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Chemical Profiling of Whiskies Using Orbitrap GC-MS

Dominic Roberts[1], Jana Hajslova[2], Michal Stupák[2], Jana Pulkrabova[2], Jason Cole[1], Paul Silcock[1]. [1]Thermo Fisher Scientific, Runcorn, UK; [2]Institute of Chemical Technology, Prague, Czech Republic.

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

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.

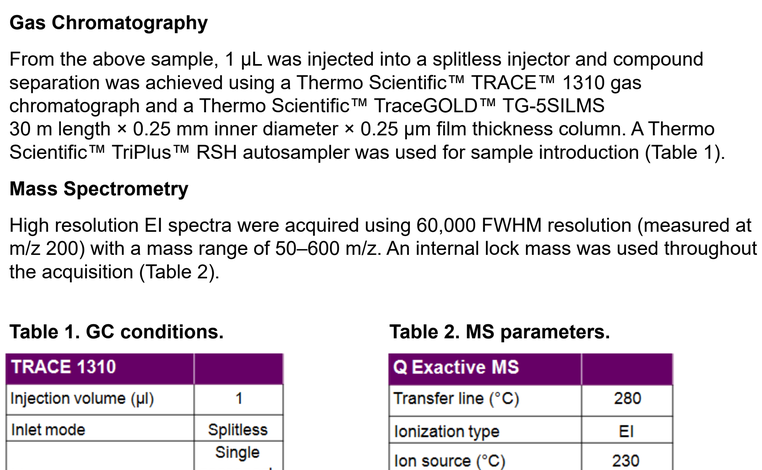
TRACE 1310	
Injection volume (µl)	1
Inlet mode	Splitless
Liner	Single gooseneck
Inlet temperature (°C)	250
Carrier gas (mL/min)	He, 1.2
Oven Program	
Temperature 1 (°C)	45
Hold time (min)	1
Temperature 2 (°C)	330
Rate (°C/min)	10
Hold time (min)	5

Table 2. MS parameters.

Q Exactive MS	
Transfer line (°C)	280
Ionization type	EI
Ion source (°C)	230
Electron energy (eV)	70
Acquisition Mode	Full scan
Mass range (Da)	50-600
Mass resolution (FWHM)	60,000
Lockmass (m/z)	207.03235

RESULTS

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

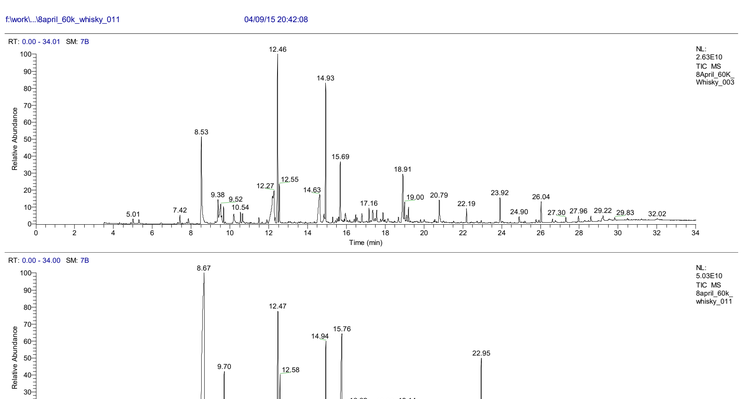


The objective of this proof-of-concept study was to analyze the whisky samples using non-targeted full-scan data acquisition and to identify, using statistical tools, whether there are any differences between the samples and to propose an identity to any differences observed.

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.

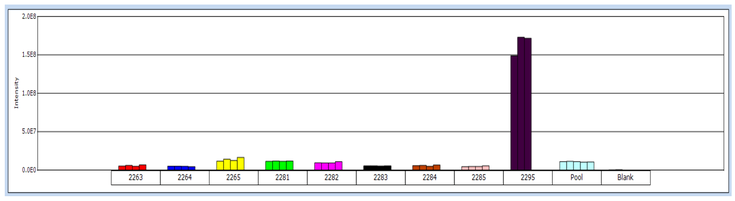
Figure 2. Principal component model of the 9 whisky samples with 4 replicate injections of each. Whiskies 2295 (bourbon) and 2265 (aged in three barrels) are different to the others, but show some similarities to each other.



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.

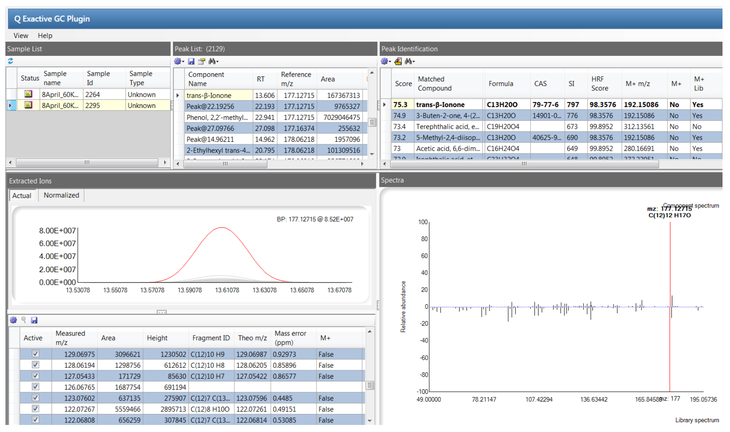
Figure 3. Trend intensity bar graph for *m/z* 177.1274 at retention time 13.6 minutes across all of the whisky samples and replicate injections. This peak is elevated in sample 2295 (bourbon).



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.



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/z* 221.18968, $[M+C_2H_5]^+$ 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).

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. EI 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.

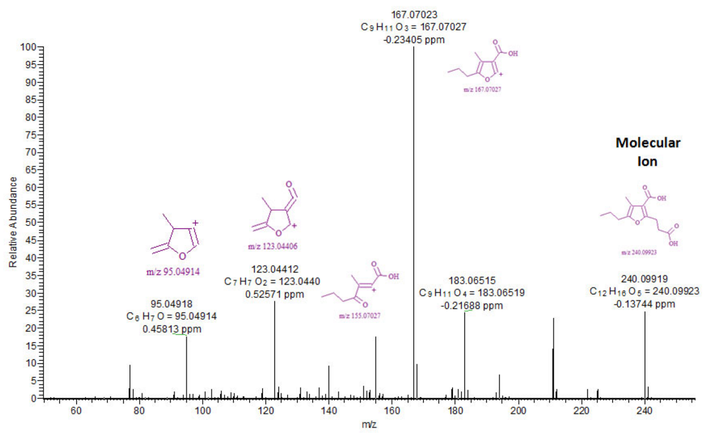


Table 3. Summary of five peaks identified as being elevated in sample 2295 and their tentative identification.

No.	Retention Time (min)	Base Peak (*m/z*)	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

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.

TRADEMARKS/LICENSING

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