Application Note

Instrument: Pegasus® BT 4D

Comprehensive Evaluation of Scotch Whisky Aroma Profiles Using SPME-GCxGC-TOFMS

Enhanced Aroma Characterization

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Key Words: Non-Target, Whisky, Aroma, GCxGC, TOFMS, Differentiation, Confidence Levels

Introduction

The UK is the largest producer of spirits within Europe, with exports worth £5.2 billion in 2016. The spirits industry is strategically important, as it makes one of the largest positive contributions to the UK balance of trade (accounting for around 20% of all UK food and drink exports). More specifically, the Scotch Whisky sector exports to 180 countries, with particularly strong growth in India and China, and contributes approximately £1 billion in taxes each year to the UK Exchequer. Across Scotland, there are 128 operating whisky distilleries, employing more than 10,000 people. The high value of the products produced by the sector provides opportunities and incentives for criminal activity (such as adulteration, substitution, or defrauding the consumer), creating safety concerns and resulting in lost revenue for genuine producers and governments. Accurate and reliable sample characterization can help to address these issues.

Whisky samples are highly complex with hundreds of aroma-critical species present at a wide range of concentrations and polarities amongst numerous interferences. Many species can coelute in the 1st chromatographic dimension (1D) when using traditional GC-MS methods.

As illustrated in the following examples, comprehensive two-dimensional gas chromatography (GCxGC) separation can significantly help to unravel this complex mixture which has low abundance aroma-active species eluting amongst more highly concentrated species such as alkanes, esters, ketones, alcohols, and acids. Coupled to time-of-flight mass spectrometry (TOFMS), which enables collection of high-quality, full mass range data at fast acquisition rates (>200 Hz), the detection and identification of these compounds is vastly improved.

Deconvolution and the use of diagnostic/unique mass fragmentation ions, in addition to Retention Indices and Mass Similarity Matching with library compounds, also plays an important role in the identification and quantitation of compounds and in attributing a confidence level to their assignment.

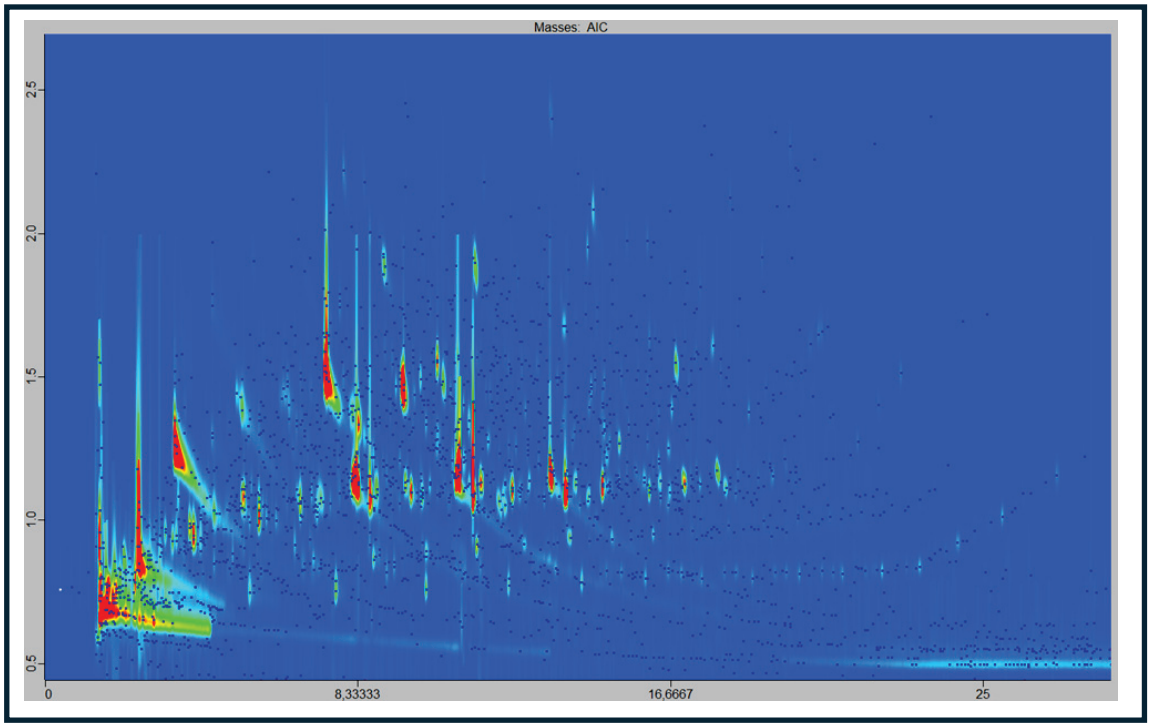

Figure 1: Representative Analytical Ion Chromatogram (AIC) Contour Plot of a sample of Glenfiddich Scotch whisky.

Experimental

Materials

A commercially available bottle of Glenfiddich Scotch whisky was obtained from a local supermarket to allow method optimization and a representative evaluation of typical aroma profiles.

n-Alkane standards (C7-C30), obtained from Sigma-Aldrich were diluted to 2 ng/mL in hexane and analyzed for calculation of retention indices.

Sample Extraction and Instrumental Conditions

The samples were prepared by loading 10 µL of neat whisky into 10 mL vials (Restek) sealed by septum caps (Restek). The sample incubation (2 min at 60 °C) was followed by extraction (20 min at the same temperature). Extraction was performed using a 1 cm DVB/CAR/PDMS fibre (Sigma-Aldrich) which was then immediately desorbed in the GC inlet for analysis with conditions listed in Table 1.

Table 1. *Pegasus* BT 4D GCxGC-TOFMS Conditions

GC	**LECO GCxGC QuadJet™ Thermal Modulator**
Injection	3 min fibre desorption with inlet temp 250 °C, split 5:1
Columns	1D: Rxi-5SilMS, 30 m x 0.25 mm i.d. x 0.25 µm (Restek)
	2D: Rxi-17SilMS, 1.3 m x 0.25 mm i.d. x 0.25 µm (Restek)
Carrier Gas	He @ 1.4 mL/min
Oven Program	50 °C (0.5 min), ramp 10 °C/min to 280 °C (5 min)
Secondary Oven	+5 °C (relative to primary oven temperature)
Modulator	+15 °C (relative to secondary oven temperature)
Modulation Period	3 s
Transfer Line	300 °C
MS	**LECO *Pegasus* BT 4D**
Ion Source Temperature	250 °C
Mass Range	35–400 m/z
Acquisition Rate	200 Spectra/s

Results and Discussion

A representative two-dimensional contour plot of the compounds sampled from Glenfiddich, using SPME, is shown in Figure 1. The complexity of this type of sample is apparent with a high number of peaks visible in the AIC. One of the benefits of coupling GCxGC separations with TOFMS is that highly comprehensive non-target data is achievable due to the enhanced peak capacity, acquisition of the whole mass range, and increased sensitivity due to the band focusing during thermal modulation. Compared with 1D GC and/or non-TOFMS approaches, richer non-target data with higher-quality mass spectra are obtained, allowing higher confidence in library matching for the discovery of a higher number of unknowns.

The extremely narrow peaks (with typical FWHH of 40-90 ms achieved in this case study) formed during the thermal modulation process used between the primary and secondary columns during comprehensive GCxGC, require fast MS detection. The *Pegasus* BT4D TOFMS detector allows high data acquisition speeds (up to 500 spectra/s), enabling the collection of sufficient data points across each chromatographic peak and thus successful and automated advanced peak finding via spectral deconvolution algorithms.

These features also enable the high numbers of both matrix and chromatographic interferences, such as siloxanes, to be efficiently separated chromatographically from the components of interest and identified during data processing. They are then easily removed from the data set via filtering, further aiding and providing a superior interpretation process compared to 1D GC-MS approaches.

Following the removal of the interferences, further filtering was then performed using ChromaTOF® software by applying thresholds of S/N>50, measured Retention Index (RI) values within 30 units of the Library RI Value, and Mass Spectral Similarity scores >600. Via these measures, a total of 375 compounds were identified in the Glenfiddich whisky sample. Then, in order to add further confidence levels to the data evaluation, these 375 compounds were classified based on additional retention index and/or mass spectral similarity criteria.

RI was divided into 3 classes, based on how close they were to the library value:

M: Match (Library R.I. +/- 10)

Pr: Probable (Library R.I. +/- 10.1 to +/- 20)

Po: Possible* (Library R.I. +/- 20.1 to +/- 30)

**Evaluated as priorities for further investigation*

Of the 375 compounds:

- 298 (79.4 %) were a Match (M)
- 53 (14.1 %) were classified as Probable (Pr)
- 24 (6.4 %) were classified as Possible (Po)

The Mass Spectral Similarity was also divided into three categories: >800, 700 to 799, and 600 to 699, respectively. The results for each of the above classes of compounds, based on the Mass Spectral Similarity, are summarized in Table 2 and Table 3.

Table 2: Number of compounds found in each class (based on their Retention Index) further classified by their mass spectral similarity

MS Similarity Score vs NIST	Number of Compounds per Class		
	M	Pr	Po
> 800	189	30	9
700 - 799	84	13	7
600 - 699	25	10	8
Total	298	53	24

Table 3: Percentage of compounds in each class (based on their Retention Index) further classified by their mass spectral similarity

MS Similarity Score vs NIST	Percentage of Compounds per Class		
	M	Pr	Po
> 800	63	57	38
700 - 799	28	25	29
600 - 699	8	19	33
Total	100	100	100

Of the 298 classified as a Match (M) by RI alone:

- 189 (63.4 %) had a MS similarity of > 800
- 273 (91.6 %) had a MS similarity of > 700

Of the 53 classified as a Probable (Pr) by RI alone:

- 30 (56.6 %) had a MS similarity of > 800
- 43 (81.1 %) had a MS similarity of > 700

Of the 24 classified as Possible (Po) by RI alone:

- 9 (37.5 %) had a MS similarity of > 800
- 16 (66.7 %) had a MS similarity of > 700

Noted, there are several reasons for compounds having a poor MS similarity value, especially for the more volatile ones, where library data may not be complete.

We were also able to demonstrate that the measured Retention Index for over 220 of the compounds were within +/-5 of the Library RI value and that there was a Gaussian frequency distribution if we classified them in 5 unit bins. There was no apparent bias within the chromatogram.

In addition to the quantitative assessment of the confidence levels in the compounds identified in the analysis, the following chromatograms (Figures 2, 3, and 4) further illustrate the value of optimized comprehensive 2D GCxGC methods in resolving peaks that would coelute using standard single dimension GC-MS methods.

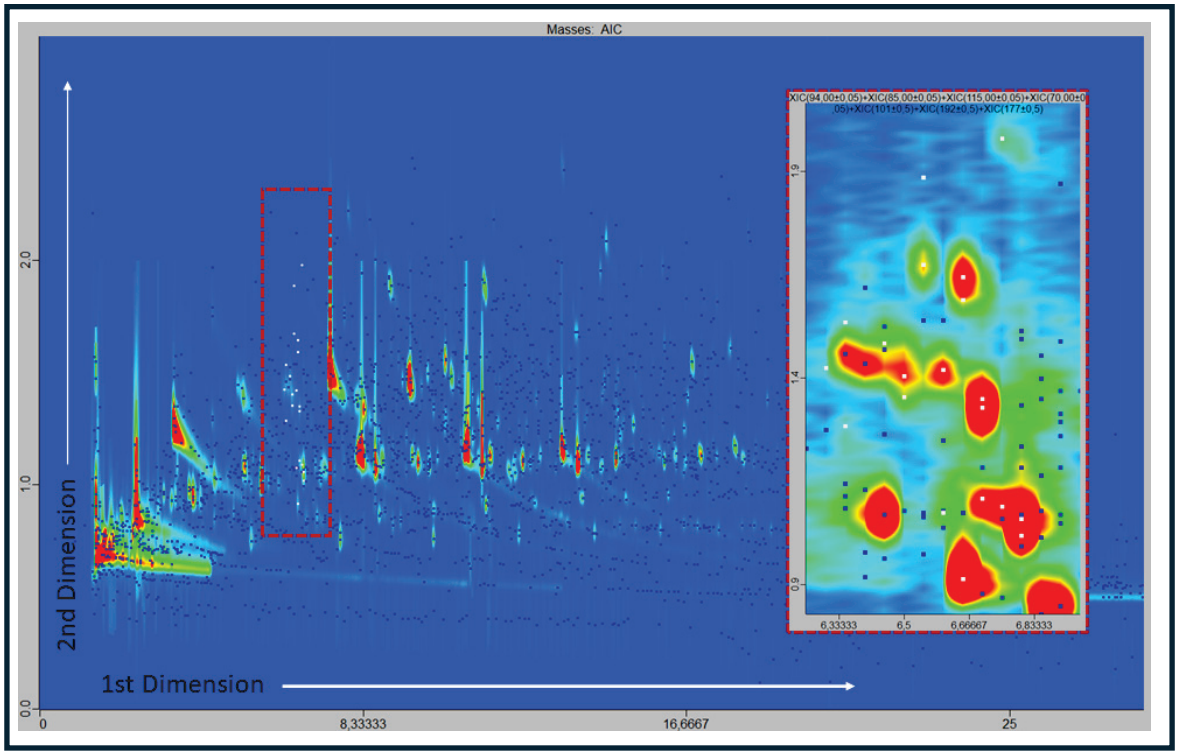

Figure 2: Exploring specific regions of interest to evidence the separation of coeluting compounds by GCxGC and the use of diagnostic fragmentation ions to identify the compounds.

Here, the chromatographic separations seen in the y-axis of the contour plot (i.e. polarity separation) are clear. It can be seen that in a one-dimensional separation, a number of components would completely coelute, having identical 1D retention times (x-axis). They are sufficiently resolved thanks to the 2nd dimension column separation, so these peaks could be correctly found, their spectra successfully deconvoluted, and excellent spectral similarities achieved. Further evidence can be found in the Contour and Surface Plots shown in Figures 3 and 4, for a different region of the chromatogram.

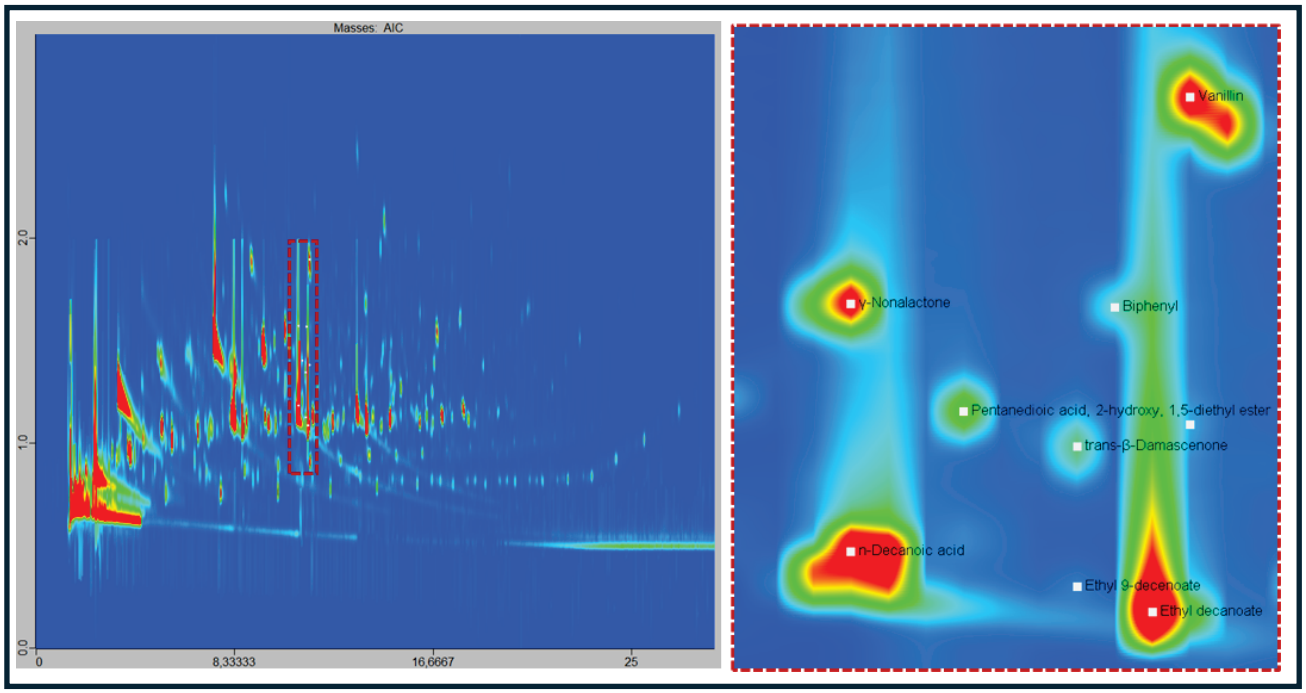

Figure 3: Resolution of low level aroma species from higher abundance components, from another region of the chromatogram.

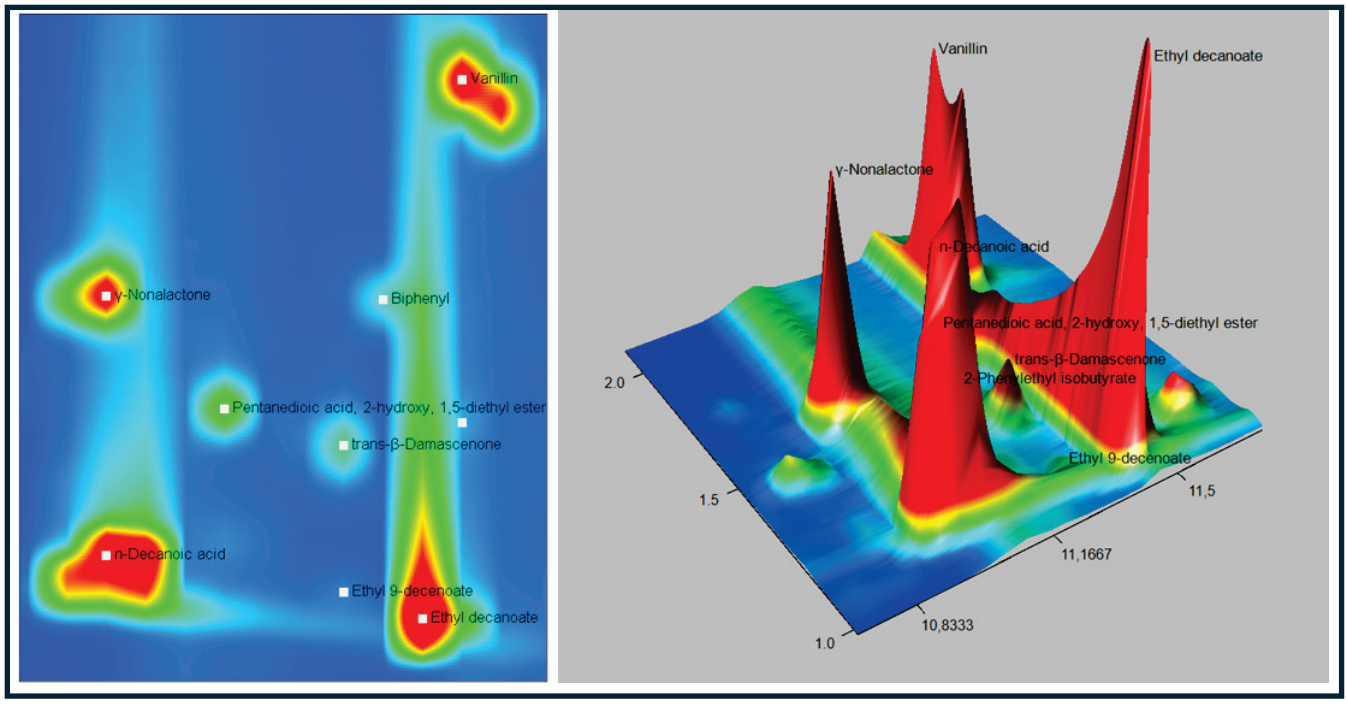

Figure 4: Surface plot and associated Contour plot for the same region of the chromatogram, further illustrating the resolving power of the GCxGC method to separate low abundance aromatic compounds from larger coeluting species.

The excellent Mass Spectral Similarities (NIST Library) for compounds, even with the same retention index, are summarized in Table 4.

Table 4: Retention Indices and Mass Spectral Similarities (NIST) for the Compounds highlighted in Figures 3 and 4

Name	NIST MS Similarity	Retention Index	Lib. RI
Pentanedioic acid, 2-hydroxy, 1,5-diethyl ester	871	1374.9	1377 ± 0(1)
n-Decanoic acid	890	1363.7	1373 ± 6(97)
γ-Nonalactone	739	1363.7	1363 ± 5(82)
Ethyl 9-decenoate	854	1386.1	1387 ± 2(13)
trans-β-Damascenone	860	1386.1	1386 ± 5(187)
Biphenyl	929	1389.8	1381 ± 4(43)
Ethyl decanoate	926	1393.5	1396 ± 2(67)
2-Phenylethyl isobutyrate	763	1397.3	1396 ± 2(13)
Vanillin	939	1397.3	1404 ± 7(120)

In the examples above, the correctly found and deconvoluted peaks' apexes were well separated from neighbouring entities. For even more closely eluting and even almost fully coeluted peaks, as shown in Figures 5 and 6 and in Tables 5 and 6 (below), the combination of high resolving GCxGC separation and fast data acquisition speeds is critical, allowing efficient spectral deconvolution to resolve not only differences in analyte species from chemical noise and background (e.g. GC column bleed) but also coeluting whisky component spectra.

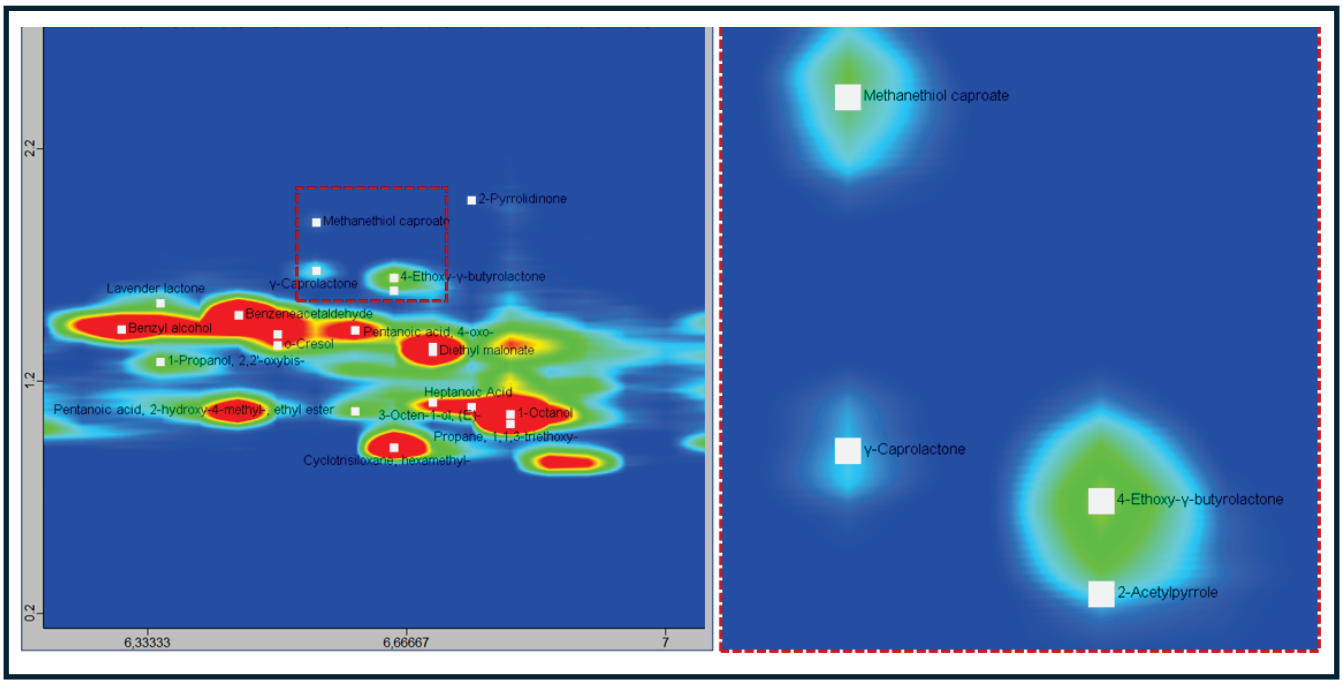

Figure 5: The benefits of GCxGC Resolution and Deconvolution to resolve even 1D and 2D coelutions.

Table 5: Excellent Mass Spectral Similarity (with NIST) for compounds that coelute in 1D and 2D, shown in Figure 5.

Name	NIST MS Similarity	Retention Index	Lib. RI
γ-Caprolactone	951	1053.8	1057 ± 8(24)
Methanethiol caproate	795	1053.8	1063 ± 0(1)
2-Acetylpyrrole	771	1060.4	1064 ± 5(36)
4-Ethoxy-γ-butyrolactone	836	1060.4	1067 ± 0(1)

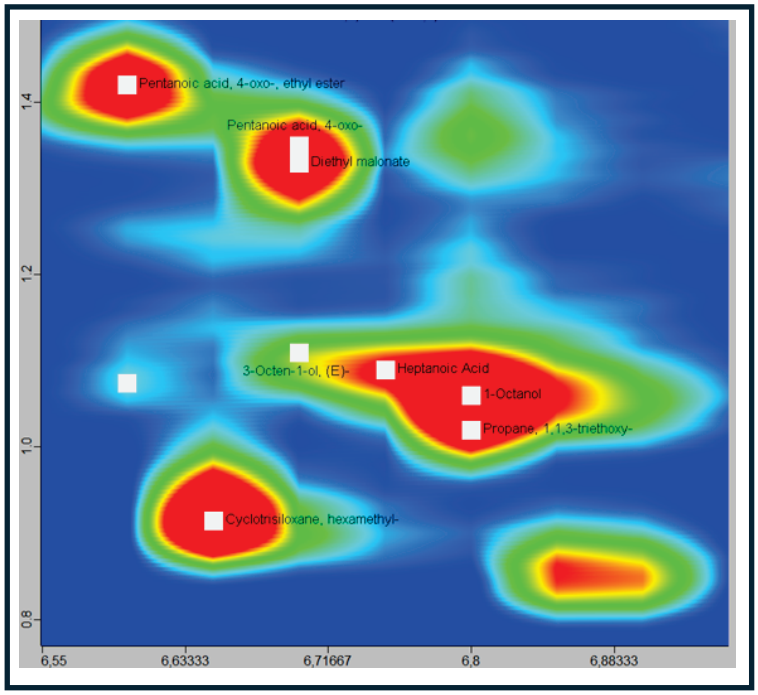

Figure 6: A Contour Plot (Extracted Ions) demonstrating the complexity of the whisky sample.

Table 6: Further examples of excellent Mass Spectral Similarity for compounds that coelute in 1D, shown in Figure 6.

Name	NIST MS Similarity	Retention Index	Lib. RI
Pentanoic acid, 4-oxo-, ethyl ester	679	1057.1	1045 ± 25(2)
Cyclotrisiloxane Species TBC	818	1060.4	TBC
3-Octen-1-ol, (E)-	677	1063.7	1066 ± 0(1)
Diethyl malonate	814	1063.7	1069 ± 0(9)
Pentanoic acid, 4-oxo-	686	1063.7	1063 ± 0(1)
Heptanoic Acid	850	1067.0	1078 TBC
Propane, 1,1,3-triethoxy-	866	1070.3	1076 ± 1(4)

These examples of using GCxGC for complex sample analysis clearly show how many more analytes can be uncovered and identified, compared with using traditional single-dimensional GC separations and non-TOFMS detection, which can struggle to provide sufficient data quality for particularly complex samples.

Conclusion

This study demonstrates how an enhanced solution for characterization of complex aroma compounds in a representative Scotch Whisky sample (Glenfiddich) was possible by applying GCxGC-TOFMS together with the analytical features built into LECO *ChromaTOF* software.

Deconvolution provided additional separation in cases of chromatographic coelution of peaks. The ease of use of LECO GCxGC-TOFMS hardware and benefits associated with *ChromaTOF* software enabled a time efficient and high-quality workflow to be applied, compared to those of a standard analysis.

A Joint Project Between LECO and The Open University

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