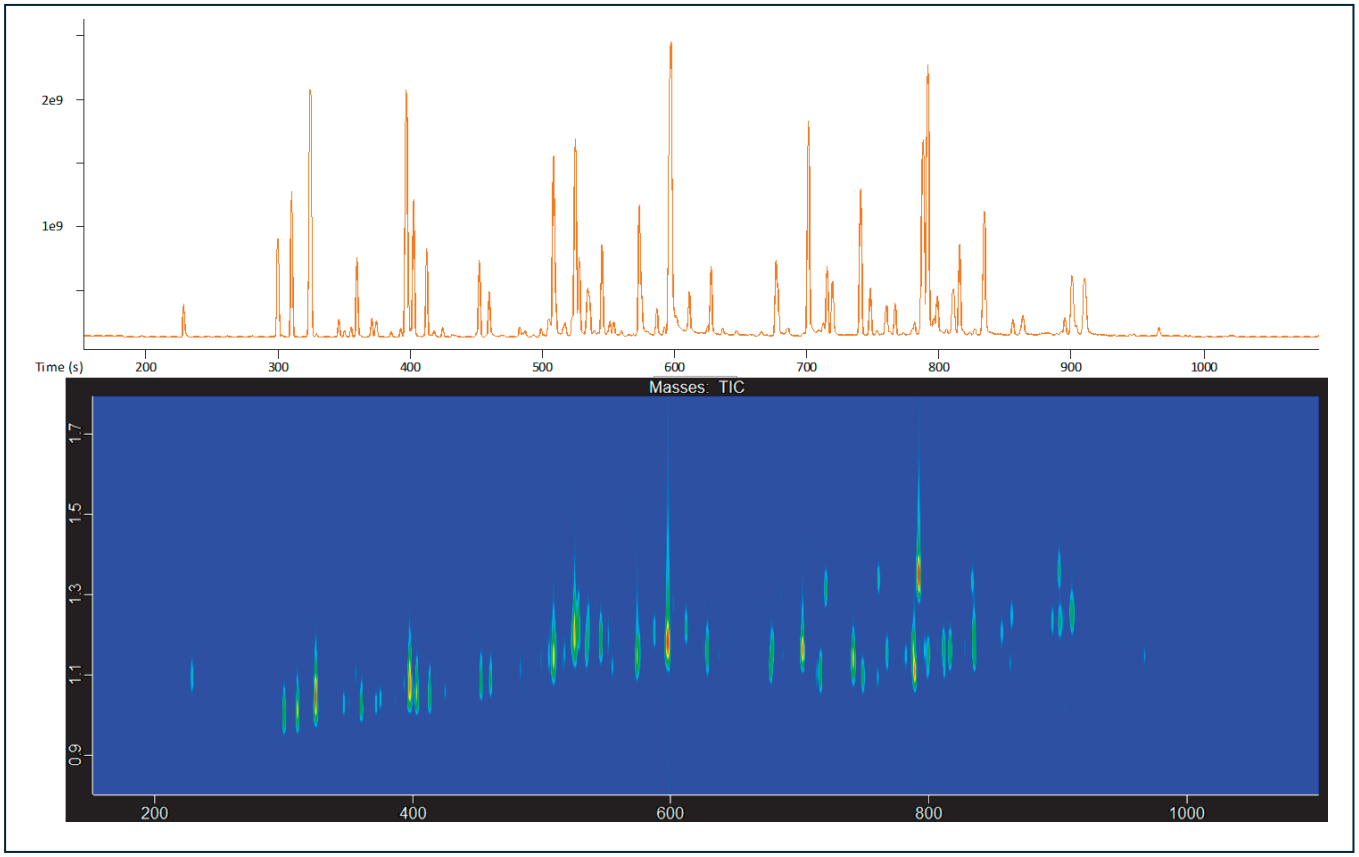

Figure 1. GC and GCxGC Total Ion Chromatograms (TIC) for citronella essential oil.

Experimental

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

Table 1. Instrument (Pegasus® BT) Conditions

Auto Sampler	**LECO L-PAL 3 Autosampler**
Injection	1 µL, split 100:1
Gas Chromatograph	**LECO GCxGC Quad Jet 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	10 spectra/s (GC) and 200 spectra/s (GCxGC)

Results and Discussion

Representative chromatograms for the GC and GCxGC separations of the citronella essential oil are shown in Figure 1. The primary separation matches between the methods, but with GCxGC analytes are also spread out in the second dimension. This can reveal more about your sample compared to GC, because it increases the peak capacity, or chromatographic resolution, and it provides additional context on the chemical structure based on the position analytes elute in the GCxGC separation space.

An example of the added context about chemical structure is shown in Figure 2. A section of the chromatograms from Figure 1 has been zoomed and enlarged with nine representative analytes labeled. A variety of chemical structures are represented in these nine analytes. There are 3 aromatic compounds (eugenol, 4-vinylveratrole, and methyl eugenol), 2 esters (cis-geranyl acetate and geranyl acetate), 3 terpenes (copaene, β-bourbonene, and β-elemene), and an alkane (tetradecane). These identifications are tentative, but were determined from spectral matching to NIST library databases and from retention index verification. GC similarity scores ranged from 660 to 950, while GCxGC similarity scores ranged from 820 to 952. Better separation and higher S/N are the likely causes for improved similarity scores with GCxGC. Retention index in the first dimension also supported the identifications. 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. This gives structural context because the least polar analyte (the alkane) elutes with the earliest second dimension retention time while the most polar analytes (the aromatic compounds) elute with the latest second dimension retention times. The terpenes and esters elute in order between these bands, as indicated on Figure 2. These elution bands related to chemical structure are helpful for general characterization of the samples and add helpful context for identifications. These nine analytes were found with both GC and GCxGC, but the structure information was only clearly apparent by retention position in the GCxGC data.

In addition to the structure context that is readily apparent with GCxGC, the added peak capacity in the second dimension can often separate analytes that coelute in the first dimension. For example, eugenol and cis-geranyl acetate, shown in Figure 2, chromatographically overlap in the GC data, but chromatographically separate in the second dimension with GCxGC. Extracted Ion Chromatograms (XIC) traces for these analytes are shown in Figure 3. In this case, deconvolution successfully separated the first dimension coelution in the GC data. In other cases, though, GC coelutions may exceed deconvolution capabilities (with perfect coelution, for example). In these instances where coelutions exceed deconvolution, the analytes can sometimes be separated with GCxGC providing new information, as shown in Figures 4-6.

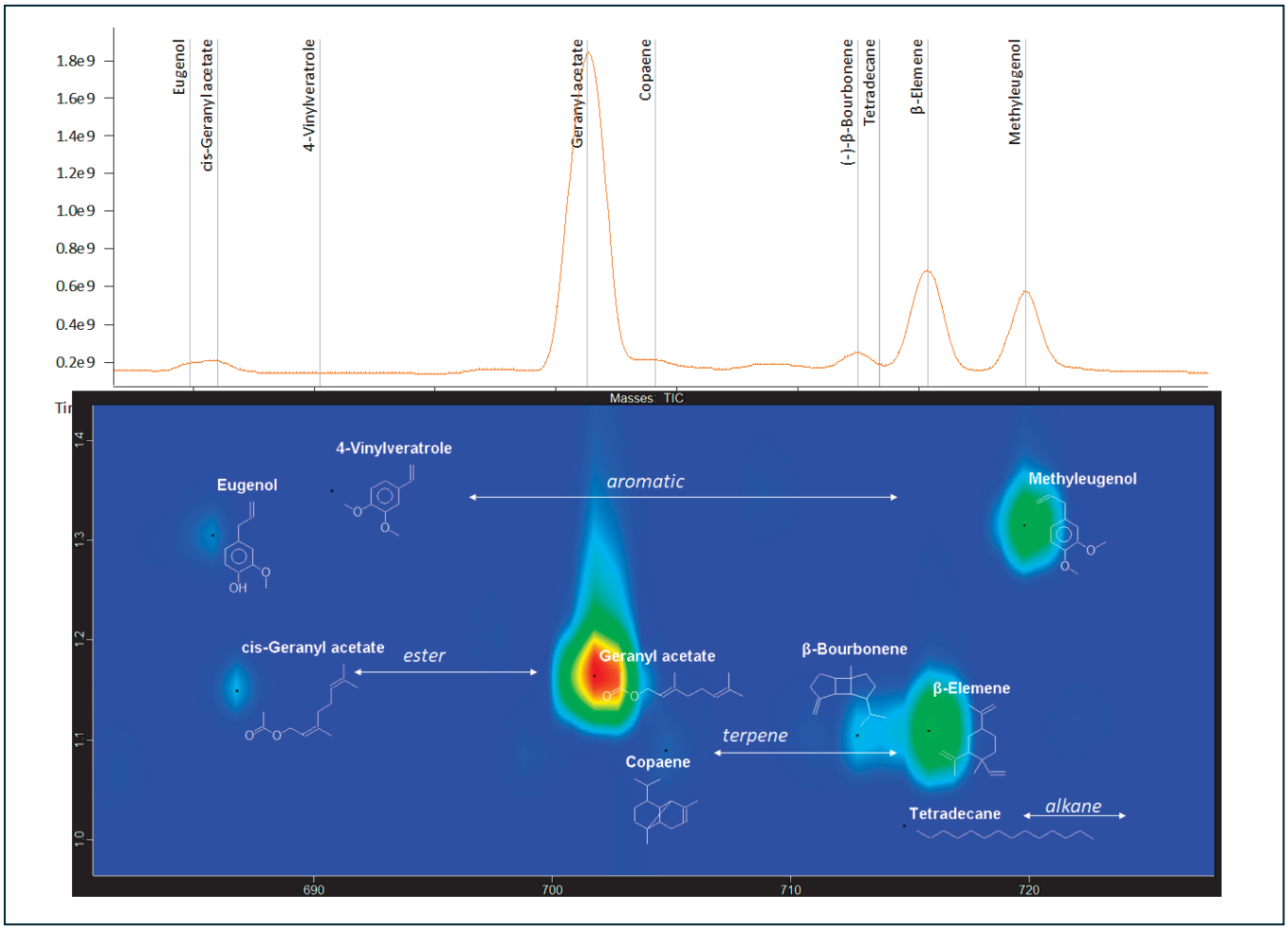

Figure 2. GCxGC separates the samples based on two mechanisms, providing insight to chemical structure based on retention in the second dimension.

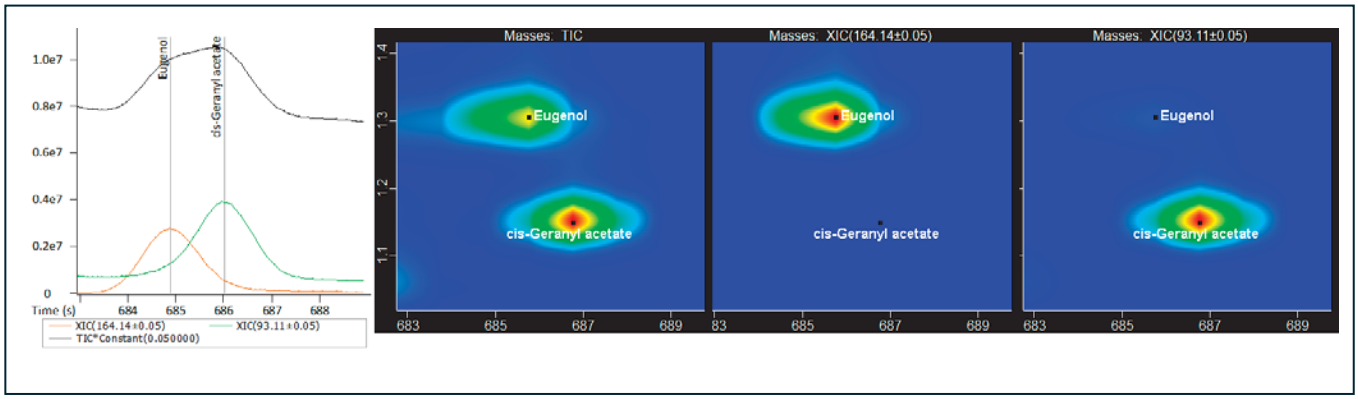

Figure 3. TIC and XIC traces associated with the first two peaks in Figures 2. These analytes coelute with GC, but are separated in the second dimension with GCxGC.

Figure 4 shows another zoomed-in section of the GC and GCxGC data. There appears to be a single peak in the TIC view of the GC data, and at least two distinct peaks in the GCxGC data. Peak markers, determined with deconvolution, indicate that two analytes were determined with GC and three analytes were determined in the GCxGC data. The spectral information for these five peaks is shown in Figure 5. With the GC data, one analyte, 2,6-dimethyl 2,4,6-octatriene, was identified with a similarity score of 860. The other analyte did not have a reliable identification because the similarity score was only 708 and there are notable discrepancies between the observed spectrum and the library spectrum. For example, m/z 69, 139, and 154 are all observed in the data, but not in the library spectrum. GCxGC results support the identification of the first analyte and clarify that the second peak is actually two analytes that had been combined to a single peak marker. When these analytes were better separated in the second dimension and with deconvolution applied also in that second dimension, the m/z that were combined to one peak with GC are separated and identified as trans-rose oxide and α-campholenal with similarity scores of 814 and 858, respectively. The degree of coelution can be observed by plotting XICs as shown in Figure 6 where a representative m/z for

each of the three analytes is plotted in the GC and GCxGC data. In the GCxGC data, each XIC is a clearly distinct peak in the contour plot. In the GC data, however, there are only two distinct peak shapes as m/z 139 (trans-rose oxide) and 108 (α-campholenal) perfectly coelute and apex together. Information on these perfectly coeluting analytes was hidden without the additional separation dimension provided by GCxGC.

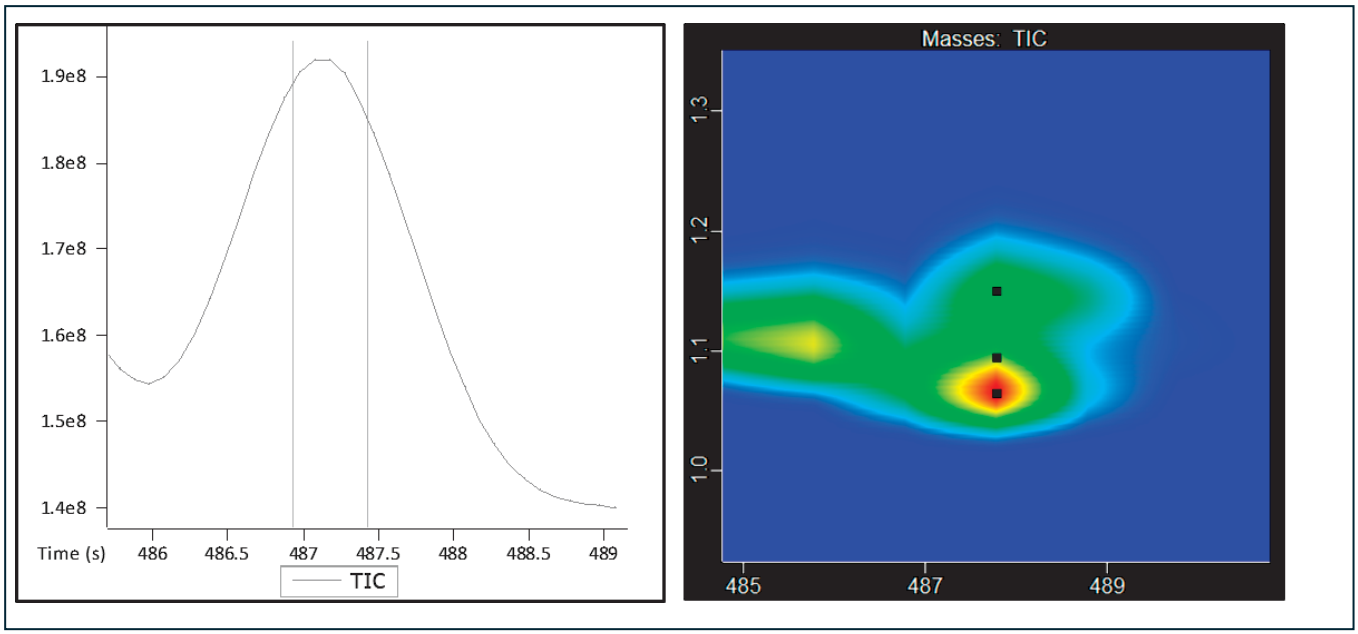

Figure 4. GCxGC can chromatographically separate some analytes that coelutes in the first dimension, increasing the peak capacity in instances of perfect coelution. What was determined to be two peaks in the GC data, was found to be three in the GCxGC data.

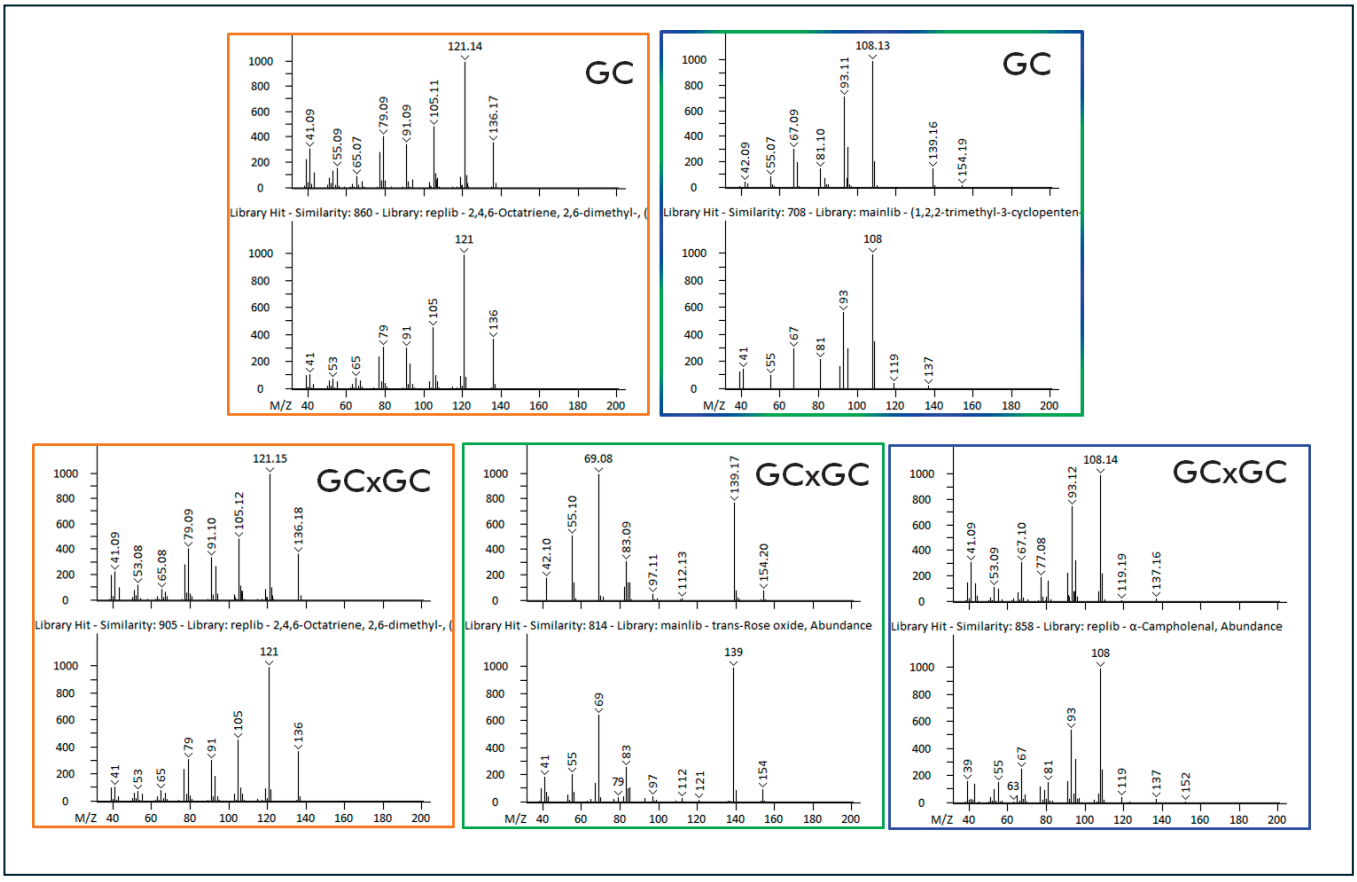

Figure 5. Spectral information for analytes that separated with GC and GCxGC. With GC, two of the three peaks perfectly coelute and are combined in a single peak marker and spectrum.

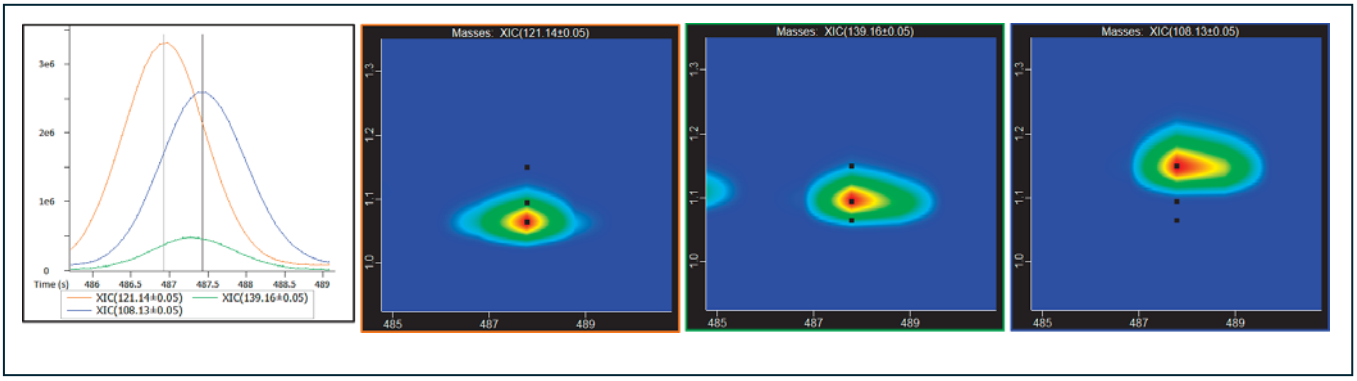

Figure 6. XIC traces associated with the peaks in Figures 4 and 5. Two of the analytes that perfectly coelute in GC are separated in the second dimension with GCxGC.

Conclusion

In this work, we have demonstrated the application of GC-MS and GCxGC-MS for the characterization of a citronella essential oil. GCxGC provided important benefits for the characterization and understanding of these samples. The structural context from the nature of the GCxGC separation space is helpful for sample characterization. The additional peak capacity of GCxGC is helpful for chromatographically separating coelutions in the first dimension, providing improved information in some cases and new information in others. GCxGC reveals even more about your sample showing you what you were missing with your GC analysis.

LECO Corporation | 3000 Lakeview Avenue | St. Joseph, MI 49085 | Phone: 800-292-6141 | 269-985-5496
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