

Chemical Profiling and Differential Analysis of Whiskies Using Orbitrap GC-MS

Dominic Roberts,¹ Jana Hajslova,² Jana Pulkrabova,² and Paul Silcock¹

¹Thermo Fisher Scientific, Runcorn, UK

²University of Chemistry and Technology, Prague, Czech Republic

Key Words

Chemical profiling, whisky, Q Exactive GC, Orbitrap mass spectrometry, differential and statistical analysis, identification, accurate mass

Introduction

Whisky is a premium spirit beverage that is distilled by following long established methods which involves a complex aging process. It is produced by the mixing of various grains with water to form a mash that is fermented with yeast, distilled to generate an alcoholic distillate, and finally matured in wooden barrels or casks.¹ This is a complex and traditional process that results in a beverage that has both a high value and high degree of variability. For example, whiskies produced on the West coast of Scotland often have a very smoky flavor, while those from the Speyside region can have flavors characteristic of honey, vanilla and fruit.² The production technology plays a significant role in the chemical composition and hence the flavor characteristics of the final whisky product.

As a result of these distinguishing features and the rising global demand, whisky has become an economically important commodity in many regions of the world. The entire whisky industry is a major source of both employment and tax revenues in these regions. For example, the whisky market is worth ~£5 billion to the UK economy,³ and in the USA, distilled spirits are collectively worth \$120 billion.⁴ As whisky has a high retail price, counterfeiting and/or adulteration is commonplace and is a threat to the integrity of the industry. The adulteration can take many forms and can occur on both small and large scales. For example, one of the most extensive forms of adulteration is to add the main chemical constituents of whisky to an alternative cheaper spirit to create an “artificial” whisky. This is of



particular concern as there is no safety control over which chemicals are being added, their quality or concentration. Another form of adulteration includes the labeling of bottles with more expensive brands and falsely claiming the age for which the whisky was matured in the barrel. The latter type of adulteration can be performed either by the addition of artificial colors or by heating during the aging process to speed up the coloration to mimic the aging process. The bottles are then mis-labeled claiming that the whisky was aged for an extended period in the barrel, which then justifies a higher price. Both processes can appear to achieve in a few months or days what otherwise would have taken many years.

innovatek 



Cra 21 No. 41 – 26
PBX (57) 338 0711

Bogotá, Colombia
www.innovatek.com.co
contacto@innovatek.com.co

Thermo
SCIENTIFIC

Therefore, it is essential that whisky producers use the available analytical technology to accurately and comprehensively characterize their products so that adulteration can be confidently identified and action taken to protect their product and brands. It may also be beneficial for whisky producers to chemically profile their products as part of their quality control procedures to enable comparison of different production batches and detection of any changes in the production process over time. This will help ensure that the whisky they bottle consistently contains the particular signature flavors and characteristics expected by the consumer.

Gas chromatography mass spectrometry (GC-MS) has been widely used to characterize whisky as it provides analytical advantages of chromatographic resolution, reproducibility, peak capacity, and, importantly, extensive spectral libraries to aid in identification of volatile and semi-volatile chemical constituents. In this proof-of-concept study, we seek to take advantage of the performance of the Thermo Scientific™ Q Exactive™ GC Hybrid Quadrupole-Orbitrap™ Mass Spectrometer for the profiling of whisky of different origins, ages and types. Another aim is to evaluate the application of a complete untargeted chemometric workflow using the Q Exactive GC system to detect and identify chemical components in whisky. It will also show the process of identifying chemical differences in whiskies of different origins. Samples were analyzed using a full scan non-targeted acquisition and high mass resolving power to obtain accurate mass measurements. This is important to enable elucidation of the elemental composition and discrimination of co-eluting and isobaric compounds. Fast scan speeds in combination with a high in-scan dynamic range and high sensitivity facilitates the detection of both low and high intensity components. These features in combination with unique software algorithms for automated deconvolution and sample comparison create a powerful solution for comprehensive characterization, quality control, and product brand protection.

Experimental Conditions

Sample Preparation

A total of nine whisky samples were included in the study, the details of each sample are shown in Table 1. The samples 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 evaporated 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, or composite, sample was prepared by pipetting 50 μ L of each whisky extract into a single GC vial. A blank ethyl acetate was analyzed to eliminate background systematic peaks. Each sample, including the pool, was injected 4 times and analyzed in a random order.

Table 1. Details of whisky samples included in the study.

Sample ID	Type	Age	Country of Origin	Region
2263	Single	12	Scotland	Lowlands
2264	Single	18	Scotland	Lowlands
2265*	Single	NAS	Scotland	Lowlands
2281	Single	10	Scotland	Campbeltown
2282	Single	15	Scotland	Campbeltown
2283	Single	15	Scotland	Campbeltown
2284	Single	12	Scotland	Highland
2285	Single	18	Scotland	Highland
2295	Bourbon	—	USA	Kentucky

NAS – No age statement. * Aged in three different casks.

Instrument and Method Setup

In all experiments, a Q Exactive GC system was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH Autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m \times 0.25 mm I.D. \times 0.25 μ m film capillary column with a 10 m integrated guard. (P/N 26096-1425). Additional details of instrument parameters are displayed in Tables 2 and 3.

TRACE 1310 GC Parameters	
Injection Volume (μL):	1 Splitless
Liner:	Single gooseneck
Inlet ($^{\circ}\text{C}$):	250
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 ($^{\circ}\text{C}$):	45
Hold Time (min):	1
Temperature 2 ($^{\circ}\text{C}$):	330
Rate ($^{\circ}\text{C}/\text{min}$):	10
Hold Time (min):	5

Table 3. Mass spectrometer conditions.

Q Exactive GC Mass Spectrometer Parameters	
Transfer line ($^{\circ}\text{C}$):	280
Ionization type:	EI
Ion source ($^{\circ}\text{C}$):	230
Electron energy (eV):	70
Acquisition Mode:	Full scan
Mass range (Da):	50–600
Resolving power (FWHM):	60,000 (m/z 200)
Lockmass, column bleed (m/z):	207.03235

The Q Exactive GC system was operated in EI full scan mode using 60,000 (FWHM at m/z 200) resolving power. Additional experiments were run using positive chemical ionization (PCI) with methane as reagent gas (1.5 mL/min) to obtain information on the molecular ions and to support the identification of unknown component peaks.

Data Processing

Data was acquired using the Thermo Scientific™ TraceFinder™ software. This single platform software package integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. TraceFinder also contains spectral deconvolution and spectral matching functionality. Statistical analysis was performed using SIEVE™ 2.2 and Mass Frontier™ 7.0 was used for structural elucidation.

Results and Discussion

The objective of this proof-of-concept study was to analyze the whisky samples using a non-target full scan data acquisition and to identify, using statistical tools, if there were differences between the samples and to propose an identity to any differences observed. In addition, the aim was to also quickly characterize which compounds are present in an individual sample using accurate mass deconvolution and spectral matching. The workflow used to achieve these objectives is summarized in Figure 1.

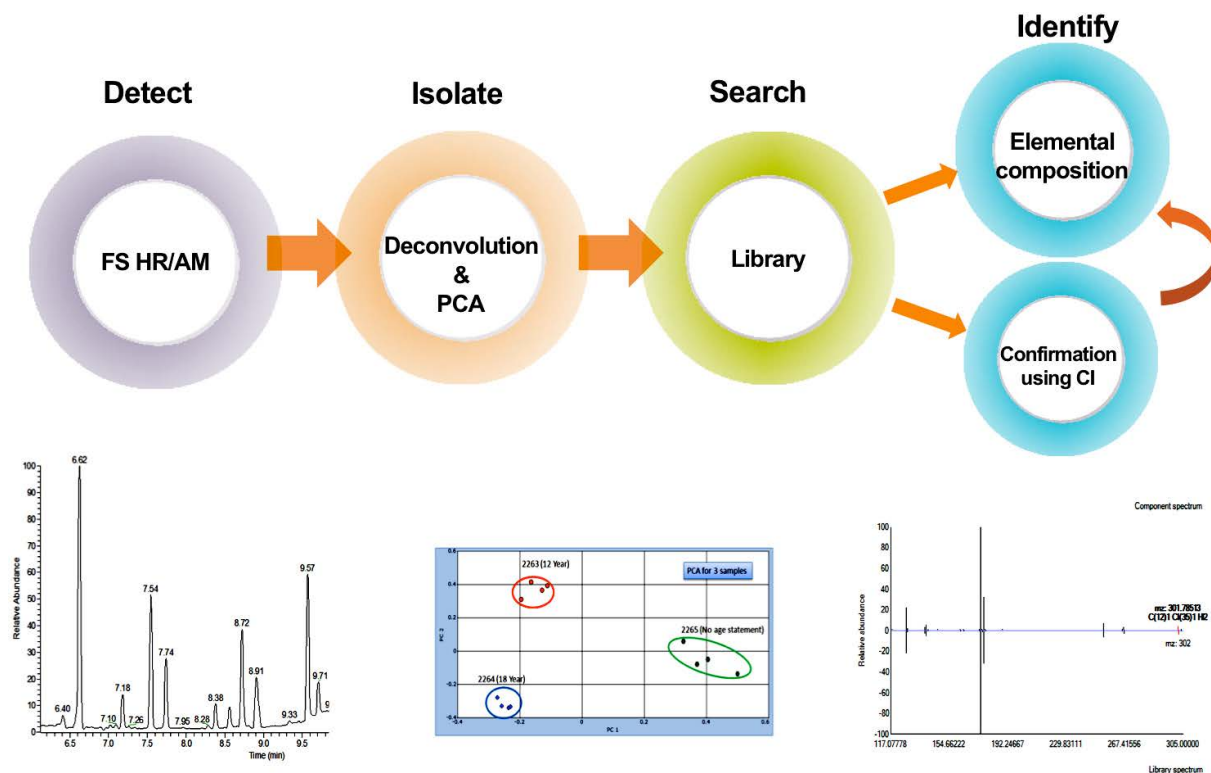


Figure 1. Workflow for the Q Exactive GC system for chemical profiling and marker identification.

Discovering Differences Between Samples

Four replicate full scan chromatograms were obtained for each sample and an example of the total ion chromatograms (TICs) are shown in Figure 2 for a single malt and a bourbon whisky. The TICs show a large number of peaks that are present at varying intensities and retention times. Importantly, for chemical profiling, the Q Exactive GC system provides a wide dynamic range to accurately capture components that are present at both trace and very high concentrations. The complete data set, including all nine 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 3, which shows a principal component analysis (PCA) of all the samples and replicates. From visual analysis of the PCA, it is clear that all of the whisky technical replicates cluster together, and as expected, the pooled sample lies towards the center of the PCA plot.

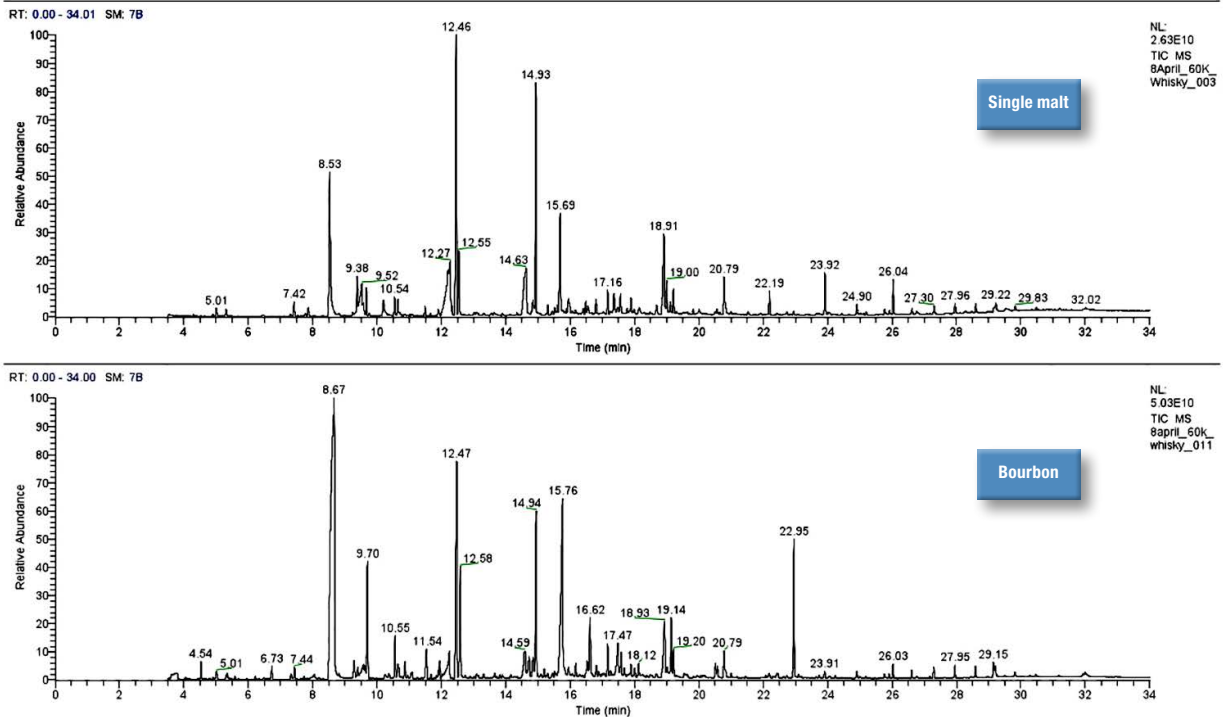


Figure 2. GC-MS total ion chromatograms of a single malt whisky (sample 2265) and a bourbon whisky (sample 2295).

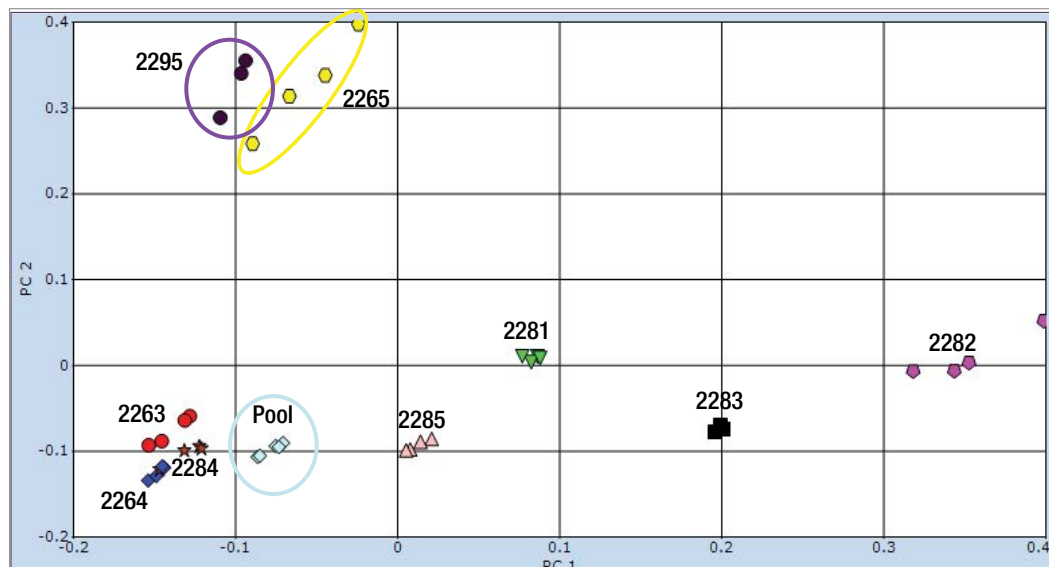


Figure 3. Principal component model, from SIEVE, of the 9 whisky samples with 4 replicate injections of each. Note that the replicates cluster appropriately. Whiskies 2295 (bourbon) and 2265 (aged in three barrels) are different to the others, but show some similarities to each other.

Isolating Unique Components

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 observed between the different sample types. One observation from the PCA was that samples 2295 and 2265 were significantly different from the other whiskeys. The 4841 component list (containing retention time and exact mass pairs) was sorted. This was achieved by ordering the list based on the p value (<0.05) for statistical significance. This revealed a number of component peaks were either unique or elevated in intensity in sample 2295 and were consistent across the technical replicates. A summary of the top five statistically different peaks were investigated and the results are shown in Table 4.

Taking a look at this process in more detail, and as indicated by the trend intensity graph from SIEVE 2.2 (Figure 4), a peak at retention time 13.6 minutes was significantly elevated in sample 2295 as compared to all other samples. The next step is to propose an elemental composition using the accurate mass to attempt to identify the chemical structure of the component. This can be a very difficult process and to successfully achieve this it is helpful to use both the EI spectrum and the PCI spectrum. The EI spectrum can be used to search against commercially available spectral libraries, such as NIST, and to propose a tentative compound identification. The accurate mass information can then be used to support this identification. In cases where there is no library match from the EI spectrum, the PCI data can be used to deduce the possible elemental composition of the molecular ion. Excellent mass accuracy is essential to limit the list of possible chemical formulae and to increase confidence when a proposed identification is made.

Table 4. 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	Mass Accuracy Base Peak (ppm)	Mass Accuracy Molecular ion (ppm)
1	13.6	177.12736	Trans β ionone	$C_{13}H_{20}O$	0.84	0.31
2	11.54	139.11180	Furanone	$C_9H_{16}O_2$	0.22	0.12
3	10.87	137.05974	Phenol, 4 ethyl -2 methoxy	$C_9H_{12}O_2$	0.29	0.08
4	16.16	194.09037	2,3-dimethoxy-4-phenol	$C_{11}H_{14}O_3$	0.15	0.15
5	18.00	167.07028	Furan propanoic acid	$C_{12}H_{16}O_5$	0.23	0.13

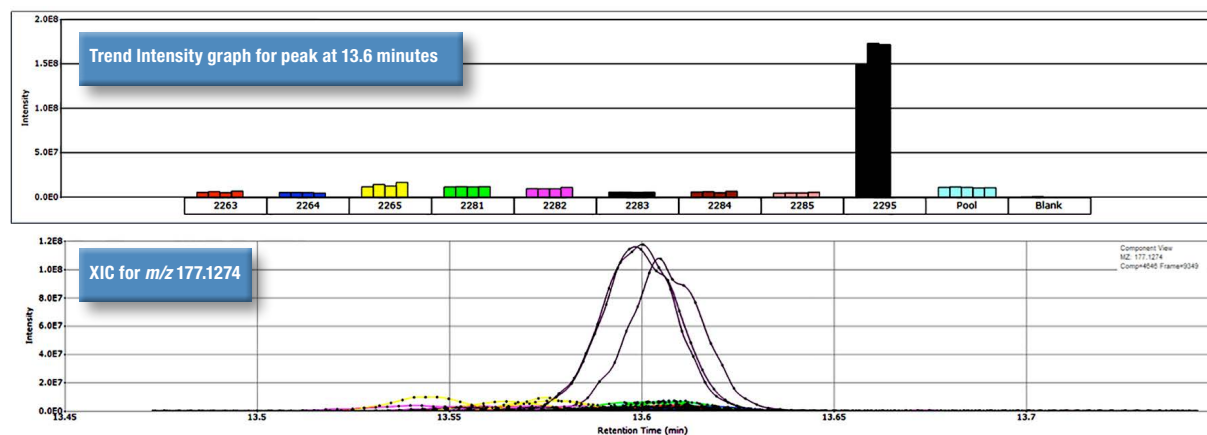


Figure 4. Trend intensity bar graph and extracted ion chromatogram for m/z 177.1274 of peak number 4646 at retention time 13.6 minutes across all of the whisky samples and replicate injections. This peak is elevated in sample 2295.

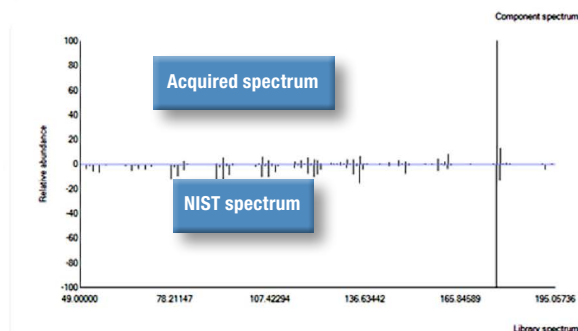
To simplify the identification of the peak at 13.6 minutes the raw data was deconvoluted in TraceFinder to provide a cleaned spectrum that was then matched against NIST14 (Figure 5). The subsequent hits from the NIST library are scored based on a combination of the search index (SI) score and high-resolution filtering (HRF) value. The HRF value is the percentage of the spectrum that can be explained by the chemical formula in the library search. For the top hit, trans β ionone, 98.4% of the spectrum can be explained based on accurate mass of the ions in the spectrum. The fragments observed can also be matched to the elements in the proposed compound to add confidence in the identification and to explain how

the fragments can form based on the hit proposed. This gives a combined SI and HRF score of 75.3%. Figure 6 shows the fragments in the EI spectrum labeled with the proposed formulas and mass errors. All of the fragments are measured with sub-1 ppm mass accuracy providing increased confidence in the assignment. Elevated levels of trans β ionone, a breakdown product of carotene, were found in sample 2295 which was a bourbon whisky. Significantly, one of the differences between bourbon and Scotch whisky is that bourbon is brewed from corn rather than barley. Corn is naturally higher in beta-carotene than barley (53 $\mu\text{g}/200\text{g}$ compared to 7 $\mu\text{g}/200\text{g}$ for barley,⁵ which would explain the elevated levels observed.

Peak Identification

Score	Matched Compound	Formula	CAS	SI	HRF Score	M+ m/z	M+	% Elements	Libra
75.3	trans- β -ionone	C ₁₃ H ₂₀ O	79-77-6	797	98.3576	192.15086	No	100	main
74.9	3-Buten-2-one, 4-(2,6,6-trimethyl-)	C ₁₃ H ₂₀ O	14901-0	776	98.3576	192.15086	No	100	main
73.4	Terephthalic acid, ethyl 2-iso...	C ₁₉ H ₂₀ O ₄		673	99.8952	312.13561	No	100	main
73.2	5-Methyl-2,4-disopropylphe...	C ₁₃ H ₂₀ O	40625-9	690	98.3576	192.15086	No	100	main
73	Acetic acid, 6,6-dimethyl-2-...	C ₁₆ H ₂₄ O ₄		649	99.8952	280.16691	No	100	main
72.9	Isophthalic acid, ethyl tridec...	C ₂₃ H ₃₂ O ₄		648	99.8952	372.22951	No	100	main

NIST hits filtered based on high resolution filtering



Trans β Ionone

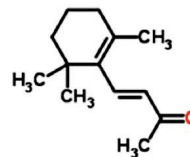


Figure 5. Identification of peak at 13.6 minutes as Trans β Ionone. Screenshot of the deconvoluted data and library match in TraceFinder 3.3. List of library hits, top hit 75.3% of spectrum explained for trans β ionone (upper). Acquired and library spectra mirror image (lower).

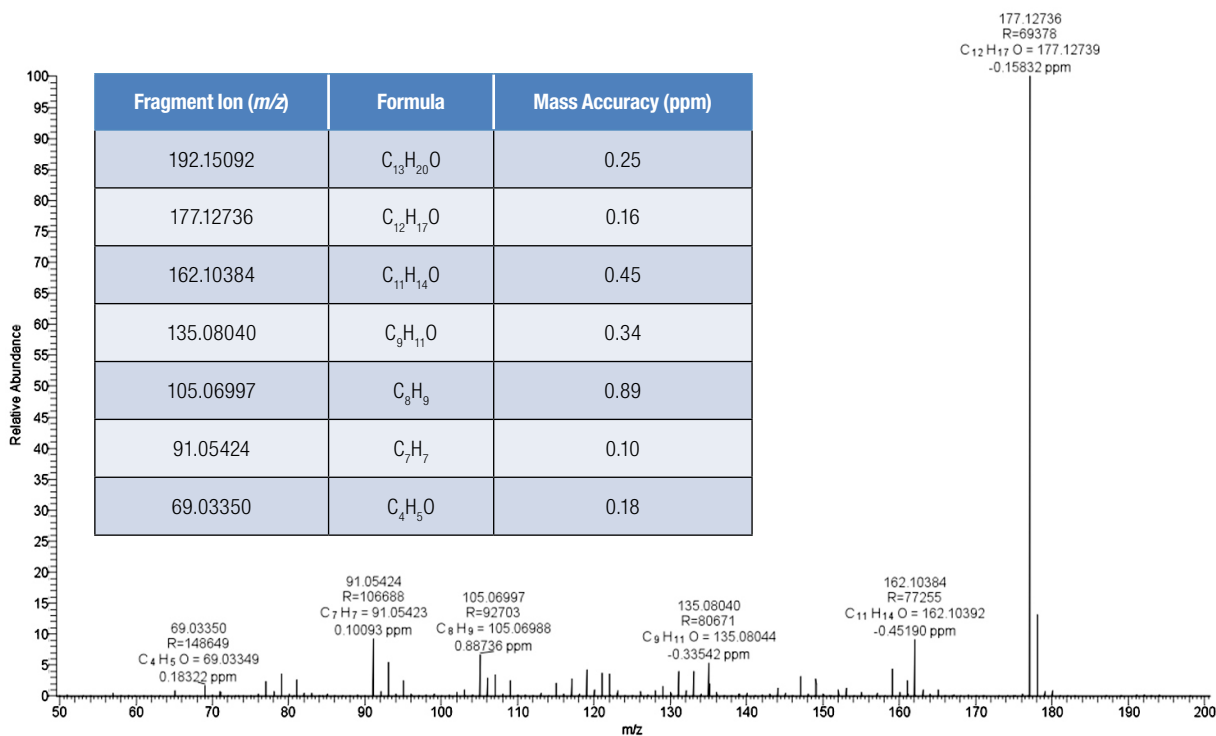


Figure 6. EI spectrum labeled with formula and mass error (ppm) for the fragments of trans β ionone. The molecular ion and the 6 most intense fragments support identification with < 1 ppm mass accuracy. R = Resolution.

Identifying Compounds with No Spectral Library Match

When there is no match using spectral libraries the process of identification is more complicated. For example, the PCA showed elevated levels of the peak at 18 minutes in sample 2295. The EI and PCI spectra (Figure 7) were used to isolate the molecular ion and propose an elemental formula. The $[M+H]^+$ and the $[M+C_2H_3]^+$ 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 will substantially limit the number of possible chemical formulae. For example, when a 10 ppm mass accuracy window was used, nine possible formulae were 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) and Chlorine (1-10). By contrast, the use of a 1 ppm mass accuracy window resulted in only one possible formula, $C_{12}H_{17}O_5$. This level of mass accuracy 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 molecular ion m/z 240.09924 seen in the EI spectrum (0.1 ppm 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. Mass Frontier 7.0 was used to theoretically fragment proposed compounds and match these to the measured fragments in the EI spectrum (Figure 7). 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-1 ppm mass accuracy allows compounds to be quickly excluded or included and adds confidence in assignments.

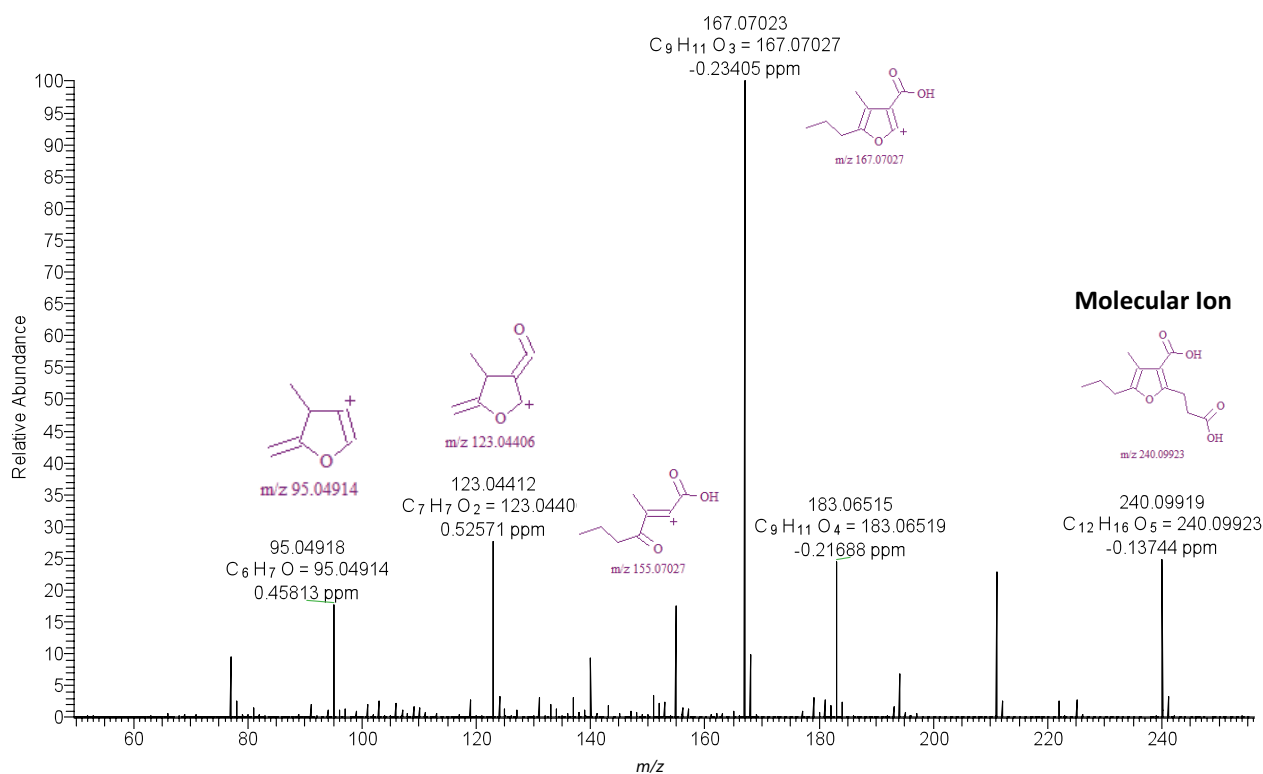


Figure 7. EI spectrum for peak at 18.00 minutes where no library match was made. Peaks are labeled with structure, formula and mass error in ppm. The sub 1ppm mass errors provide high confidence in the proposed identification of 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid. Peaks are annotated with structures identified in Mass Frontier 7.0.

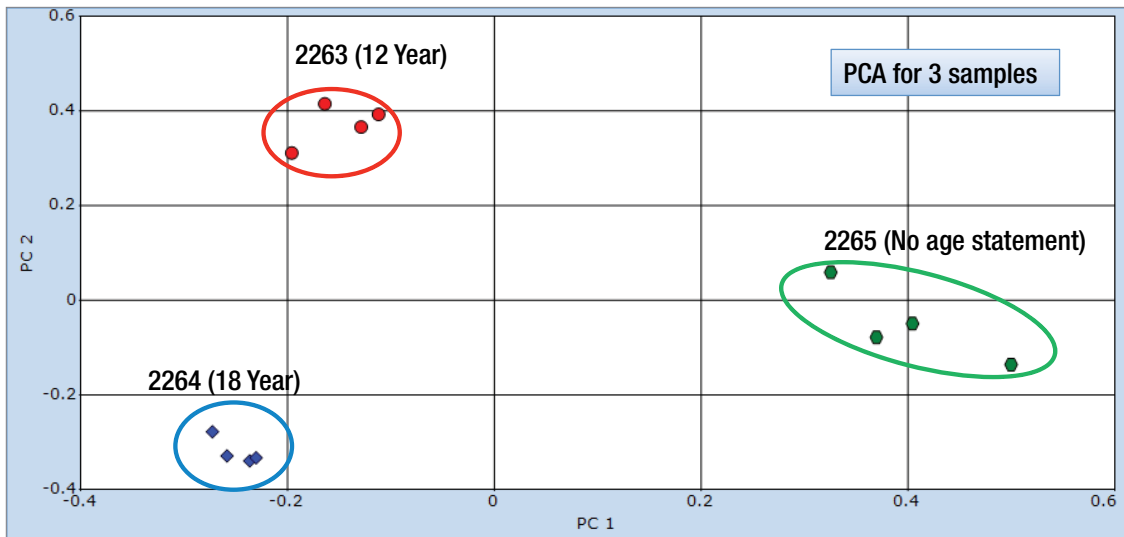


Figure 8. PCA chart generated with SIEVE for whiskies from the same distillery but with a different age declaration. Technical replicates agree.

Differences Between Whiskies from the Same Distillery

A further investigation of the dataset was to look at differences between whiskies from a single distillery, but with either a different age statement or different aging process. The replicate data for samples 2263 (12 years), 2264 (18 years) and 2265 (no age statement, aged in three barrels) was processed using SIEVE. The PCA showed that clear differences between these three samples existed and that the replicate injections were in agreement (Figure 8). The compounds that contribute to these differences were quickly isolated in TraceFinder. The deconvoluted peak list is presented as a heat map (Figure 9) to show how the intensities of peaks vary between the samples, thereby quickly isolating the peaks of interest.

A proposed identification of hydroxymethyl furfural was made using TraceFinder (Figure 10) which used spectral matching and accurate mass to sort the list of suggested compounds. This was based on the search index (>600) and scoring the proportion of the spectrum that could be explained by the suggested compound based on accurate mass. The presence of hydroxymethyl furfural (HMF) in whisky is not unusual as it is formed during the dehydration of sugars.^{6,7} The elevated levels seen in sample 2265, which was aged in three different barrels including high sugar containing ex sherry and bourbon barrels, could possibly be explained by the interaction of the whisky with the wood surfaces. In particular, the heat charring of oak bourbon barrels is known to generate high levels of HMF.

Data Review - Whisky Lowlands [Unknown]						
Heat Map						
	Retention Time	M/Z	8April_60K_Whisky_003 MS Area	8April_60K_Whisky_004 MS Area	8April_60K_Whisky_005 MS Area	
12	10.43	97.03	369,099	302,490	6,641,407,475	
13	15.69	182.06	5,724,487,496	5,798,208,026	5,693,394,891	
14	14.64	87.04	3,961,360,796	4,556,568,200	5,684,275,725	
15	14.94	73.03	4,094,135,138	5,884,332,870	5,122,210,007	

Figure 9. TraceFinder heat map showing peak area differences between the three aged whiskies. The peak at 10.43 minutes with a base peak of m/z 97.03 has a significantly higher response in the no age declared whisky compared to the 12 and 18 year old.

Peak Identification									
Score	Matched Compound	Formula	CAS	SI	HRF Score	M+ m/z	M+	% Elements	
94.2	5-Hydroxymethylfurfural	C6H6O3	67-47-0	717	99.6216	126.03114	Yes	100	
69.4	4-Ethyl-2-hydroxycyclopent...	C7H10O2	28017-6...	708	88.0651	126.06753	No	100	
68.5	2-Butyn-1-ol diethyl acetal	C8H14O2	2806-97...	662	88.0651	142.09883	No	100	
67.9	Cyclopentanecarboxylic acid...	C13H16O2	55229-4...	630	88.1169	204.11448	No	100	
67.3	Cyclopentanecarboxylic acid...	C13H22O2		602	88.1169	210.16143	No	100	
67.2	Cyclopentanecarboxylic acid...	C12H13NO4		612	99.7754	235.0839	No	75	
45.3	4-Hepten-3-one, 4-methyl-	C8H14O	22319-3...	699	28.2184	126.10391	No	100	
44.5	4-Hexen-3-one, 4,5-dimethyl-	C8H14O	17325-9...	658	28.2184	126.10391	No	100	
44.3	Furan, 2,3-dihydro-4-(1-met...	C8H14O	34379-5...	650	28.2184	126.10391	No	100	

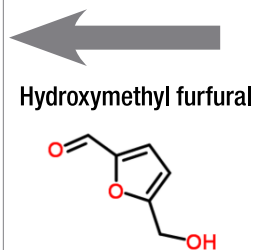


Figure 10. Identification of peak at 10.43 minutes as hydroxymethyl furfural. Screenshot of library match in TraceFinder. List of library hits are sorted based on a combination score (column 1) of spectral matching and high resolution filtering. Other acceptable spectral hits can be quickly eliminated based on accurate mass.

Conclusions

The results of this proof-of-concept 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 characterization 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 system an ideal platform for chemical profiling of complex samples.
- Routine resolving power of 60,000 FWHM and a wide dynamic range eliminates isobaric interferences, combined with consistent sub-1 ppm mass accuracy and excellent sensitivity, increases confidence in the identification of compounds, especially in complex matrices.
- SIEVE 2.2 and TraceFinder software facilitated fast and comprehensive characterization of the whisky samples, isolating and identifying compounds with confidence.
- 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 using Mass Frontier 7.0.

References

1. Poisson, L & Schieberle, P. Characterization of the most odor-active compounds in an American bourbon whisky by application of the aroma extract dilution analysis. *Journal of Agricultural and Food Chemistry*. 2008, 56, 5813-5819.
2. Alcoholic beverages: Sensory Evaluation and Consumer Research. Woodhead Publishing. 2012, 379-392.
3. Scotch Whisky Association. [online] 2015. www.scotch-whisky.org.uk.
4. Distilled Spirits Council of the United States. [online] 2015. www.discus.org.
5. LaRoe E.G & Shipley, P.A. Whiskey composition: formation of alpha and beta ionone by the thermal decomposition of beta carotene. *Journal of Agricultural and Food Chemistry*. 2002, 18.
6. J.R Piggott, Gonzalez-Vinas, J.M Conner, S.J Withers, A. Paterson. Effect of chill filtration on whisky composition and headspace. *Woodhead pub*. 1996, 319-324.
7. Hui, Y, Meunier-Goddik, L., Solvejg Hanson, A., Josephsen, J., Nip, W., Stanfield, P.S., Toldra, F. Handbook of food and beverage fermentation technology. *Taylor & Francis*. 2005.

www.thermofisher.com

©2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Canada +1 800 530 8447
China 800 810 5118 (free call domestic)
 400 650 5118

Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 10 3292 200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591

Japan +81 45 453 9100
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00

Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Spain +34 914 845 965
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
UK +44 1442 233555
USA +1 800 532 4752

innovatek



Cra 21 No. 41 – 26
 PBX (57) 338 0711

Bogotá, Colombia
www.innovatek.com.co
contacto@innovatek.com.co

Thermo
 SCIENTIFIC

A Thermo Fisher Scientific Brand