GCxGC-TOFMS of the Cooling Agent WS-3 in Mint Oils and Cinnamon Flavor

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Key Words: GC-MS, GC-TOFMS, GCxGC-TOFMS, Deconvolution, Quantification, Flavors

1. Introduction

WS-3 is a physiological cooling agent used in a variety of consumer products, including chewing gum, candy, toothpaste, mouthwash, throat drops, tobacco (as a flavor agent), beverages, gelatin desserts, ice cream, popsicles, aftershaves, lotions, bath oils, fragrances, cosmetics, and more. It was designed by Wilkinson Sword in the 1970s and its chemical name is N-ethyl-5-methyl-1-2-(1-methylethyl)-cyclohexanecarboxamide. WS-3 is often used in conjunction with mint oils (which have natural cooling agents like menthol and eucalyptol) to provide freshness and longer lasting flavor to orally consumed products. However, some buyers of mint oils, especially in Europe and Japan, do not want mint oils that contain WS-3, and require their suppliers to certify that WS-3 is not present in the product at levels as low as 50 ppb. Determination at such low levels is complicated by the complexity of the mint oils, and even though gas chromatography–mass spectrometry (GC-MS) is a powerful analytical technique, it can still fail to provide both qualitative and quantitative answers in this situation.

A more powerful way to handle complex sample analysis is by the use of comprehensive two-dimensional GC combined with time-of-flight mass spectrometry (GCxGC-TOFMS). In GCxGC, two independent separations are applied to a sample in one chromatographic run, which provides for a substantial increase in peak capacity (separating power). TOFMS provides extra characterization, and the proper peak definition for GCxGC (where peaks can be as narrow as 100 ms), because of its ability to record full mass range data at up to 500 spectra/second. This note describes the analysis of mint oils and flavors for WS-3 by GCxGC-TOFMS. Advantages offered by automated peak find and spectral deconvolution routines are discussed, and the benefit of enhanced peak capacity for complex samples is shown. Calibration curves were generated for samples spiked with WS-3.

2. Experimental Conditions

Samples

Peppermint oil, cinnamon flavor, spearmint oil, and the WS-3 were obtained from a flavor company. Due to their viscosity, which causes problems with autosampler syringe filling, and to minimize chromatographic overload, the oils and flavor were diluted by 10 times with acetone prior to GCxGC-TOFMS analysis.

GC-MS (quadrupole)

Column:
  60 m x 0.25 mm x 0.25 μm DB-5 (J&W Scientific)
Carrier:
  Helium at 1 mL/minute, constant flow
Injection:
  2 μL split at 270°C, split ratio 150:1 (neat oil)

Oven Program:
  150°C (1 minute), 0.5°/minute to 165°, 38°/minute to 280° (16.7 minutes)
Run Time:
  Approximately 50 minutes
MS:
  Quadrupole operated in selected ion recording mode

GCxGC-TOFMS

GCxGC:
  Agilent 6890 gas chromatograph equipped with a LECO GCxGC thermal modulator and secondary oven
Column 1:
  30 m x 0.25 mm x 0.25 μm Rtx-1 (Restek)
Column 2:
  1 m x 0.18 mm x 0.18 μm Rtx-200 (Restek)
Carrier:
  Helium at 1.0 mL/minute, constant flow
Injection:
  1 μL splitless at 250°C, valve time 60 seconds
Oven 1 Program:
  80°C (1 minute), 10°/minute to 300°C
Oven 2 Program:
  5°C offset from oven 1
Modulation time:
  4 seconds
Run Time:
  45 minutes

MS: LECO Pegasus® TOFMS
Ionization: Electron ionization at 70 eV
Source Temp.:   225°C
Stored Mass Range: 45 to 450 u
Acquisition Rate: 200 spectra/second

Data Processing

LECO ChromaTOF® software featuring automated peak find and spectral deconvolution—fully integrated processing for GCxGC-TOFMS data.

3. Results and Discussion

Even though GC-quadrupole MS conditions are listed in this note, due to the overwhelming complexity of the samples, it was not possible to accurately analyze for WS-3 with this system at the desired concentration level, so no additional discussion will be provided.

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Figures 1-3, reconstructed one-dimensional GC-TOFMS chromatograms, illustrate the complexity of the mint oils and cinnamon flavor. Under these GC conditions, the WS-3 would elute at about 1050 seconds, and at only 50 ppb (5 pg on column in the diluted samples), the matrix background would be overwhelming and cause problems with both qualitative and quantitative determination of WS-3.
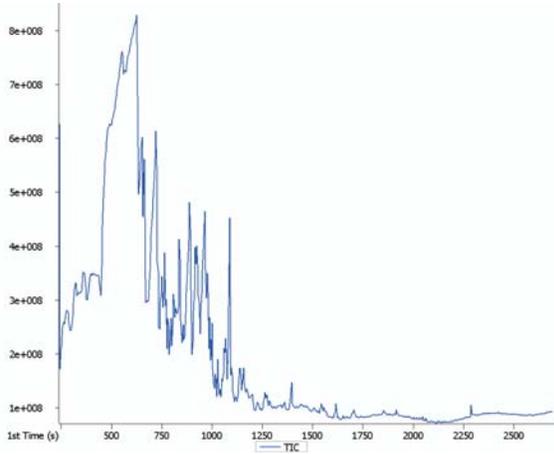
Figures 4-6 are contour plots, or GCxGC chromatograms, of the same samples. A contour plot presents the GCxGC data as the first dimension retention time on the X-axis, the second dimension retention time on the Y-axis, and a color scheme which indicates peak intensity (in this case, blue is baseline, and red is highest intensity). Although it can be hard to tell with samples this complex (especially where the plot is just a "smear"), each "spot" on the contour plot represents a peak, which can be a single compound, or perhaps coeluting compounds. The black circle on each contour plot in Figures 4-6 indicates the elution area for WS-3. What is important is that the WS-3 elutes later in the second dimension due to the selectivity of the Rtx-200 column, which means a better chance for accurate determination of low levels of WS-3.

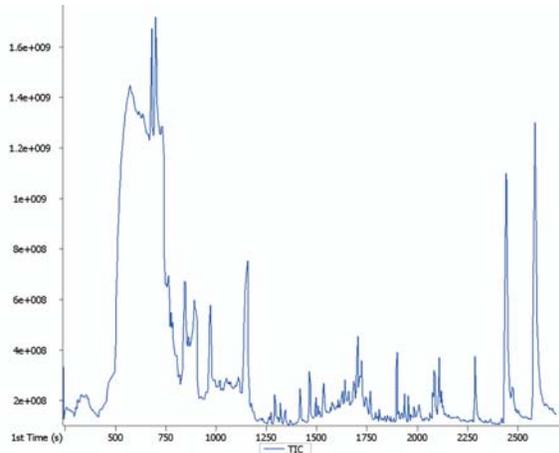Figure 1. One-dimensional chromatogram (TIC) of peppermint oil.

Figure 2. One-dimensional chromatogram (TIC) of cinnamon flavor.

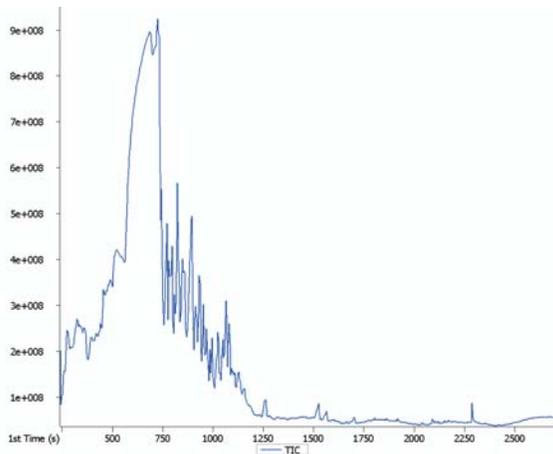

Figure 3. One-dimensional chromatogram (TIC) of spearmint oil.
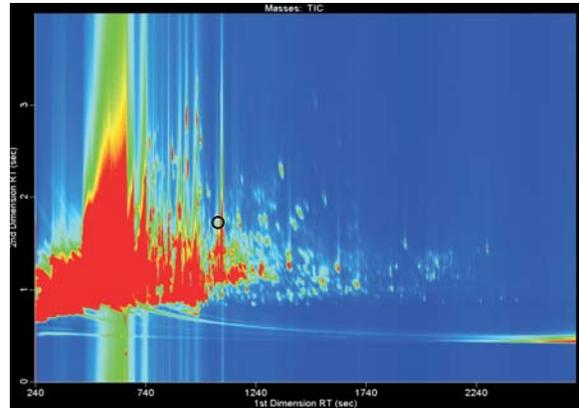

Figure 4. Contour plot of peppermint oil analyzed using GCxGC-TOFMS. The black circle indicates the elution area for WS-3.
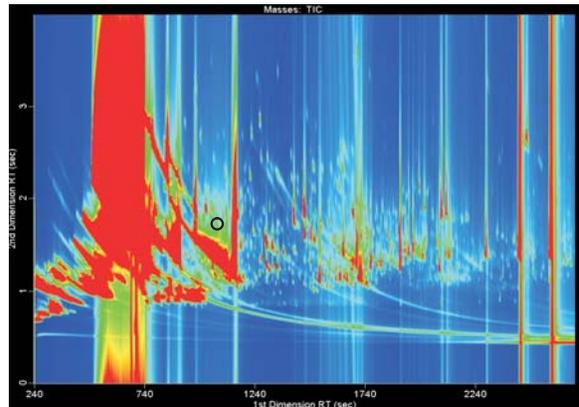

Figure 5. Contour plot of cinnamon flavor analyzed using GCxGC-TOFMS. The black circle indicates the elution area for WS-3.
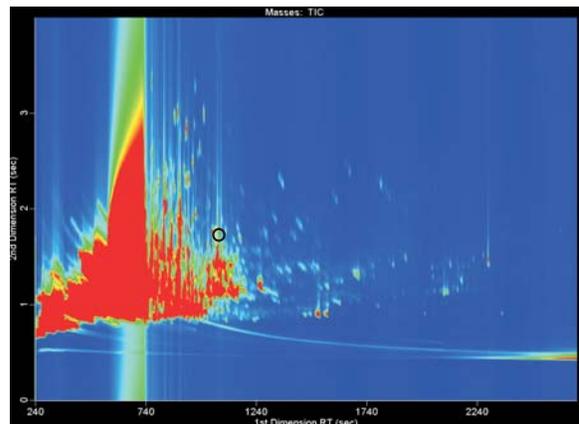

Figure 6. Contour plot of spearmint oil analyzed using GCxGC-TOFMS. The black circle indicates the elution area for WS-3.

To demonstrate that GCxGC-TOFMS could be used to quantitatively determine WS-3 in these complex samples, sets of matrix-matched standards (with Tetrabromothiophene as an internal standard) were prepared where WS-3 was spiked into the diluted samples at levels as low as 2 pg/μL (which corresponds to 20 ppb in the original sample, below the target level of 50 ppb). These standards were then used to generate Calibration Curves using m/z ion 211 for WS-3 as the quantification mass (see Figure 7 for mass spectrum and additional information on WS-3). Even though ion 211 is of less intensity than other ions in the WS-3 mass spectrum, a higher m/z ion will almost always be subject to less interference than a lower m/z ion in complex samples.

Calibration curves using the approach described above are shown as Figures 8-10. For the spearmint oil in Figure 10, the low point is 5.5 pg (or 55 ppb in the original oil). This spearmint oil actually contained WS-3, as verified by analyzing the unspiked oil using GCxGC-TOFMS. Across the ranges studied, approximately 20 to 500 ppb, the curves are quite linear, and confirm the ability of GCxGC-TOFMS to accurately quantify the WS-3 in mint oils and cinnamon flavor.
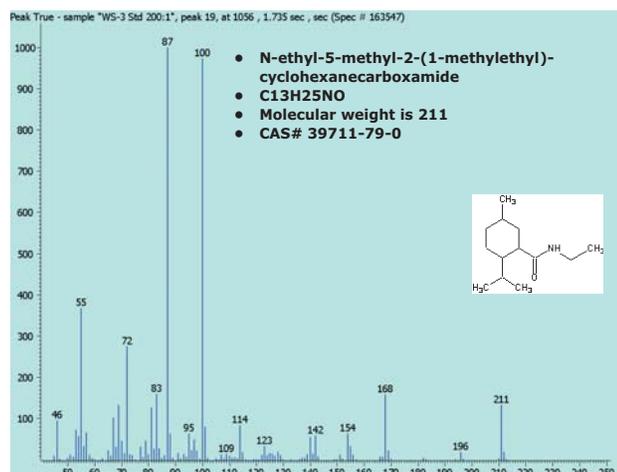

2 to 54.5 pg

y= +0.00251749x + 0.00405485
r = 0.99963

Figure 9. Calibration curve of WS-3 in cinnamon flavor from 2 to 54.5 pg. This represents a range of 20 to 545 ppb in the original flavor sample.

5.5 to 55.5 pg

y= +0.00372783x - 0.00225741
r = 0.99635

Figure 10. Calibration curve of WS-3 in spearmint oil from 5.5 to 55.5 pg. This represents a range of 55 to 555 ppb in the original oil.

Peak True - sample "WS-3 Std 200:1", peak 19, at 1056 , 1.735 sec , sec (Spec # 163547)

- N-ethyl-5-methyl-2-(1-methylethyl)-cyclohexanecarboxamide
- C13H25NO
- Molecular weight is 211
- CAS# 39711-79-0

Figure 7. TOF mass spectrum of WS-3, and other information, including structure.
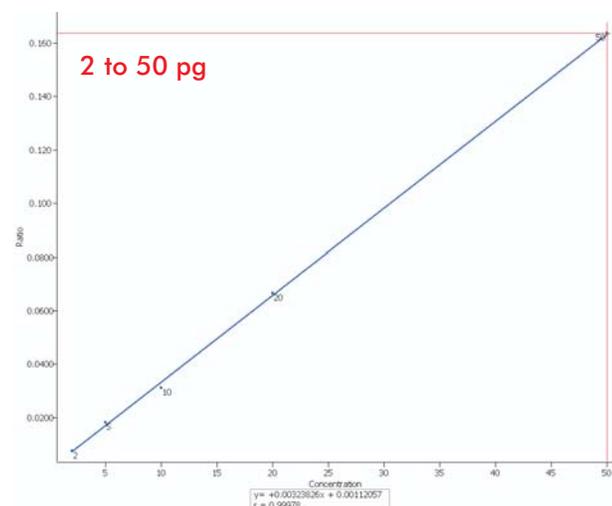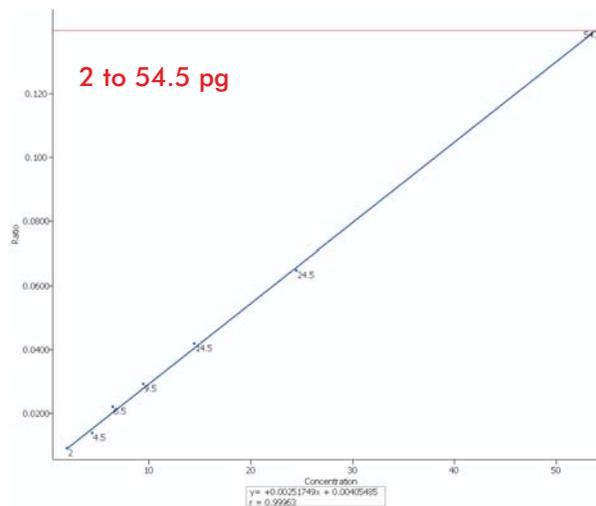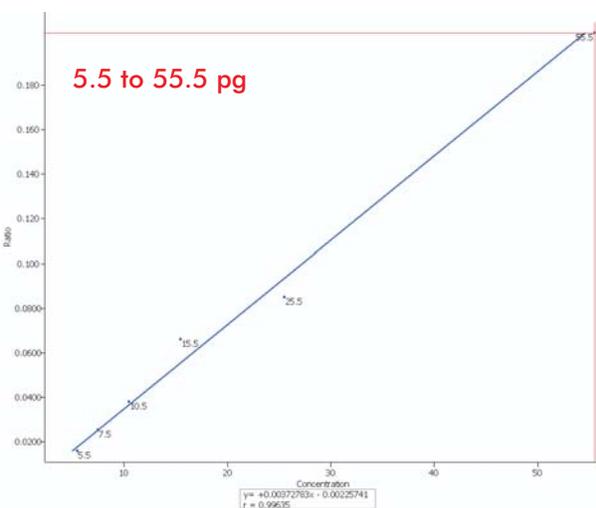

2 to 50 pg

y= +0.00328826x + 0.00112057
r = 0.99978

Figure 8. Calibration curve of WS-3 in peppermint oil from 2 to 50 pg. This represents a range of 20 to 500 ppb in the original oil.

The linear appearance of the preceding calibration curves is a quick visual way to illustrate the efficacy of the GCxGC-TOFMS method for WS-3 in complex samples. A more thorough way is to plot the results in tables, such that the calculated concentrations from the generated curves can be inspected. This is done in Tables 1-3. As can be seen from the data, the calculated concentrations are generally quite close to the certified (or spiked) concentrations.

Table 1. Calibration data for WS-3 in peppermint oil.

Certified Concentration	Calculated Concentration	Percent Difference	Response Factor
50	50.2	0.35	0.655
20	20.2	1.1	0.666
10	9.25	7.5	0.622
5	5.27	5.4	0.727
2	2.01	0.65	0.762

Table 2. Calibration data for WS-3 in cinnamon flavor.

Certified Concentration	Calculated Concentration	Percent Difference	Response Factor
54.5	53.8	1.2	0.514
24.5	24.0	2.0	0.528
14.5	15.0	3.4	0.577
9.5	9.96	4.9	0.614
6.5	7.14	9.9	0.678
4.5	3.85	15	0.610
2	1.99	0.38	0.904

Table 3. Calibration data for WS-3 in spearmint oil.

Certified Concentration	Calculated Concentration	Percent Difference	Response Factor
55.5	54.6	1.7	0.733
25.5	23.2	9.0	0.666
15.5	18.2	17	0.852
10.5	10.8	3.0	0.725
7.5	7.46	0.47	0.676
5.5	4.99	9.3	0.581

Quantification of "real world" mint oil samples and flavors can be accomplished after generation of the matrix-matched calibration curves.

To further illustrate the power of GCxGC in allowing a bias-free determination of WS-3 in complex mint oil samples, Figure 11 is a contour plot showing how WS-3 is nicely resolved from potential interfering compounds with the Rtx-200 column in the second dimension.
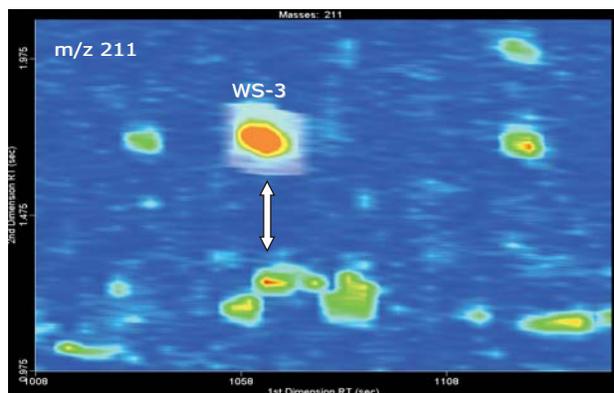

Figure 11. Contour plot of m/z 211 showing WS-3 and one-dimensional GC interferences that have been resolved using GCxGC. Without the extra peak capacity of GCxGC, the accurate determination of WS-3 in this peppermint oil at 50 ppb would be impossible.

Another way to view GCxGC data is through the use of surface plots, as shown in Figure 12 for 55 ppb WS-3 in spearmint oil. Again, the power of the second dimension separation is what allows this determination.
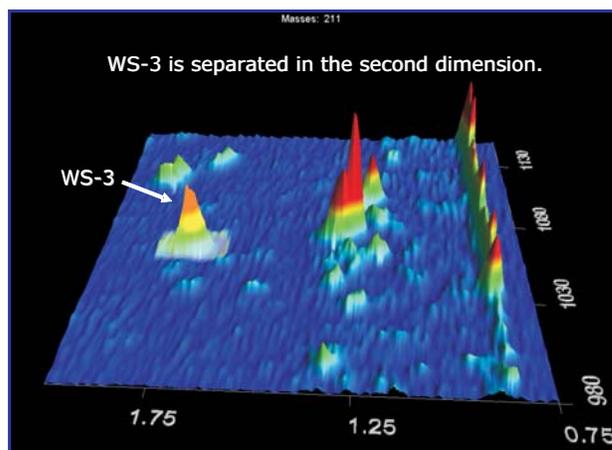

Figure 12. Surface plot of m/z 211 showing 55 ppb WS-3 in spearmint oil and one-dimensional GC interferences that have been resolved in the second dimension using GCxGC. The second dimension in this view is across the bottom of the plot.

Although it has not been discussed here (the reader can find additional information on GCxGC at www.leco.com, and from technical representatives at LECO), peaks are not passed whole as they elute from the first dimension column through the modulator. Depending on the modulation time and their first dimension peak widths, they are sliced numerous times. Even so, because of the thermal modulation process which narrows the peaks and makes them taller, a sensitivity enhancement is often seen. Although it is not compared to GC-MS here, since GC-MS alone does not have the selectivity to do this work, it can be seen how sensitive GCxGC-TOFMS is for WS-3 by looking at Figure 13. Figure 13 represents 5 pg of WS-3 in peppermint oil that had been "sliced" three times by the modulation process. Even with this slicing, the peaks, especially the "base slice", slice 2, are quite intense. It is obvious that lower levels can be determined, perhaps even sub pg amounts of WS-3.

Even with the peak capacity increase afforded with GCxGC, spectral deconvolution can still be necessary in these types of complex samples (i.e. chromatographic coelutions still occur). Fortunately, TOFMS, because of its rapid data acquisition capability and non-skewed mass spectra, permits the use of powerful (and automated) peak find and spectral deconvolution algorithms. These algorithms are integral to LECO's ChromaTOF software. Figure 14 is an example of the need for spectral deconvolution where WS-3 (10 pg) coelutes with a large interfering peak in peppermint oil. The raw (or caliper) spectrum, even though taken at the WS-3 peak apex, is more representative of the coeluting peak than WS-3 due to its large size. However, the deconvoluted spectrum (or Peak True), matches well with the Reference Spectrum, and leads to an accurate qualitative identification of WS-3 in this sample.
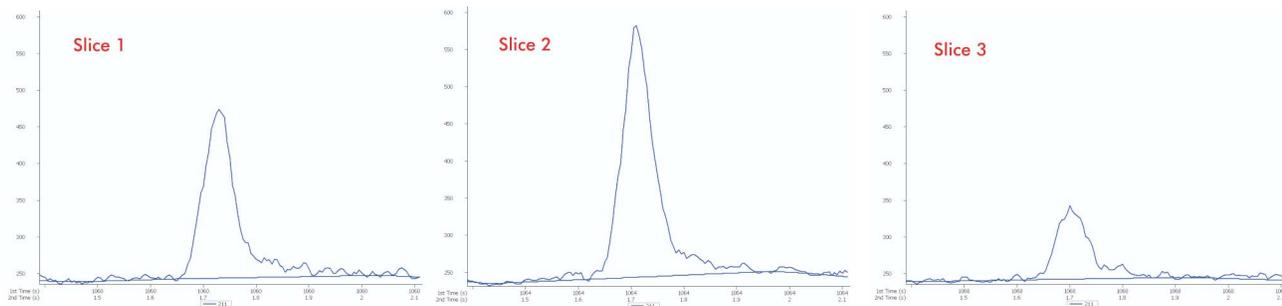
Figure 13. GCxGC slices from modulation of 5 pg of WS-3 in peppermint oil. The quantification mass 211 is plotted. The peaks, which have been narrowed to less than 200 ms by thermal modulation, have good intensity, and lower levels of analysis are possible.
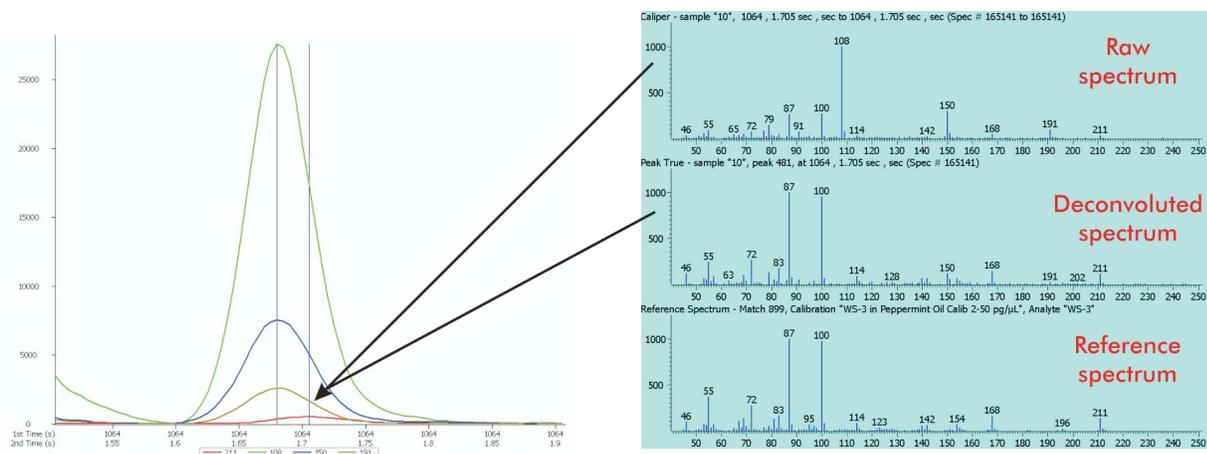

Figure 14. Spectral deconvolution is necessary to accurately identify WS-3 (10 pg) in peppermint oil, even under GCxGC conditions. The raw spectrum is taken at the peak apex for WS-3 and is mainly indicative of the large coeluting peak from the peppermint oil. The deconvoluted spectrum matches very nicely (899 out of 1000) with the Reference Spectrum, and is almost devoid of the interfering ions seen in the raw spectrum.

4. Conclusions

GCxGC-TOFMS is a powerful technique for determining WS-3 (and other cooling agents or target compounds) in complex mint oils and flavors, due to automated peak find and spectral deconvolution, and because of the peak capacity increase afforded with GCxGC. Calibration curves were generated to as low as 2 pg (20 ppb in the original samples), and could likely go lower based on observations of GCxGC peak heights for WS-3. TOFMS is the only mass spectrometer that has the acquisition speed to support GCxGC, and a full mass spectrum is always obtained.

5. References

Leffingwell & Associates web site:
http://www.leffingwell.com/cooler_than_menthol.htm

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