Characterization and Quantification of Essential Oils by GC and GC×GC with TOFMS and FID

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Introduction

In this poster, GC and GC×GC data for essential oils are presented to demonstrate the benefits of a comprehensive second separation dimension. One-dimensional GC separations with long columns and slow temperature programs to chromatographically isolate each component are commonly paired with FID detection to determine quantitative Area % information for the components of an essential oil sample. Even with the long analysis time, typical in the industry, coelutions were observed in these data leading to some inaccuracies in the Area % values when single peaks were comprised of multiple analytes. With TOFMS and related deconvolution tools, many coelutions were mathematically resolved and relative area information was determined. Without calibration, the Area % values can be impacted by variations of analyte response with MS detection. The addition of a secondary separation dimension with GC×GC-FID chromatographically separated many of the coelutions in the second dimension and provided more accuracy to the Area % determinations. As this relieved the requirement for all analytes to be completely resolved in the primary separation dimension, a reduction in overall analysis time was achieved with a simultaneous improvement in the isolation of individual analytes. The structured nature of the 2D separation space and the distinct visual chromatograms also provided characterization capabilities of the essential oils. Analyte characteristics could be deduced based on an unknown analyte's elution relative to the structured bands of analytes with similar functional groups and properties. The distinct visual chromatograms also provided improved chemical fingerprinting for rapid characterization and differential analysis of samples. Methods are reported and examples of these benefits are highlighted in this poster.

Methods

Chamomile essential oils (Blue German, Wild, and Roman) were prepared for analysis. Each essential oil was diluted to 5% in hexane and analyzed with LECO's Pegasus® HT for GC-TOFMS and GC-FID, and with LECO's Pegasus 4D for GC×GC-TOFMS and GC×GC-FID. Various separation methods were utilized, with conditions listed in Table 1.

Table 1. Instrument Conditions

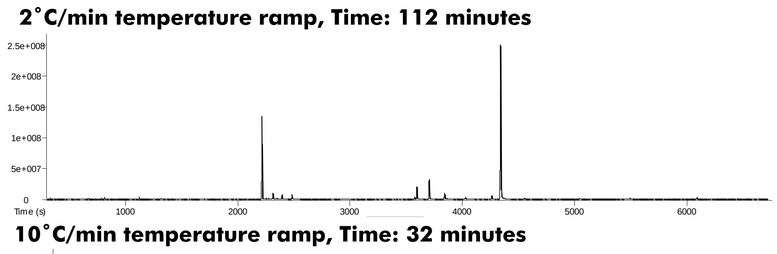
GC-TOFMS and GC-FID (Pegasus HT) Conditions	
Injection	Split 200:1, Inlet 250°C, He carrier gas
Column	Stabilwax, 30 m x 0.25 mm x 0.50 µm (Restek, Bellefonte, PA)
Slow	2 min at 50°C, ramp 2°C/min to 250°C and hold 10 min
Medium	2 min at 50°C, ramp 10°C/min to 250°C and hold 10 min
Fast	2 min at 50°C, ramp 20°C/min to 250°C and hold 10 min
MS	Collected 30-350 m/z at source temp 250°C
FID	300°C

GC×GC-TOFMS and GC×GC-FID (Pegasus HT) Conditions	
Injection	Split 200:1, Inlet 250°C, He carrier gas
Column	Stabilwax, 30 m x 0.25 mm x 0.50 µm (Restek, Bellefonte, PA) with Rxi 5Sil MS, 1.25 m x 0.25 mm x 0.25 µm (Restek)
Temp	Fast temperature program with secondary oven, 2.25 min at 55°C, ramp 20°C/min to 250°C and hold 10 min
Modulation	1.6 s with temperature maintained +20°C relative to 2nd oven
MS	Collected 30-350 m/z at source temp 250°C
FID	300°C

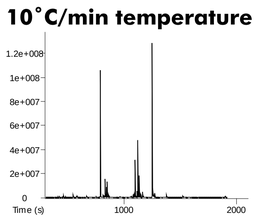
Faster Analysis Time

Separations lasting a couple of hours are common for essential oil samples. Here, more aggressive temperature programs were investigated. The incidence of coelution increased, but the analysis time was reduced to less than 20%. Representative chromatograms for the Blue German Chamomile samples, analyzed with each temperature program, are shown.

2°C/min temperature ramp, Time: 112 minutes



10°C/min temperature ramp, Time: 32 minutes



20°C/min temperature ramp, Time: 22 minutes

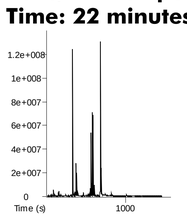


Figure 1. Blue German Essential Oil. Representative TIC chromatograms show the impact of faster temperature ramp rates.

GC×GC

Coelutions in the fast GC separation were deconvoluted in the TOFMS data for identification, but caused quantification issues in the FID data as the areas of overlapping analytes were combined. The addition of a second separation dimension allowed for the resolution of many coelutions while maintaining the shorter analysis time, shown in Figure 2.

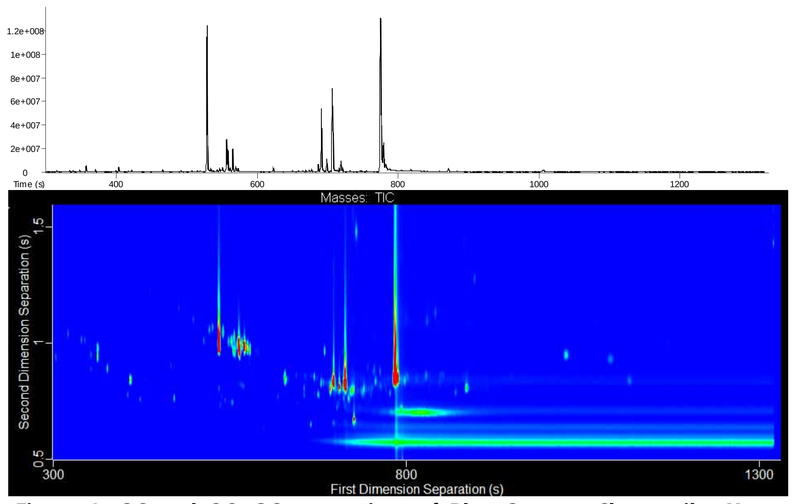


Figure 2. GC and GC×GC separations of Blue German Chamomile. Many coelutions in the GC separation were chromatographically resolved in the second dimension without increasing the analysis time.

A specific example of the increase of coelution and the ability for GC×GC to compensate is shown in Figure 3. 2-pentyl furan and trans-β-ocimene increasingly overlap until they appear as a single peak with FID. The coelution was mathematically resolved with deconvolution for the MS data with high quality spectra, but the reported Area % in the FID data was the combination of the two analytes, shown in Table 2. With GC×GC, this coelution in the primary dimension is baseline resolved in the second dimension.

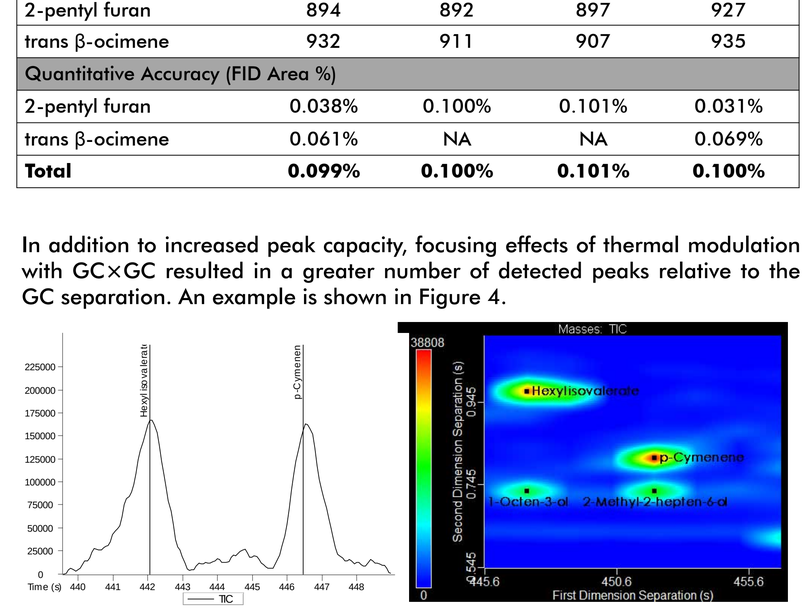


Figure 3. 2-pentyl furan (m/z 81) and trans-β-ocimene (m/z 93) are baseline resolved with a two-hour separation, but are indistinguishable by FID in the 22-minute separation. With GC×GC, this near-complete coelution in the primary dimension is baseline resolved in the second dimension presenting no problems for identification of quantification.

Table 2. Spectral Quality and Area % Comparisons

	2°C/min GC	10°C/min GC	20°C/min GC	20°C/min GC×GC
Spectral Quality with Deconvolution – Library Similarity				
2-pentyl furan	894	892	897	927
trans β-ocimene	932	911	907	935
Quantitative Accuracy (FID Area %)				
2-pentyl furan	0.038%	0.100%	0.101%	0.031%
trans β-ocimene	0.061%	NA	NA	0.069%
Total	**0.099%**	**0.100%**	**0.101%**	**0.100%**

In addition to increased peak capacity, focusing effects of thermal modulation with GC×GC resulted in a greater number of detected peaks relative to the GC separation. An example is shown in Figure 4.

	S/N		Similarity	
	GC	GC×GC	GC	GC×GC
1-octen-3-ol	--	811.7	--	925
hexyl isovalerate	28.4	925.9	880	891
2-methyl-2-hepten-6-ol	--	740.8	--	914
p-cymenene	178.8	1963.6	815	954

Figure 4. Additional analytes were detected with GC×GC relative to GC, in part due to thermal focusing at the modulator.

GC×GC-FID and MS

With faster GC separations, the number of analytes measured by FID relative to MS for a given temperature program decreased due to increased coelution. By adding a second dimension of separation to the fast GC separation, the relative coverage was restored and comparable to the slow two-hour separation, as shown in Figure 5.

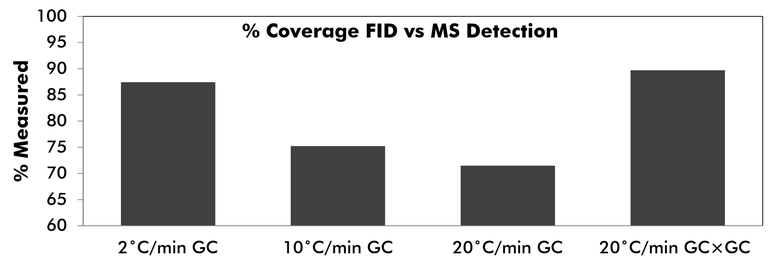


Figure 5. Relative Coverage of FID vs. MS. The number of peaks detected with FID/number of peaks with MS is shown for each method. Of note, the GC×GC separation had a greater total number of detected analytes, so some coelutions result from the ability to detect additional analytes.

The FID Area % for remaining coelutions is a combination of the overlapped analytes that were deconvoluted with GC×GC-TOFMS. The deconvoluted TIC (dTIC) Area % of the GC×GC-TOFMS data were compared to the GC×GC-FID Area %, shown in Figure 6. These were reasonably correlated, suggesting that dTIC relative areas may be useful to complement FID Area % in instances of coelution.

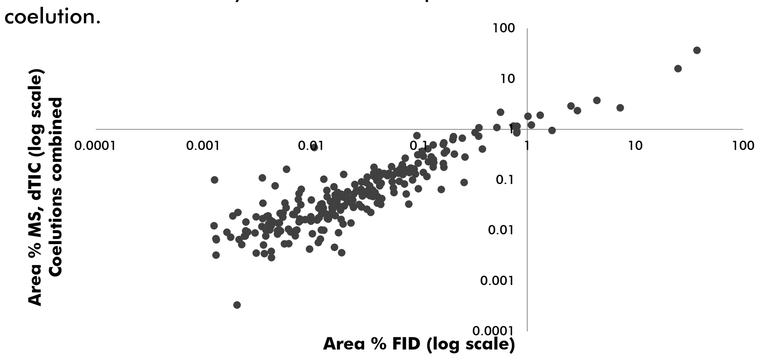


Figure 6. Area % FID vs. TOFMS. This correlation (R^2>0.95) suggests that dTIC Area % information with TOFMS deconvolution may complement and supplement FID Area % in cases of coelution.

Characterization and Differential Analysis

The structured nature of the 2D separation space and distinct visual chromatograms provide sample characterization and comparison capabilities. Differences are clear between the chamomile varieties in the 1D data, but the structured nature of the GC×GC separation offers rapid insight to these differences. For example, higher levels of esters in Roman Chamomile are visually apparent. The 2D data were also used for chemical fingerprinting with distinct sample group clustering by PCA.

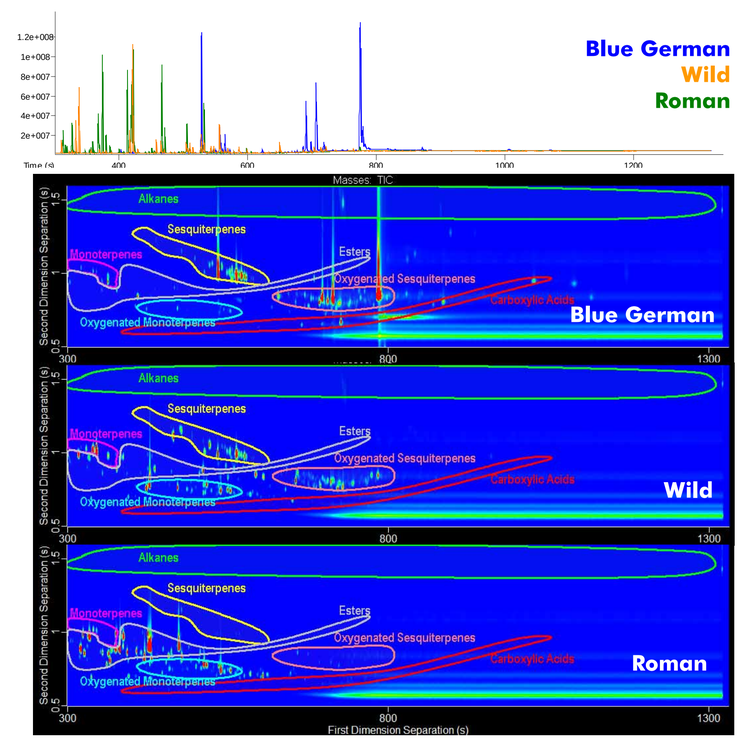
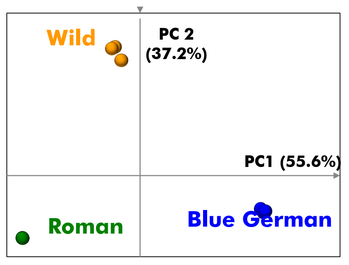


Figure 7. Differences in the chamomile varieties were readily determined with the GC×GC contour plots. The structured nature of the 2D space allows for quick comparisons of similarities and differences in functional group compositions. The distinct visual chromatograms were also used as fingerprints with PCA, resulting in clear clustering by variety.



Conclusions

Chamomile essential oils were analyzed by GC and GC×GC with both FID and TOFMS. A method was determined that reduced the analysis time from nearly two hours to just over 20 minutes without a relative increase in coelution. This was achieved by pairing a complementary secondary separation with GC×GC to a fast primary column temperature program. The use of GC×GC relieved the need for all analytes to be resolved in the primary dimension as many coelutions were separated in the second dimension. GC×GC-FID data provided Area % determinations as most analytes were chromatographically resolved. For remaining coelutions, GC×GC-TOFMS data isolated individual analytes with mathematical deconvolution. The Area % relationship between FID and dTIC was determined to be reasonably correlated (R^2>0.95). The dTIC Area % information from the MS data could complement the FID data with relative area estimates in the instances of coelution. Additionally, chamomile varieties were characterized and compared with these methods by taking advantage of the structured nature of the 2D separation space and distinct visual chromatograms.