Analysis of Menthol and Peppermint Oil Using GCxGC-TOFMS with a Chiral Column in the First Dimension

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Key Words: GC, GCxGC, TOFMS, Deconvolution, Essential Oils

1. Introduction

The quality of flavoring components is dependent on enantiomeric composition. This is particularly noticeable with menthol and peppermint. While GC analysis may be used to confirm the general composition of a mixture, it does not distinguish between enantiomers unless a chiral separation is used. With the structural similarities of compounds contained in mixtures such as peppermint oil or natural menthol, separation of enantiomers may be obtained on chiral columns, but other undesired coelutions may still occur—for example, (+)-menthol coelutes with menthyl acetate on the chiral Rt-BetaDEXsm column. GCxGC increases the peak capacity of the chromatographic system and offers a way to overcome this problem. When a chiral column is used in the first chromatographic dimension and an achiral column is used for the second chromatographic dimension, coelutions can be resolved and enantiomer ratios may be calculated free from interference. This application note shows the separation of enantiomers and resolution of other coelutions in menthol and peppermint samples.

2. Experimental Conditions

Samples

One percent solutions of (+)-menthol, (-)-menthol, and peppermint oil were made in ethanol. Aliquots of each of the three solutions were mixed to provide the dilutions shown in the following figures.

GCxGC Conditions

Inlet: Split/Splitless
Temperature: 220°C
Split Ratio: 200:1
Volume Injected: 0.05 µL
Carrier Gas: Helium at 1.4 mL/min constant flow
Primary Column: Restek Rt-BetaDEXsm
30 m x 0.25 mm x 0.25 µm
Secondary Column: Restek Rtx-17
0.75 m x 025 mm x 0.5 µm
Primary Oven Conditions #1
Rate (°C/min): Initial Temp
Target Temp.: 80°C
Duration: 1 minute
Primary Oven Conditions #2
Rate (°C/min): 5.00
Target Temp.: 220°C
Duration: 5 minutes

Secondary Oven Conditions

The secondary oven is 40°C warmer than the primary oven throughout the analysis.

Modulation

Modulator Temp Offset: 35°C
Modulation Period: 1 second

TOFMS Conditions

Mass Range Acquired: 35 to 350 u
Acquisition Rate: 200 spectra/second

Single-dimension chromatograms were obtained on the GCxGC column set by putting the modulation period at zero and acquiring data at 5 spectra/second.

Chemical compounds in the sample were identified by searching for matching spectra in the NIST mass spectral library.

3. Results and Discussion

One-dimensional chromatography of peppermint oil on the Rt-BetaDEXsm column results in coelution between menthyl acetate and (+)-menthol. Figures 1 and 2 show chromatograms of peppermint oil and peppermint oil spiked with (+)-menthol, respectively. When (+)-menthol is added to the peppermint oil, the peak identified as menthyl acetate increases in size. With the similarities in spectra between menthol and menthyl acetate (Figure 3), there is no way to determine the relative amounts of the menthol isomers and menthyl acetate present in the sample.

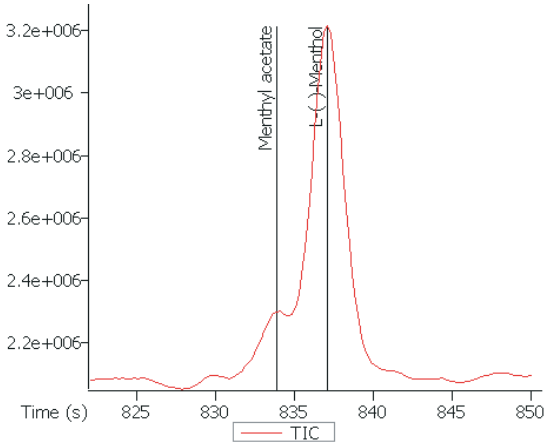

Figure 1. One-dimensional chromatogram of peppermint oil.

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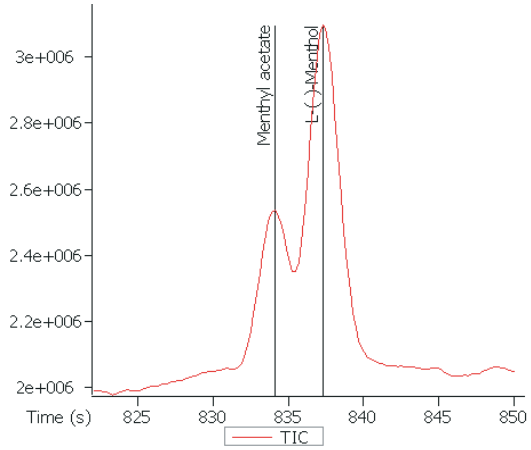

Figure 2. One-dimensional chromatogram of peppermint oil spiked with (+)-menthol. The addition of (+)-menthol increases the intensity of the peak identified as menthyl acetate.

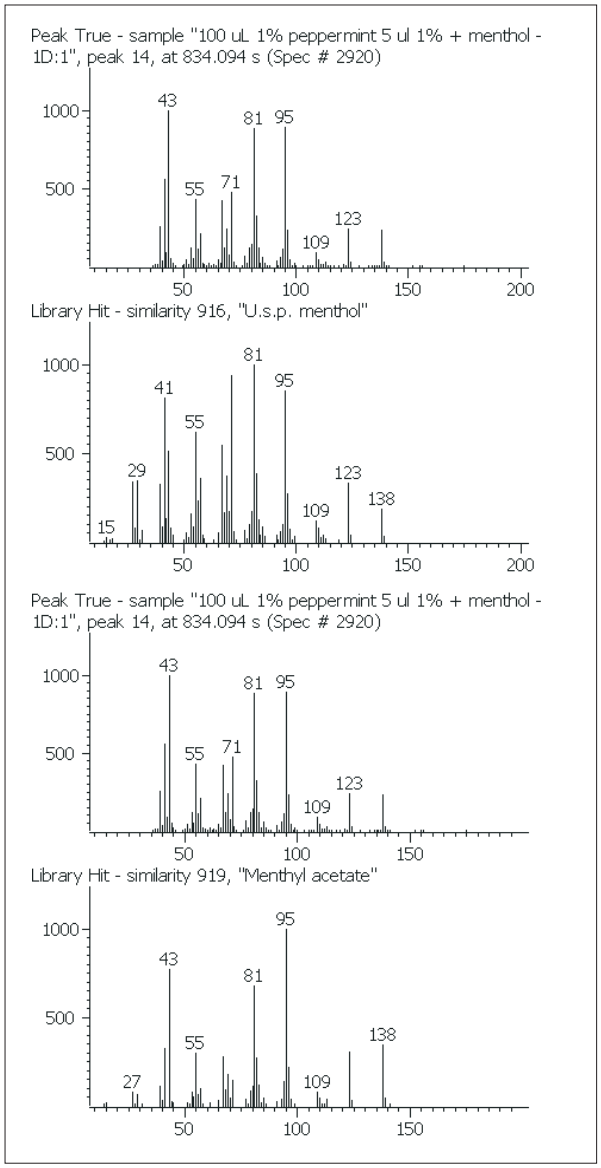

Figure 3. The spectrum of the peak resulting from the coeluting (+)-menthol and the menthyl acetate shows strong similarity to menthol and menthyl acetate spectra from the NIST library.

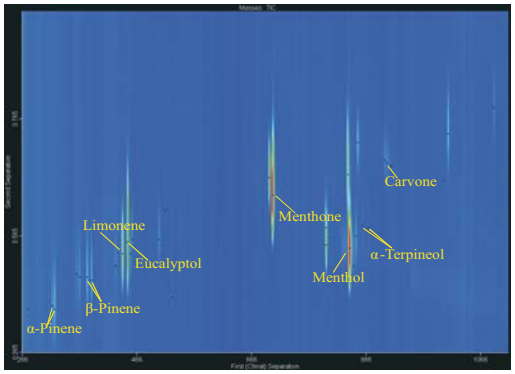

Figure 4. GCxGC separation of peppermint oil showing resolution of enantiomeric pairs. In some cases the second enantiomer is not observed in the peppermint sample.

GCxGC separation of peppermint oil is shown in Figure 4 as a contour plot, with several separated enantiomeric pairs marked; the portion of the chromatogram where menthyl acetate and (-)-menthol elute is shown in Figure 5. The same sample spiked with (+)-menthol is shown in Figure 6, where separation between menthyl acetate and (+)-menthol can be seen. This separation allows for better estimates of the quantity of the menthol enantiomers. It is clear that (+)-menthol is not detectable in the peppermint oil.

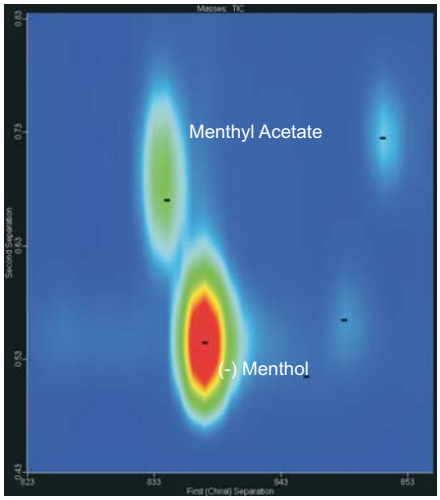

Figure 5. Contour plot of menthyl acetate and (-)-menthol in unspiked peppermint oil.

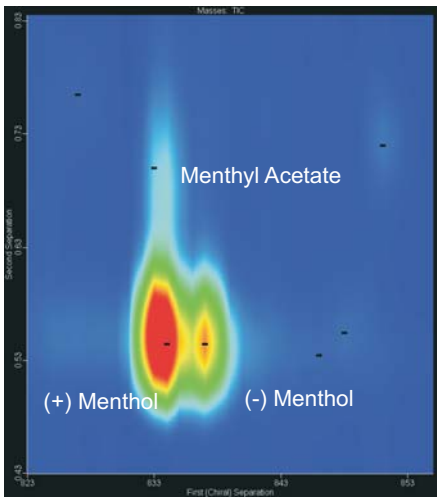

Figure 6. Contour plot of menthyl acetate, (+)-menthol, and (-)-menthol in spiked peppermint oil.

The peaks for (+) and (-) menthol are not completely resolved on the chiral column used as the first dimension in this study (Figure 7). With this coelution, the percent of the (-) isomer is overestimated when area percentages are used to estimate enantiomer percentages. A plot of area percents as a function of actual percentages of the compounds is shown in Figure 8. In a mixture of 5% (-)-menthol and 95% (+)-menthol, the (-)-menthol peak becomes a shoulder on the tail of the (+)-menthol peak. In peppermint oils, this is not an issue because the (-)-menthol isomer is the predominant isomer present.

The coelution of (+)-menthol with menthyl acetate remains a problem if there is any concern that (+)-menthol may be present in the peppermint oil. In this case, GCxGC separation must be used for the analysis.

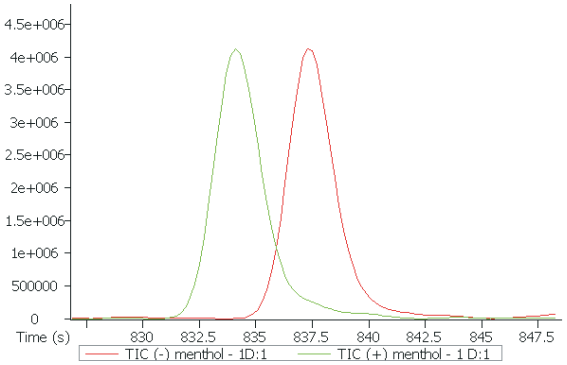

Figure 7. Overlaid one-dimensional chromatograms for (+)-menthol and (-)-menthol on Rt-BetaDEXsm. The enantiomers are not fully resolved.

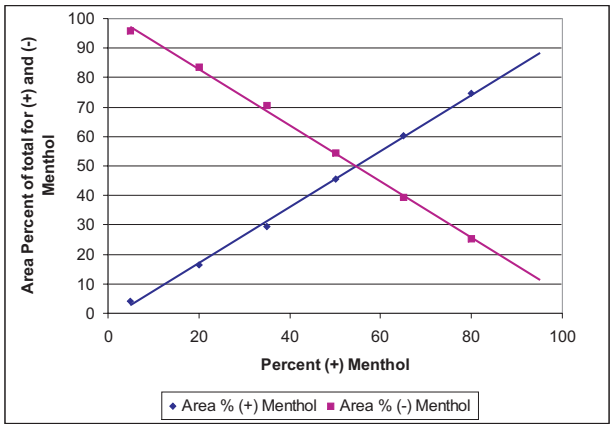

Figure 8. Response (area %) for menthol isomers as a function of weight percent for (+)-menthol, as determined by one-dimensional gas chromatography.

A portion of the linear chromatogram illustrating the "slices" from modulated (+)-menthol is shown as Figure 9. With a modulation period of 1 second, the individual menthol peaks are cut into about five slices. This modulation rate results in good peak representations (Figures 10 and 11) even when the (+)-menthol is a small fraction of the menthol present (Figure 10). At higher concentrations of (+)-menthol, quantitation of the isomer ratio becomes more difficult and would require either improved first-dimension chromatographic resolution or a higher modulation frequency to obtain good quantitation. The area percent ratios obtained for various mixtures of (+)- and (-)-menthol are shown in Figure 12.

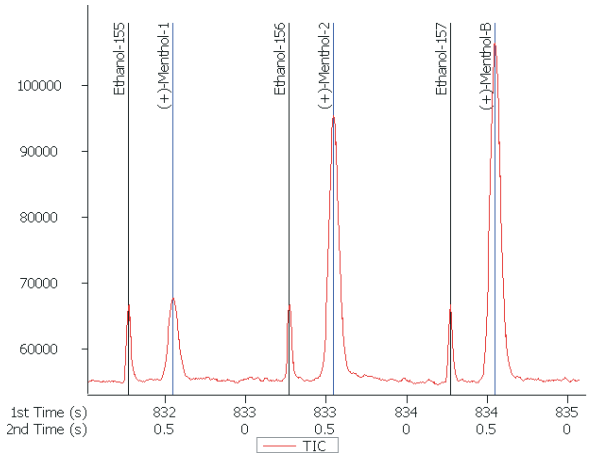

Figure 9. A portion of the GCxGC linear chromatogram through a (+)-menthol peak represents successive second dimension chromatograms of the eluent from the first dimension column.

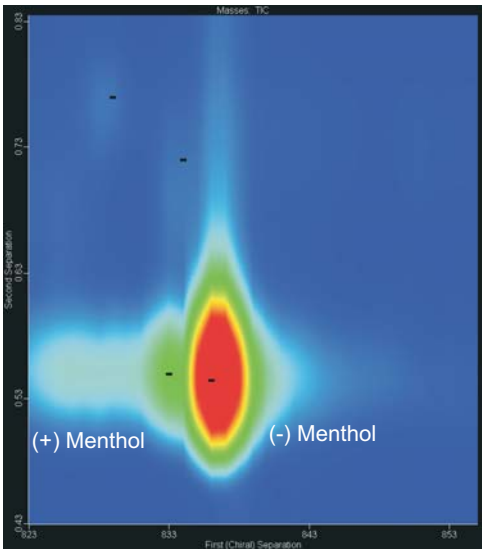

Figure 10. Contour plot of 5% (+)-menthol and 95% (-)-menthol.

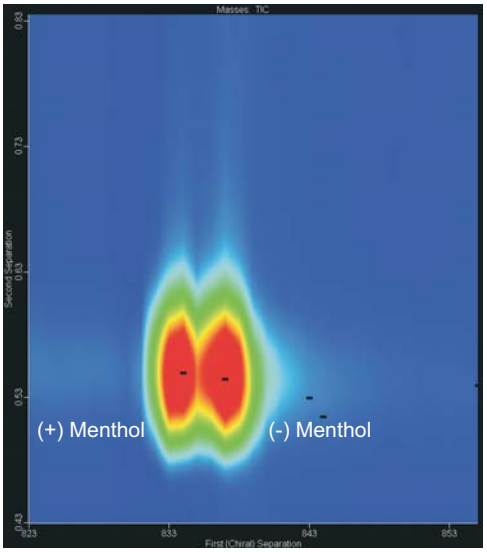

Figure 11. Contour plot of 50:50 mixture of (+)-menthol and (-)-menthol.

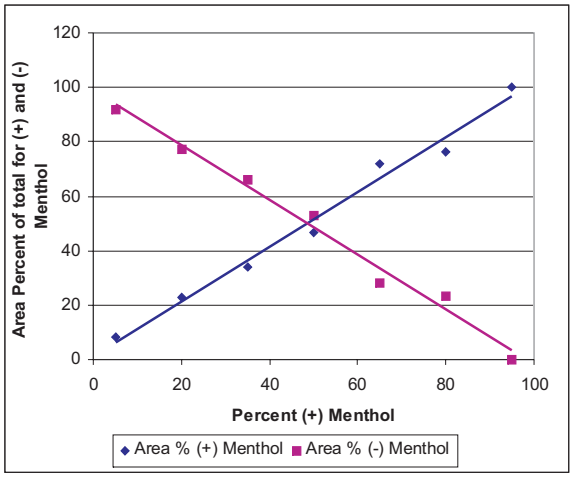

Figure 12. Response (area %) for menthol isomers as a function of weight percent of (+)-menthol, as determined using GCxGC.

For other enantiomeric pairs, the chiral separation gives sufficient resolution to yield good quantitation (Figure 13).

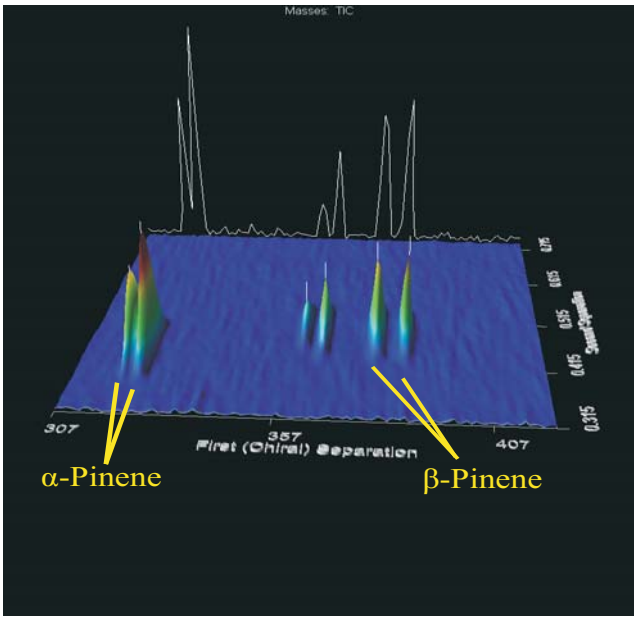

Figure 13. GCxGC surface plot of early eluting enantiomeric pairs showing good separation in the first chromatographic dimension.

4. Conclusions

GCxGC-TOFMS with a chiral first-dimension column provides improved capability for determining enantiomer ratios in complex mixtures, such as peppermint oils or natural menthol samples. GCxGC-TOFMS with a chiral column can be used to characterize flavoring ingredients, in which contamination with undesired enantiomers can adversely affect flavor quality. Selection of other chiral columns may further improve the separation of specific enantiomer pairs. GCxGC provides the benefit of increased peak capacity for resolving other possible interferences in the second dimension. Furthermore, GCxGC-TOFMS can provide a complete characterization of complex flavor samples and essential oils. Once specific compounds are identified using mass spectrometry, the method could possibly be transferred to GCxGC-FID for routine quantification of target compounds, including enantiomers.

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