# Chemical profiling of whiskies using Orbitrap GC-MS

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## ABSTRACT

**Purpose:** To evaluate the utility of Orbitrap based GC-MS technology for chemical profiling of whisky.

**Methods:** Whisky samples were extracted into ethyl acetate. The extracts, including a pool, were analysed in all experiments using a Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-orbitrap mass spectrometer. Data was acquired and processed using the Thermo Scientific™ TraceFinder™ and Sieve 2.2 ™ software.

**Results:** The results of this proof of concept study show that the Q Exactive GC 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 between 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 that is 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 which is characteristic to their individual product. This work aims to demonstrate the application of a complete untargeted chemometric workflow using the Q Exactive GC Orbitrap 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.

## METHODS

### Sample Preparation

The 9 samples from different regions and distilleries were prepared for GC analysis using the following procedure: 3 mL of whisky sample was mixed with 10 mL of distilled water and shaken with 15 mL of ethyl acetate. The organic layer was filtered through 3 g of sodium sulfate. The ethyl acetate extract was carefully eliminated by evaporation under a gentle stream of nitrogen at room temperature. The evaporated extract was re-dissolved in 0.5 mL of ethyl acetate and transferred into the GC vial. For statistical analysis a pooled sample was prepared by pipetting 50 µl of each whisky extract into a single GC vial. Each sample was injected 4 times and analysed in a random order.

### Gas Chromatography

1 µL was injected into a splitless injector and compound separation was achieved using a Trace 1310 gas chromatograph and a 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 parameters**

| TRACE 1310 | |
|---|---|
| Injection volume (µl) | 1 |
| Inlet mode | Splitless |
| Liner | Single gooseneck |
| Inlet temperature ($^0$C) | 250 |
| Carrier gas (mL/min) | He, 1.2 |
| **Oven Program** | |
| Temperature 1 ($^0$C) | 45 |
| Hold time (min) | 1 |
| Temperature 2 ($^0$C) | 330 |
| Rate ($^0$C/min) | 10 |
| Hold time (min) | 5 |

**TABLE 2: MS parameters**

| Q Exactive MS | |
|---|---|
| Transfer line ($^0$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 |

### Data Analysis

Data was acquired and processed using the Thermo Scientific™ TraceFinder™ software. TraceFinder allows easy data reviewing and data reporting. Statistical analysis was performed using Sieve 2.2 ™.

## RESULTS

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

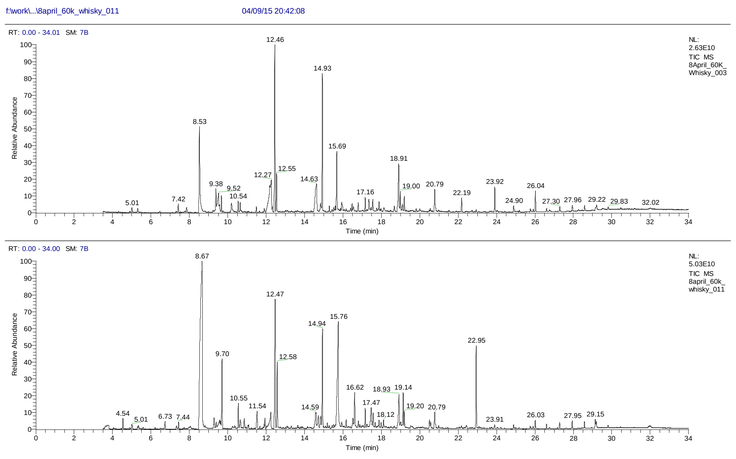


**FIGURE 1. GC-MS total ion chromatograms of a single malt whisky (sample 2265) and a bourbon whisky (sample 2295).**

### Discovering differences

The complete data set, including all 9 samples, pooled sample and replicates, was processed in Sieve 2.2 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.

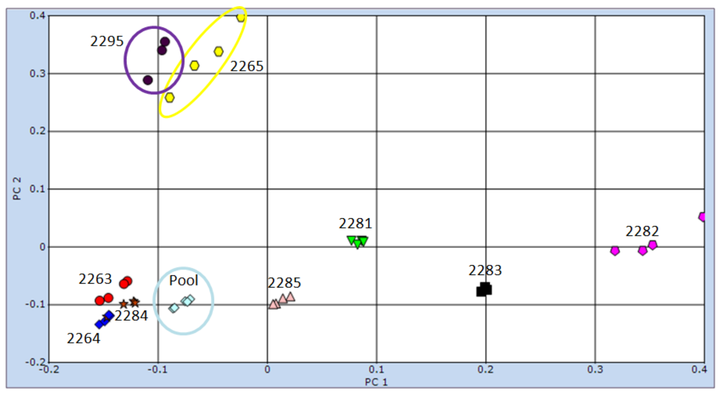


**FIGURE 2. Principal component model of the 9 whisky samples with 4 replicate injections of each. Whiskies 2295 (bourbon) and 2265 (aged in three barrels) are different to the others, but show some similarities to each other.**

### Isolating peaks of interest

From the PCA and the list of detected peaks presented in Sieve 2.2 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.

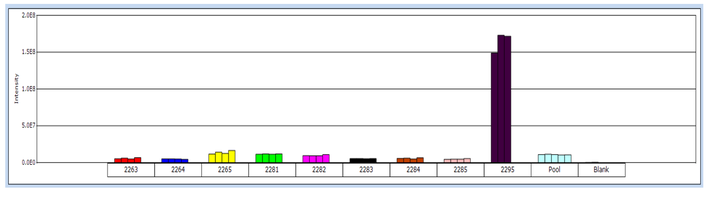


**FIGURE 3. Trend intensity bar graph for *m/z* 177.1274 at retention time 13.6 minutes across all of the whisky samples and replicate injections. This peak is elevated in sample 2295 (bourbon).**

### 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).

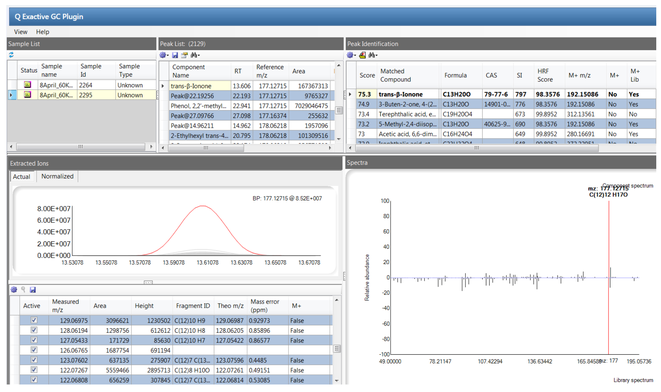


**FIGURE 4.** Identification of peak at 13.6 minutes as Trans β Ionone. Screenshot of the deconvoluted data and library match in TraceFInder 3.3.

### 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 and 6) were used to subsequently isolate the molecular ion and propose an elemental formula.

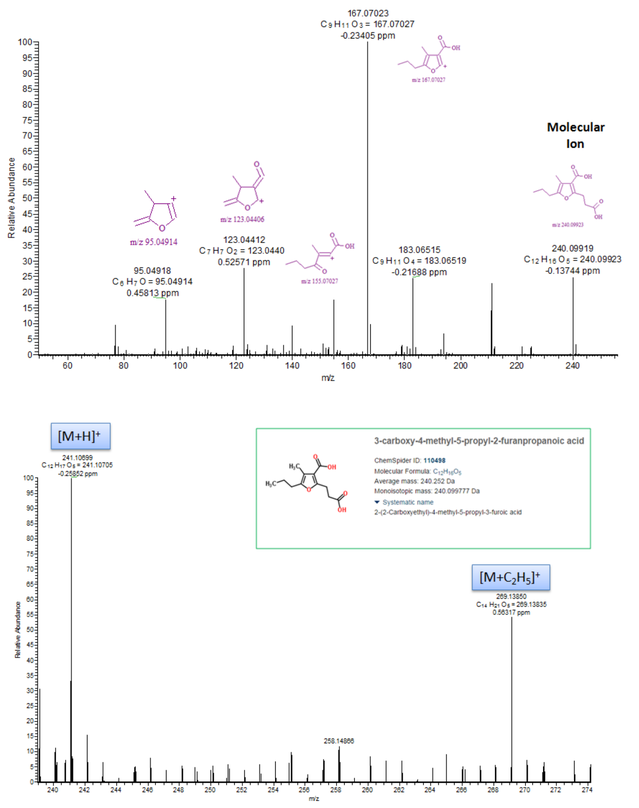


**FIGURE 5. EI spectrum (upper) for peak at 18.00 minutes. Peaks are labelled with structure, formula and mass error in ppm. PCI spectrum (lower) labelled with adducts. Inset hit from Chemspider.**

## CONCLUSIONS

- 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 2.2 and TraceFinder 3.3 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