Chemical Profiling of Whiskies Using Orbitrap GC-MS

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ABSTRACT

The results of this proof-of-concept study show that the Q Exactive GC system is an ideal analytical tool for comprehensive chemical profiling of complex matrices, offering high performance full scan analysis. Software tools enable fast and accurate differential analysis to be performed to isolate unique features of samples. Routine mass resolution of 60,000 FWHM and consistent sub-ppm mass accuracy ensures selective and confident compound detection and identification.

INTRODUCTION

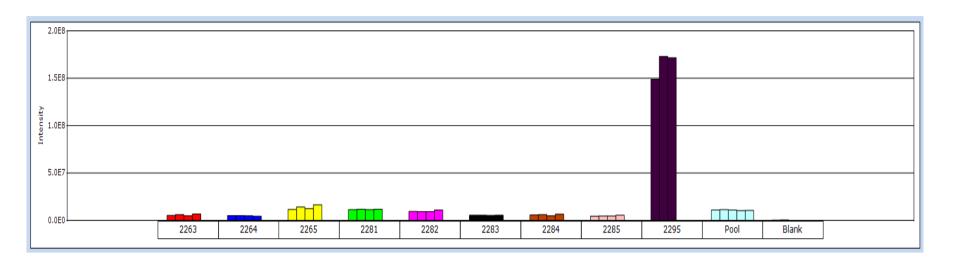
Whisky is a premium distilled spirit beverage produced using long-established methods that involve a complex aging process. These processes result in a final product that has unique characteristics, has high commercial value, and can be economically important in the regions of the world where it is produced and consumed. As such, it is essential that whisky producers are able to obtain an accurate and comprehensive chemical profile that is characteristic of their individual product. This work aims to demonstrate the application of a complete untargeted chemometric workflow using the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS to detect and identify chemical components in whisky. This proof-of-concept study also shows the process of identifying chemical differences in whiskies of different origins.

MATERIALS AND METHODS

Isolating Peaks of Interest

From the PCA and the list of detected peaks presented in SIEVE 2.2 software, it was possible to investigate which peaks contributed significantly to the differences seen between the sample types. One observation from the PCA was that the samples 2295 and 2265 were significantly different from the other whiskies. To investigate this further the 4841 component list (containing retention time and exact mass pairs) was sorted to show those components that were unique or elevated in sample 2295. This showed a peak at 13.6 minutes as being elevated in sample 2295. The Trend intensity bar graph (Figure 3) shows this in SIEVE 2.2.

Figure 3. Trend intensity bar graph for *m/z* 177.1274 at retention time 13.6 minutes across all of the whisky samples and replicate injections. This peak is elevated in sample 2295 (bourbon).



CONCLUSIONS

The results of this pilot study demonstrate that the Thermo Scientific Q Exactive GC hybrid quadrupole-orbitrap mass spectrometer in combination with TraceFinder and SIEVE 2.2 software is an extremely effective tool for the chemical profiling of complex samples. The Orbitrap mass spectrometer delivers excellent mass accuracy for all components in a sample that leads to fast and confident characterisation of samples regardless of the concentration of the component.

- Reliable and robust chromatographic separation in combination with fast data acquisition speeds make the Q Exactive GC an ideal platform for chemical profiling of complex samples.
- The consistent sub 1 ppm mass accuracy in combination with excellent sensitivity makes confident identification of all components.
- SIEVE and TraceFinder software allowed for a fast and comprehensive characterisation of the whisky samples, isolating and identifying compounds with confidence. A larger number of samples are required to draw clear conclusions on a particular whisky profile.
- The EI and PCI data obtained was used for tentative compound identification against commercial libraries. Where no library match was made the mass accuracy allowed for elemental compositions to be proposed with a high degree of confidence.
 Proposed identifications can be quickly confirmed or eliminated based on accurate mass of fragments.

Gas Chromatography

From the above sample, 1 µL was injected into a splitless injector and compound separation was achieved using a Thermo Scientific[™] TRACE[™] 1310 gas chromatograph and a Thermo Scientific[™] TraceGOLD[™] TG-5SILMS 30 m length × 0.25 mm inner diameter × 0.25 µm film thickness column. A Thermo Scientific[™] TriPlus[™] RSH autosampler was used for sample introduction (Table 1).

Mass Spectrometry

High resolution EI spectra were acquired using 60,000 FWHM resolution (measured at m/z 200) with a mass range of 50–600 m/z. An internal lock mass was used throughout the acquisition (Table 2).

Table 1. GC conditions.

 Table 2. MS parameters.

280

EI

230

70

Full scan

50-600

60,000

207.03235

Q Exactive MS

Transfer line (°C)

Ionization type

lon source (°C)

Acquisition Mode

Mass range (Da)

Mass resolution

Lockmass (m/z)

(FWHM)

Electron energy (eV)

TRACE 1310					
Injection volume <mark>(</mark> µl)	1				
Inlet mode	Splitless				
Liner	Single gooseneck				
Inlet temperature (°C)	250				
Carrier gas (mL/min)	He, 1.2				
Oven Program					
Temperature 1 (°C)	45				
Hold time (min)	1				
Temperature 2 (°C)	330				
Rate (°C/min)	10				
Hold time (min)	5				

RESULTS

Figure 1. GC-MS total ion chromatograms of a single malt whisky (sample 2265) and a bourbon whisky (sample 2295).

f:\work\\8april_60k_whisky_011	04/09/15 20:42:08	
RT: 0.00 - 34.01 SM: 7B	12.46	NL: 2.63E10 TIC MS 8April_60K_ Whisky_003
80 70 1 1 1 1 1 1 1 1 1 1 1 1 1		vvnisky_003

Identifying Compounds

Having found a peak of interest the next step is to propose an identity. This is where the combination of accurate mass and EI spectral libraries are very powerful. The EI spectrum can be used to search against existing commercially available spectral libraries, such as NIST. The accurate mass information can then be used to intelligently filter the hits based on a combination of spectral matching and the high resolution filtering (HRF) score. For the top hit trans β ionone 98% of the spectrum can be explained based on accurate mass of the ions in the spectrum (Figure 4).

Figure 4. Identification of peak at 13.6 minutes as Trans β Ionone. Screenshot of the deconvoluted data and library match in Thermo Scientific™ TraceFinder™ software.

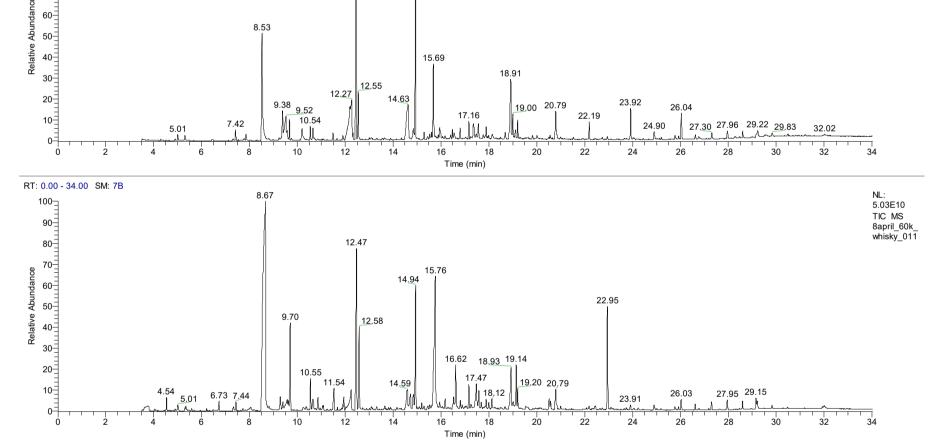
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Status	name	Sample Id	Sample Type		Component Name	RT	Reference m/z	Area		Sco	e Matched Compound	Formula	CAS	SI	HRF Score	M+ m/z	M+	M+ Lib
		2264	Unknown	•	trans-β-Ionone	13.606	5 177.12715	167367313										
	8April_60K	2295	Unknown		Peak@22.19256	22.193		9765327	1	75.3		C13H20O	79-77-6		98.3576	192.15086	No	Yes
					Phenol, 2,2'-methy	22.941		7029046475	- = 1	74.9	3-Buten-2-one, 4-(2	C13H20O	14901-0		98.3576	192.15086	No	Yes
					Peak@27.09766	27.098	177.16374	255632		73.4	Terephthalic acid, e	C19H20O4		673	99.8952	312.13561	No	No
					Peak@14.96211	14.962	178.06218	1957096		73.2	5-Methyl-2,4-diisop	C13H20O	40625-9		98.3576	192.15086	No	Yes
					2-Ethylhexyl trans-			101309516	-	73	Acetic acid, 6,6-dim	C16H24O4		649	99.8952	280.16691	No	Yes
		111)							720	Teenhthalic acid at			610	00 0050	272 22051	No	No
	8.00E+007						BP: 177.12715	@ 8.52E+007			100 80						97.127)12 H17	
	8.00E+007 6.00E+007 4.00E+007 2.00E+007 0.00E+000 13.53	178 13.5507	8 13.57078	13.9	59078 13.61078	13.6307		@ 8.52E+007 13.67078		bundance	80 60 40 20				1.h	C(12		egt spectrum O
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Activ	6.00E+007 4.00E+007 2.00E+007 0.00E+007 13.53 e Measure m/z 129.0 128.0	d Area	Heigh 96621 1: 98756 0	t 230502 612612	Fragment ID T C(12)10 H9 12 C(12)10 H8 12	neo m/z 9.06987	8 13.65078 Mass error (ppm) 0.92973 0.85896	13.67078 M+ False		Relative abundance	80 60 40 20 0 1111	чн.—— 1ı	1+1-1		'ŀłr''ŀ	C(12		
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Identifying Peaks with no Spectral Match

When there is no match using spectral libraries the process of identification can be more complicated. For example, the peak at 18.00 minutes was also identified from the PCA as being elevated in sample 2295. In order to identify the compound both the EI and PCI spectra (Figures 5) were used to subsequently isolate the molecular ion and propose an elemental formula. The $[M+H]^+$ and the $[M+C_2H_5]^+$ adducts were identified in the PCI spectrum and from this an elemental composition of the parent molecule could be proposed. This is a critical stage in the process and it is where excellent mass accuracy can be used to limit the number of possible chemical formulae. For example, when a 10 ppm mass accuracy window is used 9 possible formulae are proposed for the [M+H]⁺ ion of m/z 241.10699 using the elements Carbon (1-50), Hydrogen (1-100), Nitrogen (1-5), Oxygen (1-10), Chlorine (1-10). This is compared to a 1 ppm mass accuracy window that suggests only one possible formula, $C_{12}H_{17}O_5$. This level of mass accuracy significantly reduces the number of formulae that need to be investigated and also increases the confidence in any proposed assignment. The identification is further supported by the mass accuracy and elemental formula for the second adduct m/z221.18968, $[M+C_2H_5]^+$ in the PCI spectrum shown in Figure 8 and also from the molecular ion m/z 240.09924 seen in the EI spectrum (0.08ppm mass error).

TRADEMARKS/LICENSING

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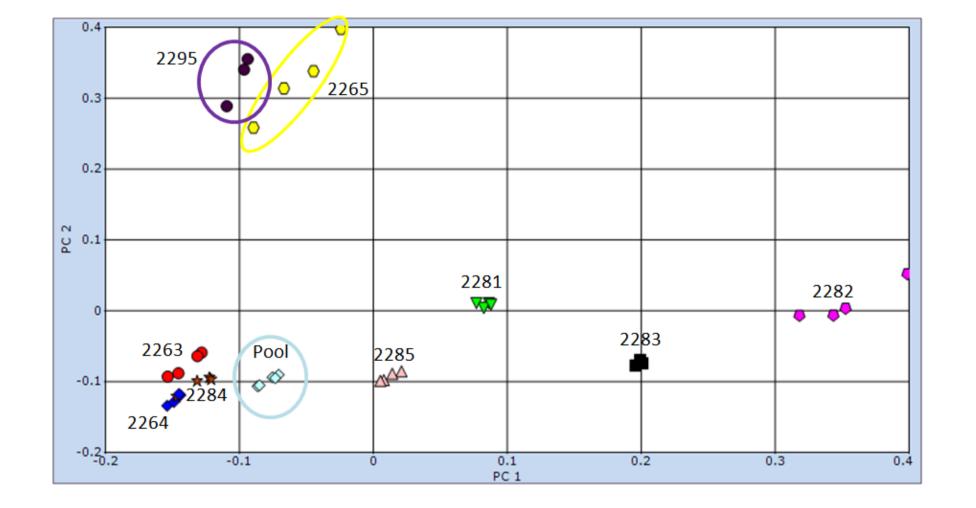


The objective of this proof-of-concept study was to analyze the whisky samples using non-targeted full-scan data acquisition and to identify, using statistical tools, whether there are any differences between the samples and to propose an identity to any differences observed.

Discovering Differences

The complete data set, including all 9 samples, pooled sample and replicates, was processed in Thermo Scientific[™] SIEVE[™] 2.2 software for component extraction and statistical analysis. This software initially performed a peak alignment to correct for any retention time variation across the batch, followed by peak detection and finally statistical analysis. The results of this are shown in Figure 2, which shows a principal component analysis (PCA) of all the samples and replicates.

Figure 2. Principal component model of the 9 whisky samples with 4 replicate injections of each. Whiskies 2295 (bourbon) and 2265 (aged in three barrels) are different to the others, but show some similarities to each other.



The proposed chemical formula for the compound $C_{12}H_{16}O_5$ was searched using the online chemical database ChemSpider. The results were investigated and the fragment information was used to either support or exclude possible suggestions. MassFrontier 7.0 was used to theoretically fragment proposed compounds and match these to the measured fragments in the EI spectrum (Figure 5). The fifth compound hit suggested by ChemSpider, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid, was the only compound that could explain the fragments measured in the EI spectrum. The sub-1ppm mass accuracy allows compounds to be quickly excluded or included and adds confidence in assignments.

Figure 5. El spectrum for peak at 18.00 minutes where no library match was made. Peaks are labelled with structure, formula and mass error in ppm. The sub 1ppm mass errors provide high confidence in the proposed identification of 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid. Peaks are annotated with structures identified in Mass Frontier 7.2.

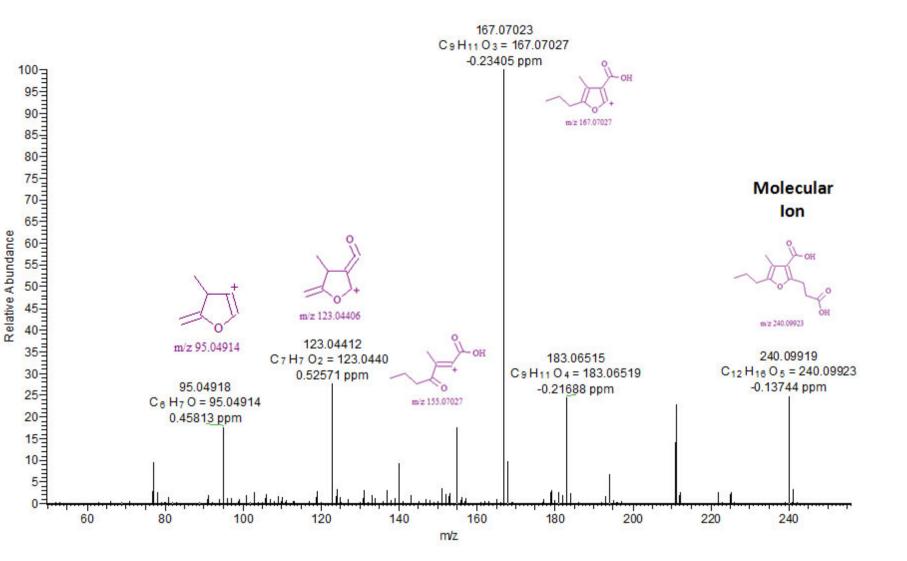


Table 3. Summary of five peaks identified as being elevated in sample 2295 and their tentative identification.

No.	Retention Time (min)	Base Peak (<i>m/z</i>)	Compound Name	Formula	NIST Forward Match	Mass Accuracy Base Peak (ppm)	Mass Accuracy Molecular ion (ppm)
1	13.6	177.12736	Trans β ionone	C ₁₃ H ₂₀ O	772	0.84	0.31
2	11.54	139.11180	Furanone	$C_9H_{16}O_2$	775	0.22	0.12
3	10.87	137.05974	Phenol, 4 ethyl -2 methoxy	$C_9H_{12}O_2$	828	0.29	0.08
4	16.16	194.09037	2,3-dimethoxy-4-phenol	$C_{11}H_{14}O_3$	747	0.15	0.15
5	18.00	167.07028	Furan propanoic acid	$C_{12}H_{16}O_5$	No Match	0.23	0.13

