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Rapid Marker Identification and Characterization of Essential Oils Using a Chemometric Approach

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Abstract

This application note describes how to provide a solution for the identification of potential markers of essential oils for the characterization of fragrances, to aid in quality control and authentication within the flavor and fragrance market.

Introduction

The flavor, fragrance, and aroma chemical market is said to be worth around $15 billion worldwide, with the essential oils market seeing significant growth backed largely by the increasing preference among consumers for more natural products.

As commodities in the fragrance and flavor market are generally high value items, the ability to characterize and compare raw materials, blends, and final goods has important financial implications.

As prices of natural products fluctuate widely depending on the raw material, determining factors such as country of origin, climate, batch, concentration, extraction method, and shelf life become paramount for quality control and to prevent product counterfeiting.

With reports of 5 to 7% of world trade being counterfeit products, amounting to a mammoth $500 billion annually, validation of factors in natural products such as essential oils become even more vital.

Currently identification and characterization of fragrances is carried out manually, a very time-consuming process that can take a trained analytical chemist many hours, with a high risk of human error.

By implementing a GC-ToF exact mass-MS solution, a powerful; information rich data set can be generated. Automated, chemometrics based software, using a statistical interpretation of patterns in multivariate data can be used for further analysis. This results in a faster and more precise assessment of composition.

The advantage of using a chemometrics with GC-TOF-MS approach is the production of a unique "fingerprint" for each essential oil. This allows a good statistical separation and facilitates the identification of differences or similarities between groups.

The following method describes essential oil characterization for a range of samples from the citrus family using a GC-TOF-MS solution complete with chemometric data analysis.

Examples of essential oil separation, marker identification, interand intra-class differentiation will be illustrated as well as database building and fragrance characterization.

Four types of essential oils from the citrus family.

Experimental

Sample

Four types of essential oils from the citrus family were studied (lemon, mandarin, orange, and bergamot). The essential oils analyzed are listed in Table 1. The samples were diluted 1000:1 in ethanol prior to analysis by GCT Premier.

	Essential oils	Origin
1	Bergamot	Ivory Coast
2	Bergamot	Italy
3	Lemon	Argentina
4	Lemon	Argentina
5	Lemon	Ivory Coast
6	Lemon	Spain
7	Lemon	Italy
8	Lemon	Italy
9	Lemon	Italy
10	Mandarin Yellow	Italy
11	Mandarin Yellow	Italy
12	Mandarin Red	Italy
13	Mandarin Red	Italy
14	Mandarin Red	Italy
15	Mandarin Red	Italy
16	Mandarin Green	Italy
17	Mandarin Green	Italy
18	Mandarin Green	Italy
19	Orange	South Africa

Column:	Restek Rtx -5 ms, 30 m x 0.25 mm 0.25 μm
Flow rate:	1.0 ml/min Helium constant flow
Injection method:	Split (split ratio of 10:1), 1 μl
Temperature ramp:	60 to 180 °C at 7.5 °C/min and to 250 °C at 50 °C/min (hold for 1.6 min)
Total run time:	20 min

MS Conditions

MS system:	Waters GCT Premier
Ionisation mode:	Electron ionisation (EI +)
Electron energy:	70 eV
Source temperature:	200 ˚C
Trap current:	200 μA
Interface temp.:	280 ˚C
Detector voltage:	2850 V
Acquisition range:	*m/z* 50 - 500
Acquisition time:	0.19 s with 0.01 s delay (2.5 spectra/s)
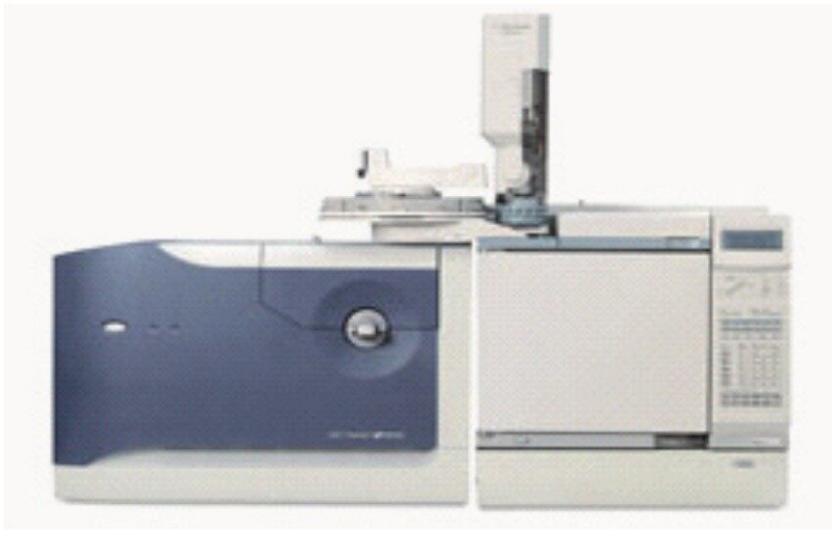
GCT Premier Mass Spectrometer

Exact mass spectra were obtained using a single-point lock mass (perfluorotributylamine, *m/z* = 218.9856) infused into the source continuously during the run. The instrument was tuned so that resolution was greater than 7000 FWHM (Full Width Half Maximum).

Acquisition and Processing Methods

The data were acquired using Waters MassLynx Software v. 4.1 and processed using MarkerLynx and ChromaLynx Application Managers.

· MarkerLynx is used for comprehensive data processing for chemometric applications, including multivariate statistical analysis and visualization

· ChromaLynx facilitates rapid detection, identification and semi quantitative determination of components in complex matrices

Results and Discussion

Figure 1 shows that differences between citrus essential oils are not easily discerned and a manual chromatogram-by-chromatogram inspection is very labor-intensive. MarkerLynx automates this process by rapidly characterizing essential oils, based on a PCA data set and will generate a list consisting of t_R - *m/z*
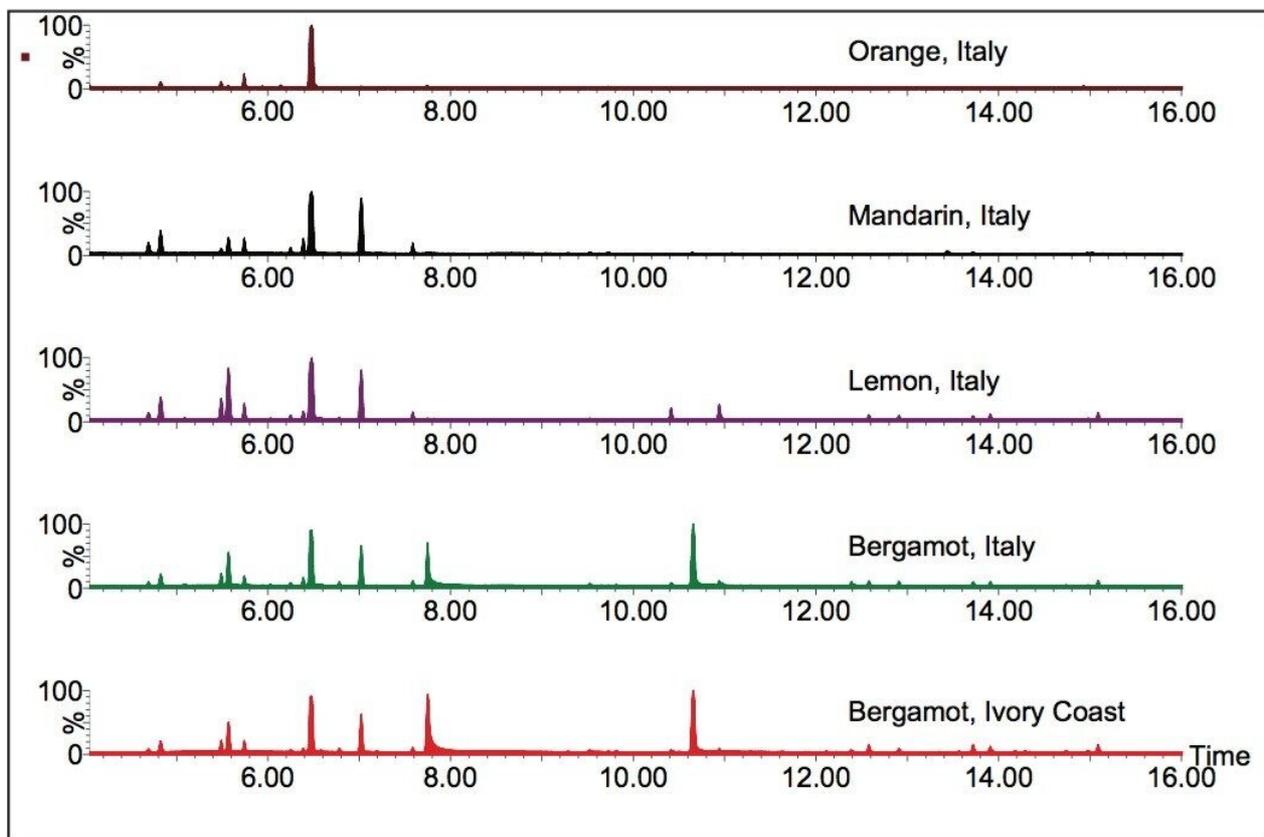
pairs ranked in order of statistical significance.

Figure 1. Total ion chromatograms of five citrus essential oils obtained using GC-TOF-MS.

Rapid inter-class citrus oil differentiation

Figure 2 illustrates the MarkerLynx scores plot after processing 25 essential oils. The results show that the three types of mandarin oils are statistically similar, while divergent from orange, lemon, and bergamot. The high analytical reproducibility of the system is demonstrated through the tight sample clustering of each class of oil.
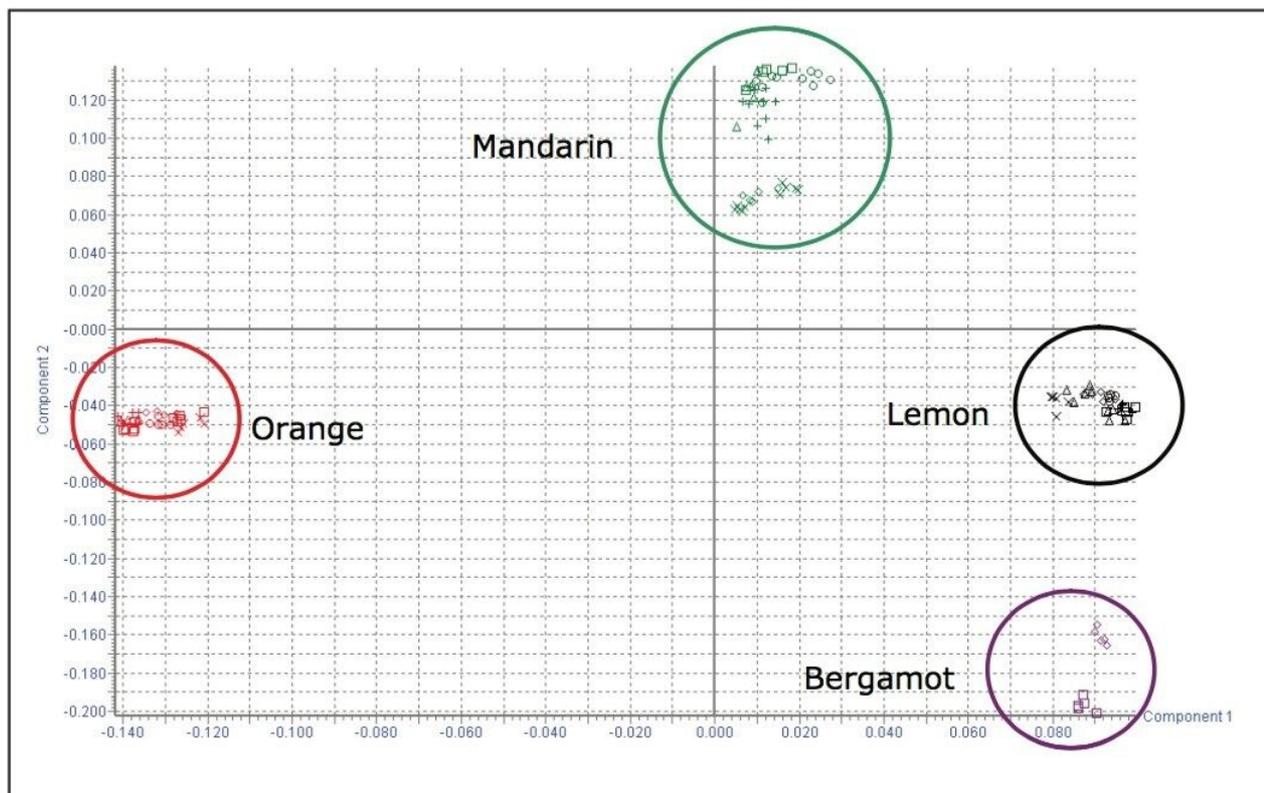
Figure 2. Scores plot obtained from MarkerLynx, illustrating group similarities between 25 essential oils.

The results show that the oils can be successfully separated. However, in order to determine the reasons for this differentiation, closer inspection of these results are required.

Figure 3 shows one of the t_R - *m/z* pairs that contributed most significantly to the separation of lemon from the rest of the oils. This trend plot showing sample number versus significance of marker to class, illustrates a potential marker for lemon oils.
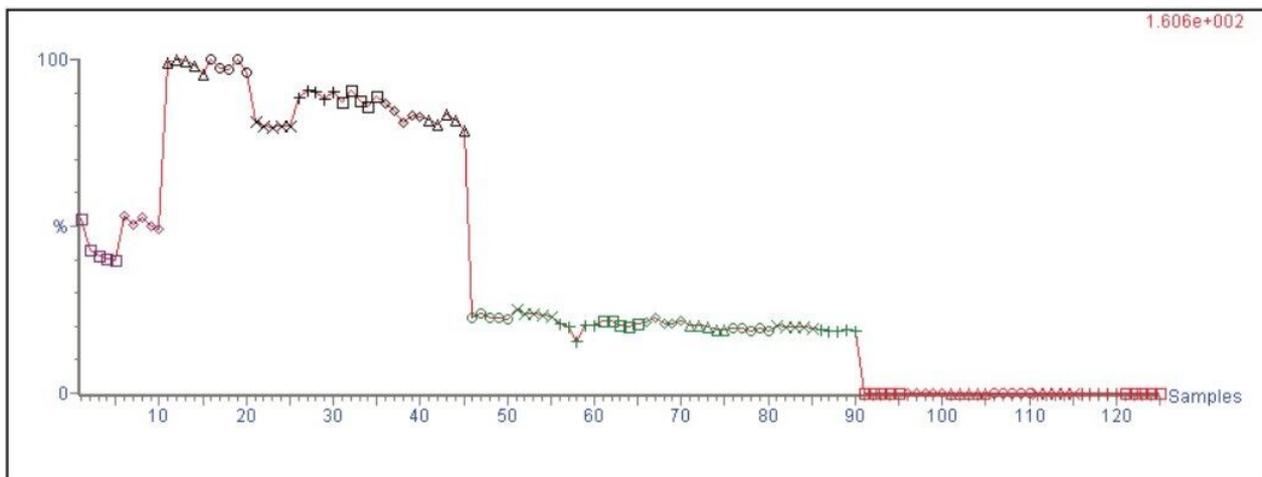
Figure 3. Trend plot of the t_R - m/z pair 12.89 - 93.0700, across 25 essential oils.

Identification of Potential Essential Oil Markers

After automated processing of the data sets using ChromaLynx, the potential marker shown in Figure 4 was identified by library searching and exact mass. The marker was identified as beta-pinene with an exact mass of 136.1256, 0.4 mDa from the calculated mass of $C_{10}H_{16}$.

If the potential marker does not exist in the library, exact mass will enable the elemental composition to be determined as a useful start point in establishing structural identification.

After potential markers have been identified, MarkerLynx can be used to create a database of compounds to help search for markers in subsequent analyses.
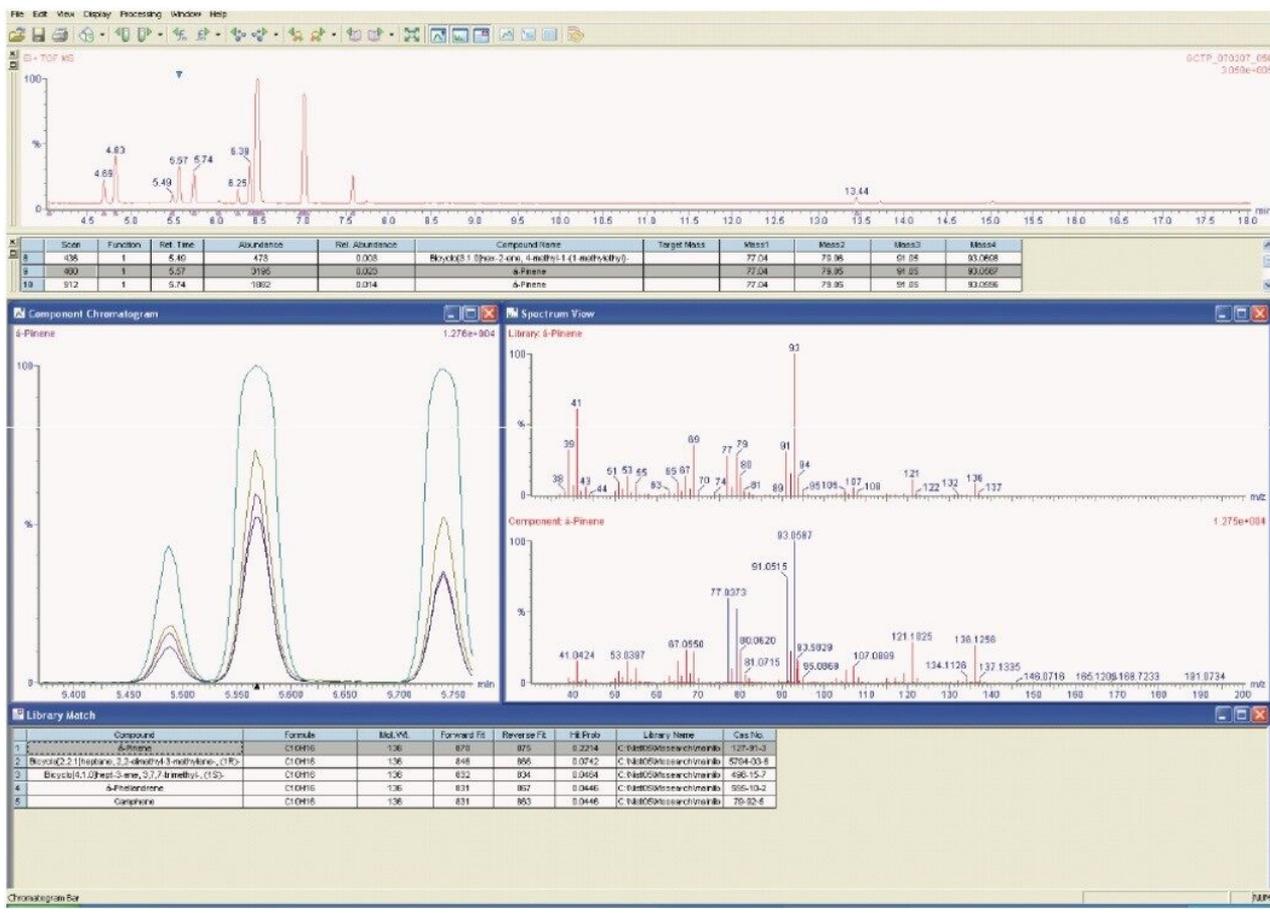
Figure 4. Identification of beta-pinene in a lemon oil using ChromaLynx.

Rapid Intra-Class Essential Oil Differentiation

It is also possible to find similarities within each class of oil using multivariate analysis. These intra-class differences include country of origin, age, quality, etc.

For example, Figure 5 illustrates the intra-class separation of mandarin red oils obtained using MarkerLynx. Differences between sample clusters relate to the year of harvest and quality of the olfactory citrus notes.
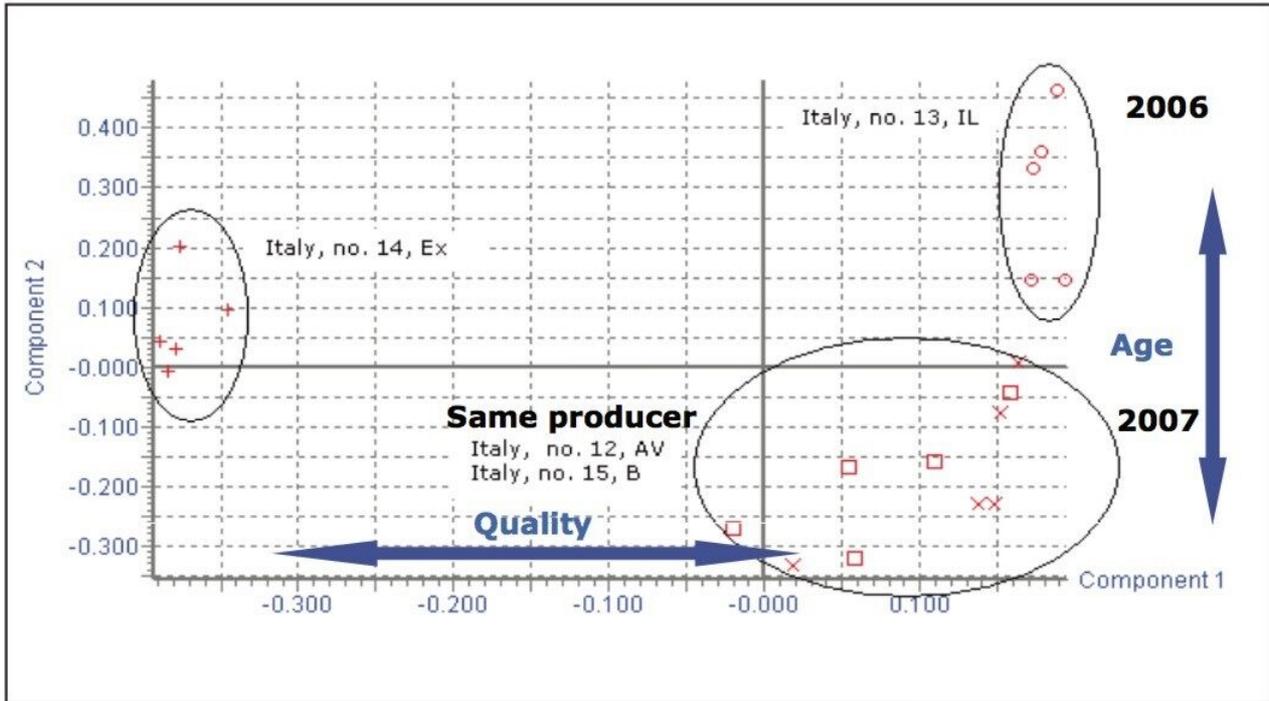
Figure 5. Scores plot obtained from MarkerLynx illustrating group similarities between four Italian mandarin red oils.

Additionally, a supplementary software package, SIMCA-P from Umetrics, can be used to search for further differences within each class. SIMCA-P identifies differences between two samples, using advanced statistical tools such as partial least squares discriminate analysis (PLSDA) and orthogonal partial least squares (OPLS), allowing researchers to visualize their data and extract more meaningful information from their experimental results.

Figure 6 shows enhanced differentiation between two of the lemon oils. The points above 80% significance (highlighted in red) were selected and used to create an additional list of potential markers, which can be imported back into MarkerLynx.
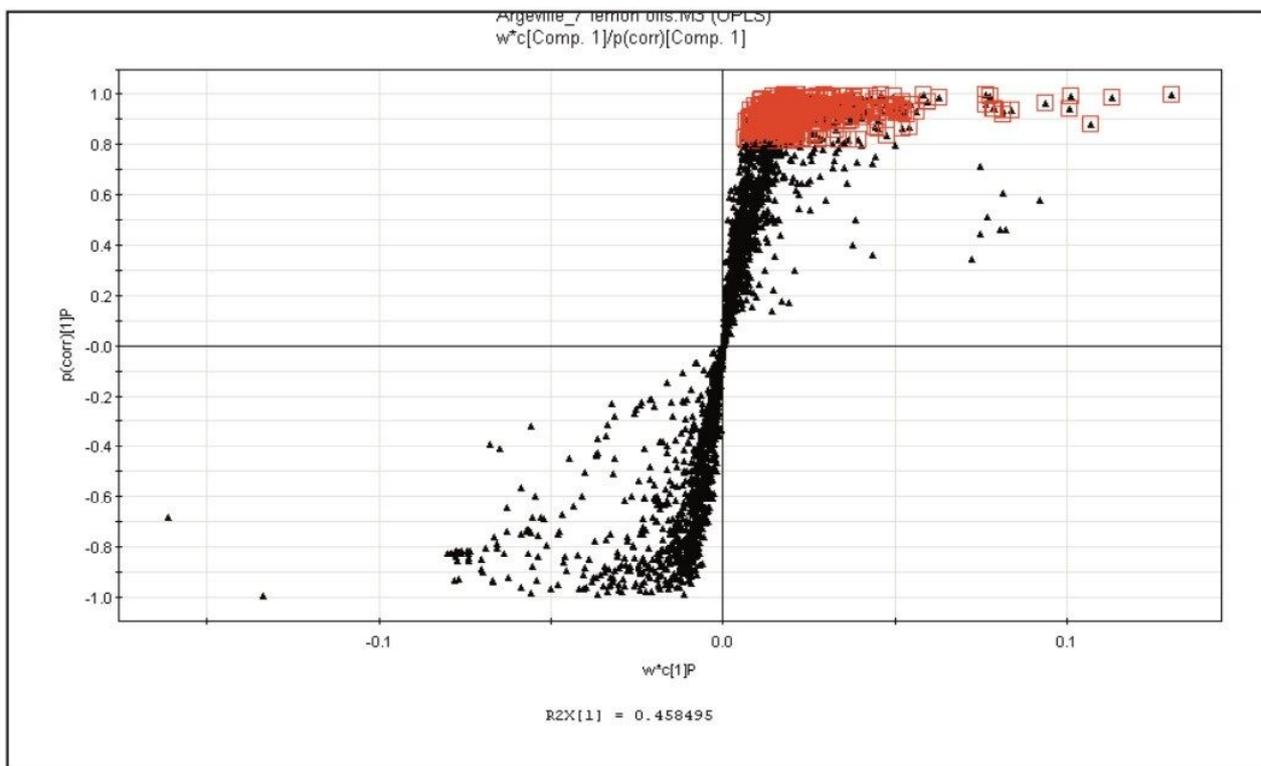
Figure 6. Scatter plot of lemon oil no. 7 vs. lemon oil no. 9, obtained from an OPLS model using SIMCA-P.

Identifying Essential Oils within a Fragrance

ChromaLynx Compare offers complementary functionality that is able to evaluate multiple samples to identify either common or unique components between them.

Figure 7 shows the comparison between a fragrance and one lemon oil. The results show unique components by retention time and mass (flagged with the dark triangles). The common components suggest that this variety of lemon oil is part of the composition of the fragrance analyzed.
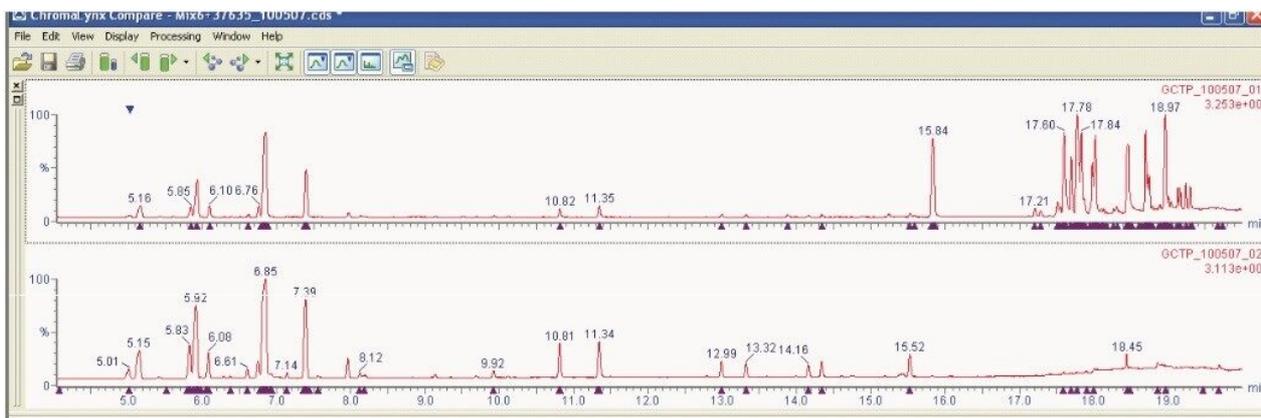
Figure 7. Comparison of a fragrance (top chromatogram) with one lemon oil (bottom chromatogram), using ChromaLynx Compare.

Conclusion

As the prices of raw materials vary greatly and product counterfeiting is a rising problem, the GCT Premier with MarkerLynx and ChromaLynx Application Managers is the ideal solution for product authentication and quality control in the flavor and fragrance market.

With an unmatched combination of capabilities, as well as high full scan sensitivity and rapid spectral acquisition rates, this total Water's solution is ideal for full characterization and profiling of components in a wide range of sample types.

Using GC-exact mass TOF-MS on the GCT Premier enabled separation and identification of potential markers in essential oil samples from the citrus family.

MarkerLynx with a chemometric approach to the interpretation of data, allowed valuable detection and identification of potential markers in these essential oils. Inter- and intra-class differentiation between samples was also achieved using SIMCA-P software with multivariate PLS or OPLS statistical methods.

ChromaLynx Compare is also a useful tool in the characterization of fragrances. It enables the comparison of unique (or common) components that have previously undergone automated library search for identification.

The GCT Premier with MarkerLynx and ChromaLynx Application Managers enables powerful data acquisition and analysis capabilities for fragrance characterisation. Plus, the automation of manually intensive processes

will result in increased productivity and efficiency within the lab.

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ChromaLynx <https://www.waters.com/513759>

Progenesis QI Software <https://www.waters.com/134790655>

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