Advances in Essential Oils Analysis Using Comprehensive Two-Dimensional GC and Time-of-Flight Mass Spectrometer (GCxGC-TOFMS) Detection

LECO Corporation; Saint Joseph, Michigan USA

Key Words: GCxGC-TOFMS, Food and Flavors, Deconvolution

1. Introduction

Rosemary (Rosmarinus officinalis) is one of the most used of aromatic herbs. Part of the Lamiacea (mint) family, rosemary is closely related to peppermint, spearmint, lavender, thyme and sage. The steam-distilled oil is obtained from the flowering tops, leaves, and soft twigs. The quality and chemical composition of the oil depend on how and where the plant was grown, harvested, and distilled. When conditions cause the plants to permanently produce variations in the chemical composition of their essential oils, these plants are known as chemotypes. There are three principal chemotypes of Rosmarinus officinalis, with the names given by one of the main constituents: camphor/borneol, cineole, and verbenone

Traditionally used for food flavoring, rosemary found its way into alternative medicine and aromatherapy as a mental and physical tonic, as well as a neuro-muscular regulator.

While GC-FID is the traditional method for essential oils quantification, GC-MS is the most common analytical method for component identification. The presence of numerous isomers (terpenes and oxygenated terpene structures), as well as the wide concentration range of the analytes (from ppb to percentage levels), adds an additional challenge in solving the puzzle created by these mixtures. Comprehensive two-dimensional gas chromatography (GCxGC) enhances the peak capacity for a chromatographic run allowing better separation in complex sample analysis. The additional peak capacity obtained when GCxGC is used combined with the deconvolution power from the TOFMS system tremendously improves the results obtained from the analysis of essential oils.

The purpose of this analysis was to demonstrate the use of GCxGC-TOFMS technology for the analysis of essential oils obtained from three different rosemary chemotypes and to compare the results with the ones obtained from one-dimensional analysis.

2. Experimental Conditions

GC-TOFMS

GC:	Agilent 6890 GC
Primary Column:	Rtx-5, 30 m, 0.25 mm id, 0.25 µm film thickness
Oven Program:	50°C (1 minute hold) to 250°C at 3°C/minute
Inlet Temperature:	250°C
Injection Size:	0.2 µl with a split ratio of 200:1
Carrier Gas:	He at a constant flow of 2 ml/minute
MS:	LECO Pegasus® III TOFMS
Ionization:	EI at 70 eV
Mass Range (u):	20 to 350
Acquisition Rate:	20 spectra/second
Source Temperature:	225°C

GCxGC-TOFMS

GCxGC:
Agilent 6890 GC equipped with a LECO Thermal Modulator (technology under license from Zoex Corporation)
Primary Column:
Rtx-5, 30 m, 0.25 mm id, 0.25 µm film thickness
Main Oven Program:
50°C (1 minute hold) to 250°C at 3°C/minute
Secondary Column:
VB-210, 2 m, 0.1 mm id, 0.1 µm film thickness
Secondary Oven Program:
5°C leading difference from main oven
Inlet Temperature: 250°C
Injection Size: 0.2 µl with a split ratio of 200:1
Carrier Gas:
He at a constant flow of 2 ml/minute
Modulator Temperature: 30°C offset from main oven
Modulation Frequency:
4 seconds with a 0.5 second hot pulse time

MS: LECO Pegasus® 4D GCxGC-TOFMS
Acquisition Rate: 150 spectra/second
The rest of the parameters were kept the same as for GC-TOFMS analysis.

3. Results

One-Dimensional Analysis (GC-TOFMS)

The one-dimensional total ion current (TIC) chromatograms for the oils obtained from three different rosemary chemotypes are presented in Figure 1.

More than 200 components were found to be present in each of the rosemary oils analyzed when the data was processed with a S/N ratio of 30. The main components are presented in Table 1. Peak identification for these 51 components was accomplished with the NIST Library and confirmed with published retention time data (Robert P. Adams). All 51 analytes have similarities with NIST library spectra higher than 700 (a similarity of 999 represents a perfect match with the library).

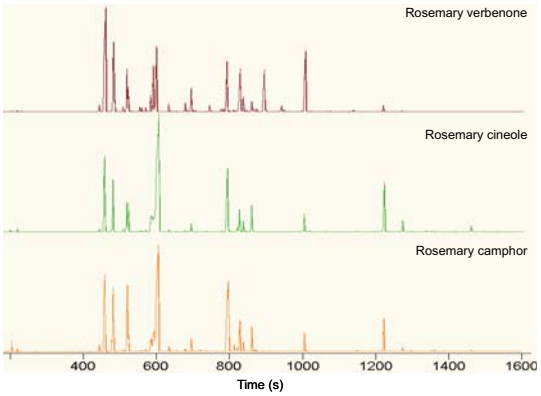

Figure 1. TIC chromatograms obtained from the GC-TOFMS analysis of the three rosemary oils.

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The three essential oil samples were compared using an automated algorithm available in the ChromaTOF® software. The algorithm allows the analyst to build a reference table from the peak table of one the analyzed samples and compare other samples against it on a peak-by-peak and spectrum-by-spectrum basis.

For this study the camphor chemotype was chosen as the reference sample. The major differences between the three rosemary chemotypes analyzed for this study are presented in Table 2. Differences in the analyte's concentrations were not the purpose of this study and are not presented in the table.

Table 1. Main components present in rosemary oil listed with retention times and library similarities.

Component	t_R (s)	Similarity
Propylene glycol	269.2	814
γ -Thujene	392.3	790
Tricyclene	443.5	846
γ -Pinene	458.7	950
2,2-dimethyl-5-methylene-norbornane	465.1	810
Fenchene	477.2	911
Camphene	481.8	942
4-benzyloxy-benzenepropionic acid	484.7	781
4,5-Nonadiene	490.9	754
γ -Pinene	519.3	919
γ -Myrcene	522.9	853
Sabinene	552.6	788
γ -Phellandrene	554.9	806
2-Carene	560.3	673
γ -Terpinene	570.9	866
p-Cymene	584.8	902
Limonene	593.0	902
Eucalyptol	604.9	959
γ-terpinene	633.7	886
cis-Sabinenehydrate	654.7	846
Terpinolene	678.6	860
2-methyl-2-propenylbenzene	686.2	723
3,3,5-trimethyl-1,4-hexadiene	691.8	716
Linalool	695.4	875
trans-Sabinenehydrate	706.2	818
2,6-dimethyl-3,7-octadien-2-ol	714.6	741
γ -Campholenal	720.3	751
exo-Fenchol	738.1	776
Pinocarveol	781.3	720
Camphor	797.1	965
Isoborneol	812.8	890
p-Meth-1en-8-ol	821.8	776
Borneol	828.4	948
4-Terpineol	837.8	872
p-Cymen-8-ol	846.2	725
Terpineol	860.8	908
Ocimenol	867.5	787
Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl	873.3	795
Verbenone	891.6	807
Linalyl anthranilate	936.4	794
1-Carvone	943.6	754
Bornyl acetate	1004.6	884
Isobornyl formate	1009.7	825
γ -Cubebene	1148.5	756
Caryophyllene	1222.2	901
Caryophyllene	1273.5	817
Copaene	1297.3	748
Farnesene	1337.3	723
Germacrene	1354.1	731
Adamantane, 1-(2-bromoethenyl)-	1354.3	707
Cadinene	1359.5	751

Table 2. Major difference in the composition of the oils obtained from the three different rosemary chemotypes.

Compound	R Camphor	R Cineole	R Verbenone
Fenchene		ND	ND
p-mentahadiene	ND	ND	
2,7-Octadiene-1,6-diol, 2,6-dimethyl-, (Z)-	ND	ND	
cis-3-Hexenyl iso-butyrate	ND	ND	
Pinocarveol		ND	
Verbenol	ND	ND	
Verbenone		ND	
10-Undecyn-1-ol	ND	ND	
Dihydrocarveol acetate	ND	ND	
2 Cubebene isomers			ND
Farnesene			ND
Germacrene			ND
Cadinene			ND
Calamanene			ND

ND – Not detected

The essential oil obtained from the verbenone chemotype of the rosemary plant seems to be more complex. Some of the 236 components found are unique to this sample. The essential oil obtained from the verbenone chemotype is the one preferred in aromatherapy and is defined as the "most skin-friendly". The cineole and camphor chemotypes are more similar to each other. The main difference between these two samples is the complete absence of verbenone from the rosemary cineole oil sample.

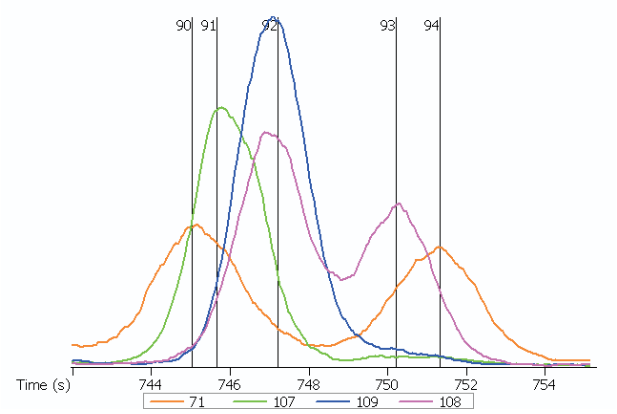

Figure 2. Unique m/z values for five rosemary oil components eluting in a twelve-second region of the chromatogram.

The high acquisition rates (up to 500 spectra/second) along with the spectral continuity along the chromatographic peak profile obtained from the TOFMS instrument allow automated peak find and spectral deconvolution capabilities for severely overlapping peaks. Figure 2 shows the power of the Peak Find algorithm. Five peaks are found in a time window span of only 12 seconds. The separation between peak apexes is as small as 0.6 seconds (peaks 90 and 91).

Two-Dimensional Analysis (GCxGC-TOFMS)

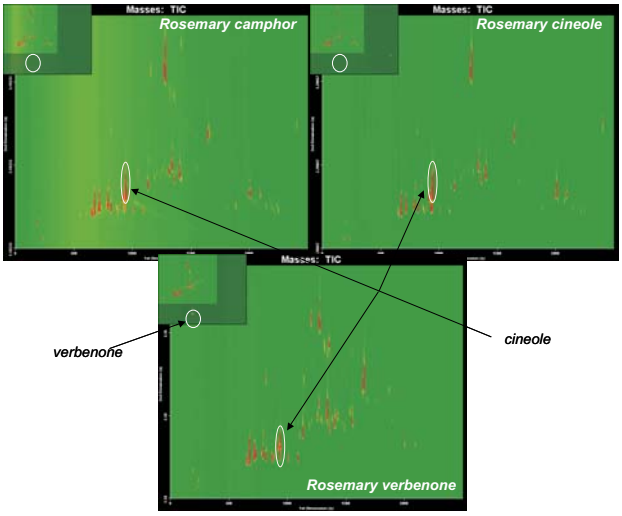

Figure 3. TIC chromatograms from GCxGC analysis presented as contour plots on selected time regions for the three rosemary chemotypes. The upper left corner of each chromatogram represents the overall view of the TIC.

GCxGC technology allows the use of two different separation mechanisms in order to increase the separation power of the chromatographic system. The combination of increased peak capacity and deconvolution present in the Pegasus 4D GCxGC-TOFMS system resulted in more than 450 peaks being found and identified in each of the three rosemary oil samples (twice as many as in the one-dimensional analysis). Figure 3 shows the TIC chromatograms presented as contour plots with retention time on the first column plotted on the x-axis and retention time on the secondary column plotted on the y-axis. Peak intensities are represented on a color scale from green to red. Major differences between the three samples can be visualized especially in the region between 1700 and 2500 seconds.

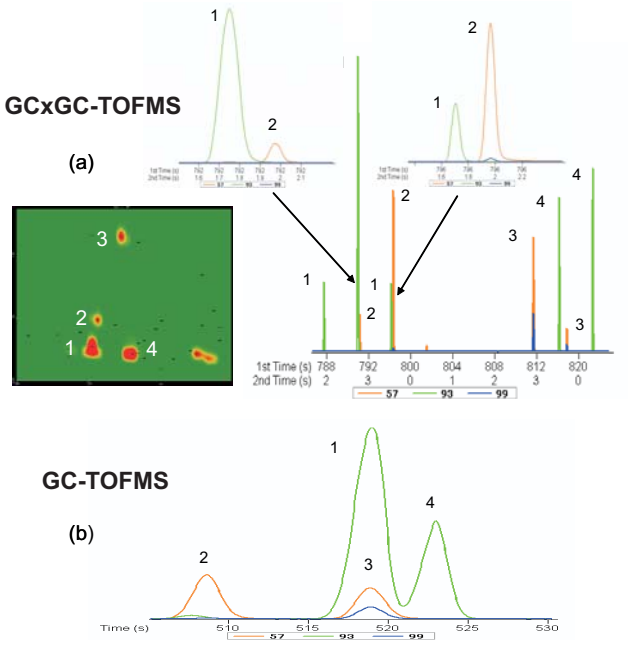

Figure 4. Chromatograms of rosemary verbenone oil. (a)–contour plot of the TIC chromatogram in a selected region and extracted ion chromatogram for the same region in a linear display. (b)–extracted ion chromatogram from a one-dimensional analysis.

The increased peak capacity obtained when using the GCxGC-TOFMS system results in increased chromatographic separation for peaks that were coeluting in a one-dimensional analysis. Figure 4 presents such an example from the analysis of the verbenone chemotype. When one-dimensional analysis was used a-pinene (1) and 3-octanone (3) perfectly coeluted and only one peak was detected. Since the octanone has a lower concentration than pinene (about 3 times lower), the peak was identified as a-pinene. The higher polarity of 3-octanone generated an increased retention time on the wax column and, consequently, a complete separation of the two compounds.

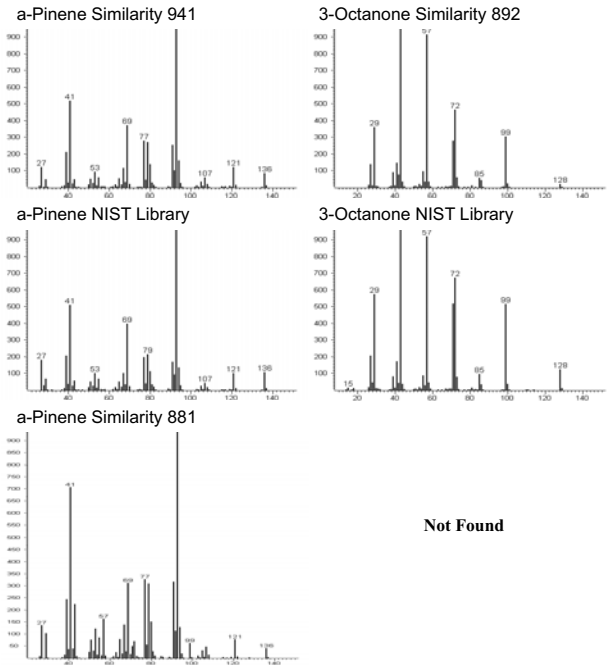

Figure 5. Mass spectra of components 1 and 3 from Figure 4. Upper part represents the data obtained from the GCxGC-TOFMS analysis, second row represents the first match from the NIST library, and the lower part represents the data obtained from the GC-TOFMS analysis.

Added chromatographic separation results in better spectral quality and better spectral matches with the library. Figure 5 shows spectral data for a-pinene and 3-octanone obtained from the one- and two-dimensional analysis. After the octanone interference was subtracted from the pinene spectrum, the library match increased from 881 to 941.

Delivering the Right Results

4. Conclusions

Three different essential oils obtained from three different chemotypes of rosemary were analyzed and compared both by GC-TOFMS and by GCxGC-TOFMS. The increase in chromatographic separation obtained from the two-dimensional analysis led to additional peaks being found and to a more thorough comparison of the samples analyzed.

The Deconvolution algorithm of the ChromaTOF software facilitated by the acquisition speed and spectral continuity obtained from the TOFMS was able to extract accurate spectral information for comparison with the NIST library in the regions where coelutions of peaks occurred.

LECO Corporation • 3000 Lakeview Avenue • St. Joseph, MI 49085 • Phone: 800-292-6141 • Fax: 269-982-8977
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