White Paper 037
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Easy-to-learn workflows for quality control of essential oils

This study demonstrates the use of ChromCompare+ software to identify significant differences across lemon oil batches for quality control and authenticity studies, as well as streamlined workflows to quickly classify samples of unknown origin.

Introduction

Robust quality control (QC) of essential oils is required to ensure purity or authenticity, prevent adulteration and maintain consistent levels of quality and safety.

Essential oils, such as citrus oils, are widely used for flavouring and fragrances, but their compositions can differ considerably due to different plant phenotypes, growing conditions and extraction techniques. Furthermore, expensive lemon oils can be subject to economic adulteration. It is therefore important to have fast analytical workflows for routine QC testing, to ensure consumers do not receive an inferior product.

While GC–MS instrumentation is constantly evolving, allowing us to gain greater insight into our samples' compositions than ever before, it often results in data processing becoming a 'bottleneck'.

ChromCompare+ is a powerful chemometrics platform to transform complex 1D or 2D GC–MS data into meaningful and usable results through automated, easy-to-use workflows.

Here, we will demonstrate the use of ChromCompare+ software to identify significant differences between batches of lemon oils using all of the raw GC–MS data.[1] This innovative approach will be shown to minimise laborious pre-processing steps, reduce the risk of missing important details and enable simple classification of unknown samples.

Experimental

Samples: Seven lemon oils of different origin were analysed in replicate (n = 5) by GC–TOF MS.

GC-MS: Instrument: BenchTOF-Select™; Mass range: m/z 40–500; Acquisition rate: 10 Hz in Tandem Ionisation® mode at 70 and 12 eV.

Software: Full instrument control by TOF–DS, with data processing in ChromCompare+.

Results

Analysis of seven lemon oils of unknown origin and authenticity was performed in replicate (n = 5) by GC–TOF MS (Figure 1). At first glance, the chromatograms seem similar, but the minor constituents varied significantly.

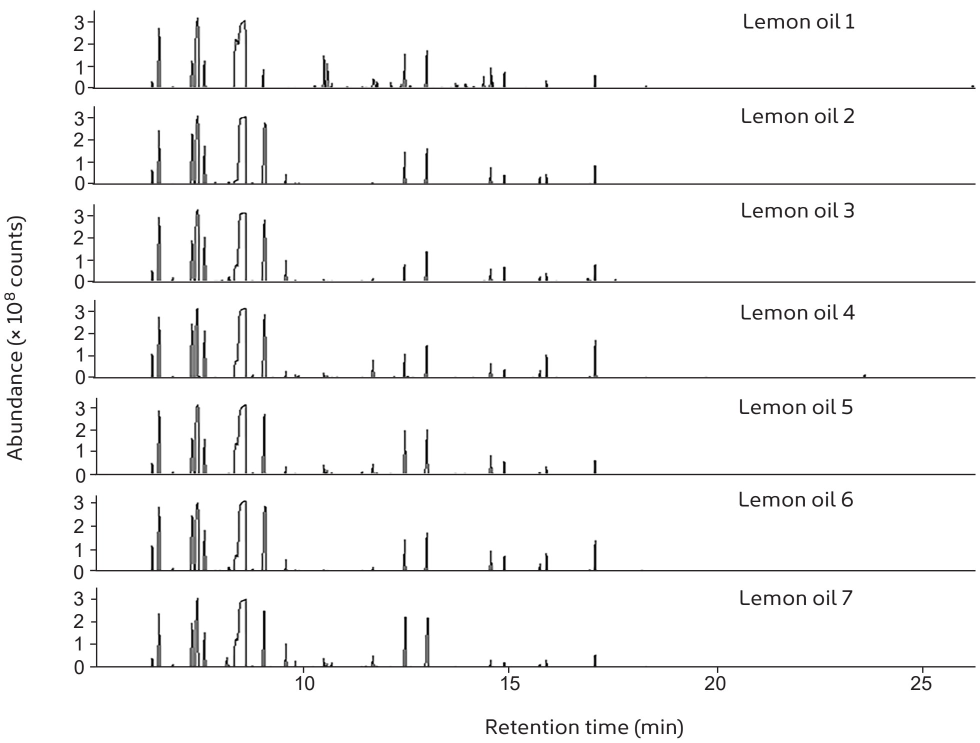

Figure 1

GC–TOF MS chromatograms for the seven lemon oils analysed in this study.

A simple test for lemon oil purity is to measure the δ-3-carene/camphene ratio. Typically, values greater than 0.13 indicate possible adulteration by addition of less expensive orange oils.[2] Figure 2 shows that four of the oils in this study exceeded this threshold so could have been adulterated. However, there are other considerations to be taken into account for product quality, as well as other methods of adulteration, so it is important to be able to look at the entire sample composition during quality control to uncover other significant differences between batches.

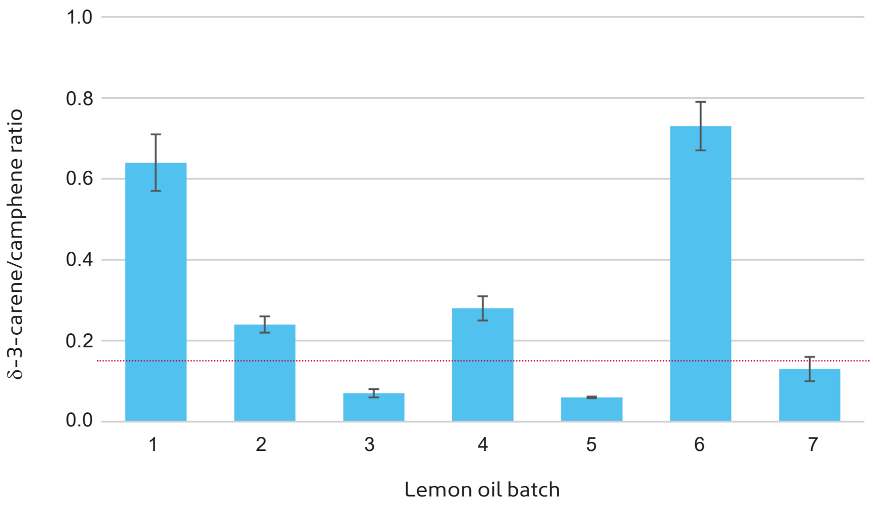

Figure 2

The δ-3-carene/camphene ratio calculated for each lemon oil (n = 5). The red line indicates the typical threshold (<0.13) used to define a high-purity lemon oil.

Therefore, ChromCompare+ software was used to investigate sample differences using all the raw data to minimise the risk of important details being overlooked. 'Feature Selection & Validation' was then performed to automatically find the most significant differences from a total of over 10,000 features and create a class prediction model (Figure 3). Class prediction is a useful statistical tool for automatically assigning unknown samples to a predefined class, for example, different qualities of essential oils.

Figure 3 shows that lemon oils 3, 5 and 7 – which exhibited low δ-3-carene/camphene ratios – cluster closely. These oils were also shown to contain higher abundances of citropten and oxypseucedanin than all other oils. Higher abundances of these compounds are known indicators of cold-pressed lemon oils (in comparison to steam distilled oils).[3] On the other hand, lemon oils 2 and 4 were found to contain significantly higher abundances of geranial. These differences were found through automatic workflows in ChromCompare+ with no manual pre-processing.

SepSolve Analytical Ltd
T: +44 (0)1733 669222 (UK) +1 888-379-3835 (USA)
E: hello@sepsolve.com

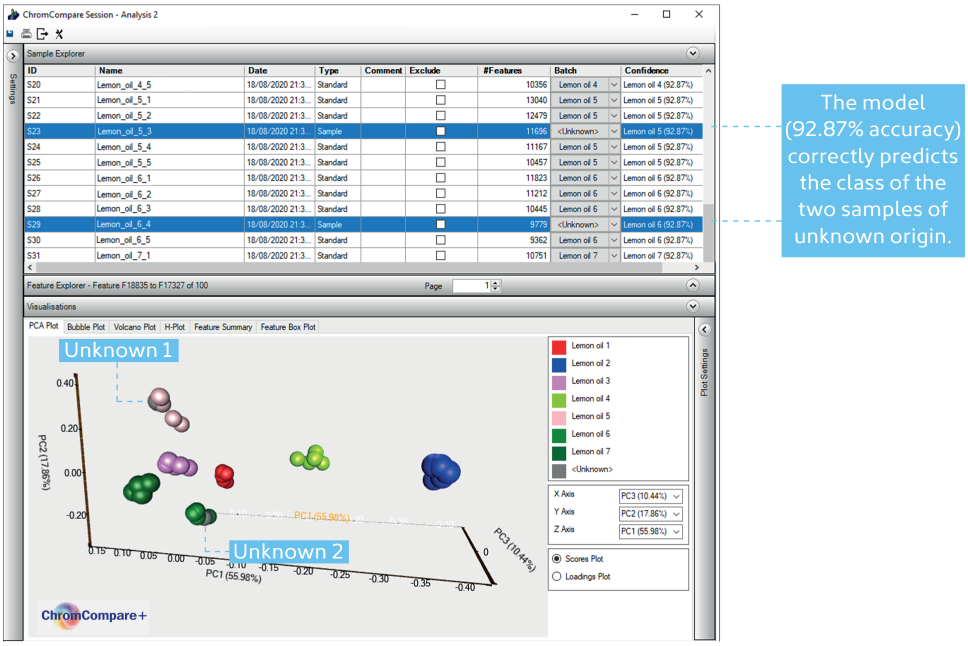

Figure 3

3D PCA visualisation of the class prediction model in ChromCompare+ allowing two ‘unknown’ lemon oils to be classified automatically.

In this study, two datafiles (replicates of lemon oils 1 and 5) were selected as ‘unknowns’ to test the ‘Random Forest’ model, which was shown to have 92.87% accuracy during cross-validation. In this case, both classes were predicted correctly. Such prediction models are essential for fast insight into samples of unknown origin and provide a routine QC measure to maintain product quality and ensure consumers do not receive an inferior product.

Minor differences between the samples were also easily viewed using feature summary charts in ChromCompare+ (Figure 4). Sample 6 was found to contain a number of analytes that were not present in any of the other lemon oils – these included triacetin and a series of acetals (namely octanal diethyl acetal, nonanal diethyl acetal and decanal diethyl acetal). These are naturally-occurring compounds, but are known flavouring additives, which could be why they are only found in high abundances in one variety of lemon oil. Figure 4 shows that the nonanal diethyl acetal was a trace difference (present at an abundance 3.5 orders of magnitude lower than the highest-loading peak) and may have been overlooked if all the raw data had not been utilised.

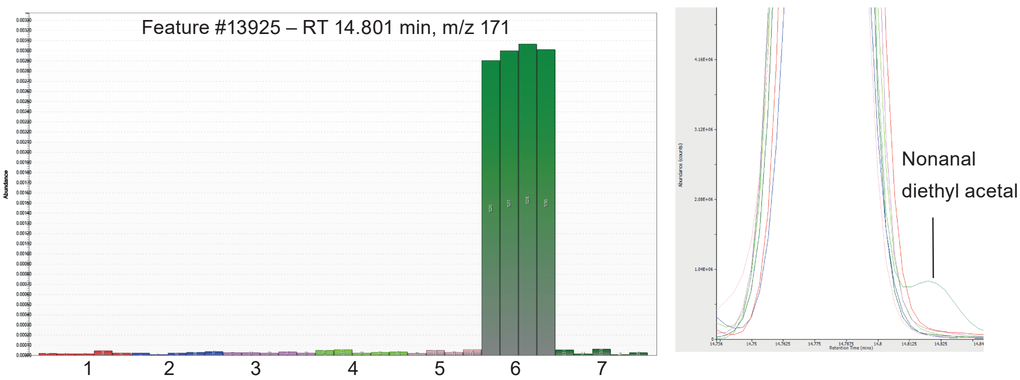

Figure 4

Feature summary chart showing a key difference uncovered using the raw data in ChromCompare+, with the corresponding section of the overlaid chromatogram shown on the right.

Conclusions

This white paper has demonstrated that ChromCompare+ is a powerful tool for chemometrics in the fragrance industry, specifically:

- Fully automated processes that minimise laborious pre-processing steps, thereby accelerating workflows.
- Importing the entire raw dataset reduces the risk of missing important trace differences.
- Prediction models provide fast and robust classification of unknown samples.

For more information on this application, or any of the techniques or products used, please contact SepSolve.

References and notes

[1] C.E. Frye, N.R. Moore and R.E. Synovec, Enhancing the chemical selectivity in discovery-based analysis with tandem ionization time-of-flight mass spectrometry detection for comprehensive two-dimensional gas chromatography, *J. Chromatogr. A.*, 2018, 1537: 99–108.

[2] G. Dugo *et al.*, High resolution gas chromatography for detection of adulterations of citrus cold-pressed essential oils, *Perfumer & Flavorist*, 1992, 17: 57–74.

[3] D. McHale and J.B. Sheridan, Detection of Adulteration of Cold-pressed Lemon Oil, *Flavour Fragrance J.*, 1988, 3: 127–133.

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Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.

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SepSolve Analytical Ltd
T: +44 (0)1733 669222 (UK) +1 888-379-3835 (USA)
E: hello@sepsolve.com