Application note | 003094

Food and beverage

Non-targeted analysis of whisky using SPME Arrow and Orbitrap Exploris GC 240 mass spectrometer

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Keywords

SPME Arrow, spectral deconvolution, Flavor and Fragrance HRAM spectral library, unknown screening, gas chromatography, Compound Discoverer software, profiling, Orbitrap mass spectrometer

Goal

The goal of this application note is to demonstrate the Thermo Scientific™ Orbitrap™ Exploris™ GC 240 mass spectrometer to characterize the chemical profile of both known and unknown whisky samples to highlight differences between distilleries and markers for whisky counterfeiting. Identification was made using the Thermo Scientific™ Orbitrap GC-MS HRAM Flavor and Fragrances Library.

Introduction

Whisky is a premium alcoholic beverage based on the tradition built into its production. It consists of a cereal-water mixture or “mash”, which undergoes fermentation followed by distillation and cask maturation, creating a unique flavor profile over a long and complex aging process. Depending on the grain used, cask condition (new vs. old, charred vs. uncharred) and distillery process, significant variability in a whisky's chemical composition can occur. As a result of these distinguishing features, whisky is a sought-after commodity both for consumption and economic purposes, where investments in whisky have far outperformed stocks and other commodities such as gold in recent years.

As the demand and retail value of whisky increases, counterfeiting has become commonplace. Through the incorporation of flavoring agents into lower-cost whiskies, profitability can be increased by imitating the characteristics of premium whiskies. Regulation exists within both the European Union and United States prohibiting additives of synthetic or non-agricultural origins and/or compounds used to alter color and taste. However, considering the complexity and variability between individual whiskies, producers require analytical technology to accurately and comprehensively characterize their products to ensure product integrity as well as confidently identify fraudulent whiskies.

In this study, the performance of the Orbitrap Exploris GC 240 high resolution accurate mass (HRAM) mass spectrometer together with the headspace solid phase micro extraction (HS-SPME) for chemical profiling of whisky is demonstrated. With high mass accuracy (<1 ppm) and resolution capabilities (up to 240,000 mass resolution full width half maximum (FWHM) at *m/z* 200), the Orbitrap Exploris GC 240 mass spectrometer provides unsurpassed selectivity towards compound identification. Differences in chemical profiles are easily visualized using the statistical tools incorporated into the Thermo Scientific™ Compound Discoverer™ software and a streamlined identification achieved using the Thermo Scientific Flavor and Fragrances HRAM library together with the NIST nominal mass library. Switching between electron impact (EI) and positive chemical ionization (PCI) for molecular ion identification/confirmation is achieved quickly without system venting using the Thermo Scientific™ NeverVent™ technology. This provides analysts accurate identification of the chemical profile in whisky for product quality control and differentiates between whiskies suspected of fraudulent activities (e.g., additives and mislabeling).

Experimental conditions

Standard and sample preparation

Four individual standards of known flavor compounds in whisky (i.e., 3-methyl-1-butanol, 2-methyl-1-butanol, isoamyl acetate, and 3-octanone) were purchased from Sigma-Aldrich (Germany) to assess headspace extraction and quantification methodology using SPME Arrow coupled to the Orbitrap Exploris GC 240 mass spectrometer. A 1,000 µg·mL^{-1} stock solution in 13% EtOH in water was prepared to construct a 7-point calibration curve, 0.25 to 40 µg·mL^{-1}. Each standard was prepared in a 20 mL Thermo Scientific™ SureSTART™ headspace vial (P/N 6ASV20-1) with a 1 mL vial volume containing 5% EtOH content with a SureSTART headspace vial screw cap (P/N 6PMSC18-ST2).

For identification purposes, 5 µg·mL^{-1} C_7-C_{40} alkane mixture (Sigma-Aldrich, Germany) was prepared in hexane to establish a retention time index. A volume of 20 µL was added to a headspace vial and analyzed in total vaporization mode using the same method conditions for sample analysis outlined in Tables 1–3.

Table 1. TriPlus RSH SMART and GC conditions

Parameter	Setting
TriPlus RSH SMART SPME Arrow parameters	
SPME Arrow fiber	Thermo Scientific™ SMART SPME Arrow 1.1 mm: DVB/C-WR/PDMS (P/N 36SA11T3-SM)
Incubation temperature (°C)	40
Incubation time (min)	10
Agitation speed (rpm)	500
Extraction time (min)	10
Needle depth in vial (mm)	30
Needle speed in vial (mm·s^{-1})	20
Fiber injection	
Injection liner depth (mm)	70
Penetration speed (mm·s^{-1})	40
Injection desorption time (min)	3
SPME fiber conditioning	
Conditioning temperature (°C)	290
Post-conditioning time (min)	10
TRACE 1610 GC system parameters	
Injector	Thermo Scientific™ iConnect™ SSL
Liner	Thermo Scientific™ SPME Arrow Liner, i.d. 1.7 mm (P/N 453A0415-UI)
Injection mode	Split
Split flow (mL·min^{-1})	60
Injector temperature (°C)	250
Carrier gas, (mL·min^{-1})	1.2
Oven temperature program	
Initial temperature (°C)	40
Hold time (min)	3
Rate 1 (°C·min^{-1})	10
Temperature 1 (°C)	270
Final hold time (min)	5
Total analysis time (min)	45

Table 2. EI source and mass spectrometer conditions

Parameter	Setting
Orbitrap Exploris GC 240 MS parameters	
Transfer line (°C)	280
Thermo Scientific™ ExtractaBrite™ ion source temperature (°C)	300
Electron energy (eV)	70
Acquisition mode and scan range (*m/z*)	Full scan, 40–600
Resolving power (at 200 *m/z*)	120,000
Emission current (µA)	50
C-Trap offset (V)	0
Internal lock mass calibration (column bleed, *m/z*)	207.02235, 281.05114, 355.06993

Table 3. PCI ion source and mass spectrometer conditions

Parameter	Setting
Orbitrap Exploris GC MS parameters	
Transfer line (°C)	280
ExtractaBrite ion source temperature (°C)	200
Reagent gas and flow (mL·min^{-1})	Methane; 1.1
Ionization mode	Positive
Acquisition mode and scan range (*m/z*)	Full scan; 50–600
Resolving power (at 200 *m/z*)	120,000
Emission current (µA)	50

For sample preparation, 100 µL of whisky (EtOH content approximately 40%) was diluted with 900 µL of ultrapure water (18.2 Ω) with a final alcohol content of 4%. Three blank samples (1 mL 4% EtOH in water) were prepared to assess background contamination and determine the limit of quantification (LOQ: 10 x standard deviation of blank response). If no analyte response was detected in the blank samples, then the variation in the lowest calibration standard was used for LOQ determination. A QA/QC sample was prepared by mixing 100 µL of each whisky together to a total volume of 1 mL. This serves as a control for assessing the statistical analysis performance for unknown identification within Compound Discoverer software.

Instrument and method setup

Headspace extraction and injection of whisky samples was performed using the Thermo Scientific™ TriPlus™ RSH SMART autosampler equipped with the SMART SPME Arrow 1.1 mm DVB/C-WR/PDMS fiber (P/N 36SA11T3-SM). Incubation and extraction were performed online followed by sample injection/desorption. After sample injection, the SPME Arrow fiber was re-conditioned at high temperature under nitrogen using a SPME conditioning station to avoid sample carryover between injections. Further details surrounding the SPME Arrow operating parameters can be found in Table 1.

A Thermo Scientific™ TRACE™ 1610 GC equipped with a Thermo Scientific™ TraceGOLD™ TG-624SilMS (30 m × 0.25 mm i.d. × 1.4 µm film) capillary column (P/N 26085-3320) was used to perform the chromatographic separation. Oven program conditions can be found in Table 1. Data acquisition was carried out in full scan analysis using both EI and PCI with the Orbitrap Exploris GC 240 mass spectrometer. Additional MS method parameters are summarized in Tables 2 and 3. External mass calibration was performed prior to analysis, while characteristic ions representing column bleed were used as lock masses when scanning in EI to perform internal mass calibration. Sample acquisition and qualitative processing were performed using Thermo Scientific™ Chromeleon™ version 7.3.2 Chromatography Data System (CDS) software. Unknown analysis and identification were performed using Compound Discoverer version 3.3 software.

Results and discussion

Targeted analysis with full scan acquisition of flavor compounds in whisky

In full scan acquisition, sensitivity is compromised as less scanning time is spent on the ions of interest, thus affecting detection over background and interfering ions. However, with HRAM, higher ion selectivity from background interference can be achieved without sacrificing trace quantification. Calibration performance for the analysis of 3-methyl-1-butanol, 2-methyl-1-butanol, isoamyl acetate, and 3-octanone can be seen in Table 4. Linear response was observed over the entire calibration range (0.25–40 µg·mL^{-1}) with the exception of 2-methyl butanol and 3-octanone. Visual inspection of the calibration curve for 2-methyl butanol shows evidence of two distinct linear calibration ranges observed ranging from 0.25 to 1 µg·mL^{-1} and from 1 to 40 µg·mL^{-1} (Appendix, Figure A1). Based on the high content of 2-methyl 1-butanol present within the samples, quantification was performed using the higher calibration range with a linear offset applied. No response for isoamyl acetate or 3-octanone was observed in blank samples. Thus, the variation in the lowest calibration curve standard was used to calculate the LOQ.

Table 4. Calibration performance of targeted flavor compounds

Name	Retention time (min)	%RSD	Slope/response factor	Correlation coefficient (R^2)	LOQ ($\mu g \cdot mL^{-1}$)
3-Methyl-1-butanol	8.96	2.3	1.3e4	0.999	0.23[c]
2-Methyl-1-butanol	9.03	9.1	8.2e4	0.994[a]	0.11[c]
Isoamyl acetate	11.45	7.0	2.5e6	0.997	0.06[d]
3-Octanone	13.82	9.1	7.5e6	0.994[b]	0.02[d]

[a]Linear calibration range from 1–40 $\mu g \cdot mL^{-1}$ with offset applied

[b]Linear calibration range from 0.25–10 $\mu g \cdot mL^{-1}$

[c]LOQ determined from blank response variation (n=3)

[d]LOQ determined from lowest calibration standard variation (n=3)

Table 5. Country of origin, age, and sample concentration ($\mu g \cdot mL^{-1}$) of 3-methyl-1-butanol, 2-methyl-1-butanaol, and isoamyl acetate for whisky sample analysis. Region of Scottish whisky origin is provided in parentheses if declared.

Sample ID	Country (region)	Age (years)	3-methyl-1-butanol	2-methyl-1-butanol	Isoamyl acetate	3-methyl-/ 2-methyl-1-butanol ratio
A	Unknown	Unknown	372	179	0.2	2.1
B	Unknown	Unknown	10,017	5,936	5.2	1.7
C	Unknown	Unknown	126	81	0.1	1.5
D	Unknown	Unknown	6,303	3,170	2.7	2.0
1	Scotland (Highland)	12	3,321	1,633	2.2	2.0
2	Scotland (Highland)	15	4,196	1,746	1.8	2.4
3	Scotland (Lowland)	Unknown	225	108	0.8	2.1
4	Scotland (Island)	12	3,613	1,922	2.4	1.9
5	Scotland (Island)	10	3,697	1,775	6.9	2.1
6	France / Brandy	Unknown	3,463	950	0.3	3.6

A total of nine different whiskies were analyzed in this study, five originating from Scotland (sample ID: 1-5) and the remaining four from an unknown origin (Sample ID: A-D). In addition to the whisky samples, one sample of Brandy (sample ID: 6) was also analyzed to serve as a negative control towards whisky classification and identification. Sample concentrations for targeted flavor compounds are reported in Table 5. Concentrations of 3-octanone are not reported as they were below the limit of quantification.

The concentrations of 3 methyl-1-butanol and 2-methyl-1-butanol as well as their ratio have been used as an analytical fingerprint for helping distinguish and authenticate whiskies. In a study by Aylott and MacKenzie, the concentrations of 3 methyl-1-butanol and 2-methyl-1-butanol were typically higher in Scotch malt whiskies, whereas their content in grain Scotch whisky was lower due to their reduction during the distillation process for grain whisky.[1] Within the same study, analysis of over 56 whiskies observed the ratio of 3 methyl-1-butanol to 2-methyl-1-butanol to range between 1.9 to 3.5 in malt, grain, and blended Scotch whiskies. Several samples within our analysis deviated outside this range, causing suspicion towards the samples' authenticity.

The ratio of 3-methyl / 2-methyl-butanol for sample 6, which served as our negative control, fell outside the range typically observed for whisky. However, this result is expected considering that it is Brandy. Ratios determined for samples B and C were also found to fall outside this range, and thus, raise suspicion regarding their authenticity. Elevated concentrations of isoamyl acetate were also observed in several samples (B and 5). Although this compound is produced naturally in whiskies, it may be added intentionally to improve a whisky's flavor profile. This highlights the challenges in using targeted approaches to access whisky authenticity, indicating the necessity for additional information on the chemical composition.

Whisky differentiation through chemical profiling

The chemical profile of each whisky sample was determined through a non-targeted analysis workflow in Compound Discoverer software. Identification of deconvoluted spectra was aided through library matching with NIST 2023 and the Thermo Scientific Orbitrap GC-MS HRAM Flavor and Fragrances Library (P/N 834-009400). A key advantage of Compound Discoverer software is the integration of various statistical tools to help users evaluate and visualize differences in samples based on their chemical profile. From the positively identified compound list detected in whisky samples, differential analysis was performed using principal component analysis within Compound Discoverer software (Figure 1).

Clear separation can be observed within the first principal component (PC1), which accounts for over 45% of the data variance. The origin of the loading plot represents an average of the chemical composition measured in all samples. As our QA/QC sample is a mixture of all samples analyzed, it verifies that the sample variances have been accurately accounted in the PCA. Differences observed between samples could be attributed to various factors such as country/region of origin, age, grain type, grain preparation (malted vs. un-malted), cask type (new vs. old) / preparation (charred/tanned), or adulteration. Closer investigation into the chemical profiles can lend insight into the cause for these differences.

Malt vs. un-malted whisky

Several whisky distinctions exist and are useful to define to avoid confusion:

- Single malt – Produced from a single distillery made solely from malted barley.
- Single grain – Produced from a single distillery but consists of other grains such as wheat, corn, or rye in addition to barley. These grains can also be malted or un-malted.
- Blended – Consists of a mixture of both malt and grain whiskies. Blended whiskies represent the most common type of whisky produced in Scotland.

To help distinguish between malt and un-malted whisky, identification of chemical tracers for malted cereals is useful. One such chemical tracer is furfural, which is present in higher amounts in malted grains, particularly if they have been roasted.[2] The presence of furfural can also originate from cask charring. This process promotes the breakdown of cross-linked sugar molecules within the wood. These can be degraded further to form furan derivatives that migrate into the whisky during storage giving it an almond-like flavor and dark caramel coloring. Positive identification of furfural was achieved through identification of the molecular ion exact mass (*m/z* 96.02055) at a mass accuracy of 0.28 ppm (Figure 2).

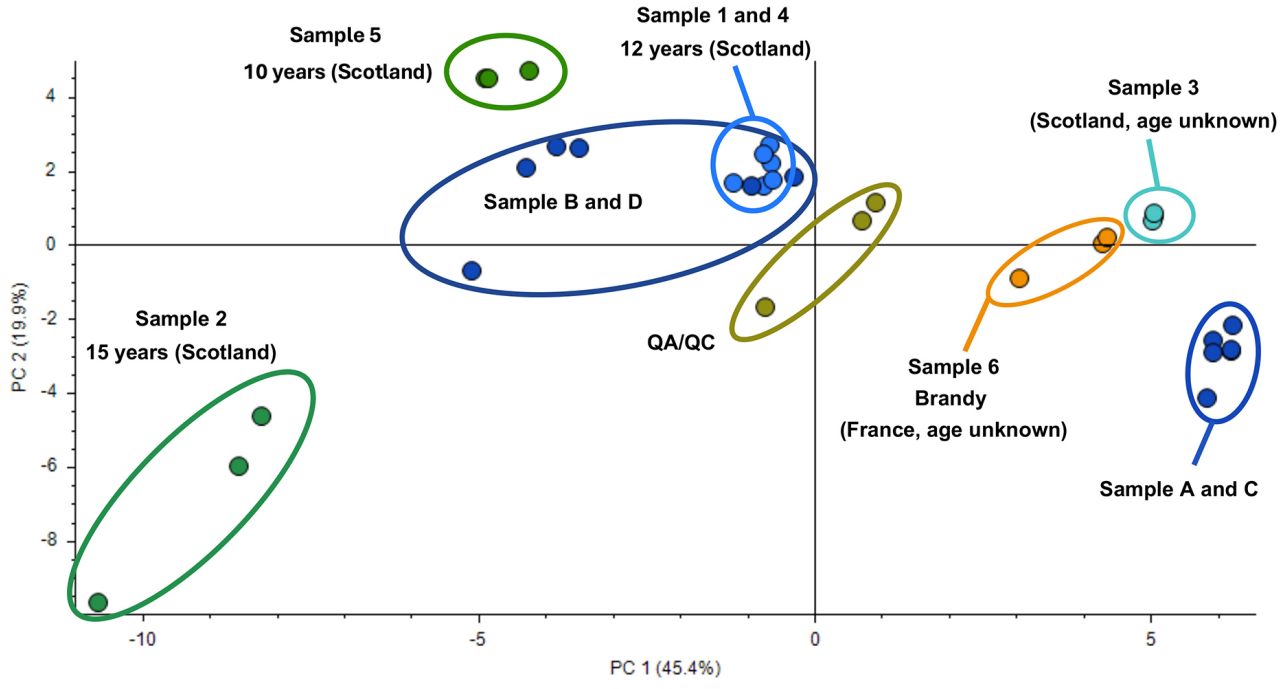

Figure 1. Principal component analysis loading plot of whisky samples based on identified chemical profile from Compound Discoverer software. Whisky age and country of origin (if provided) have been displayed as additional explanatory variables.

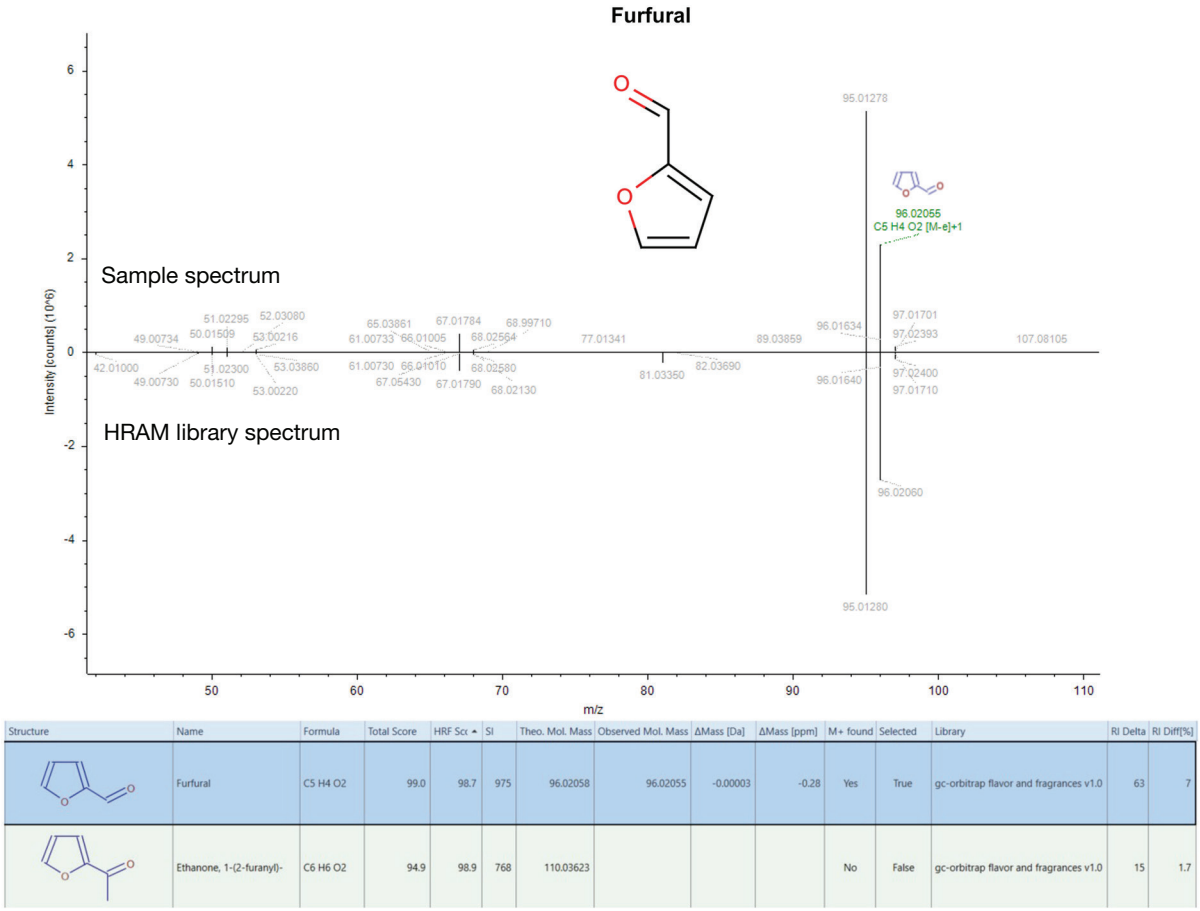

Figure 2. Sample mass spectra and matching HRAM library hit of furfural with library search results table

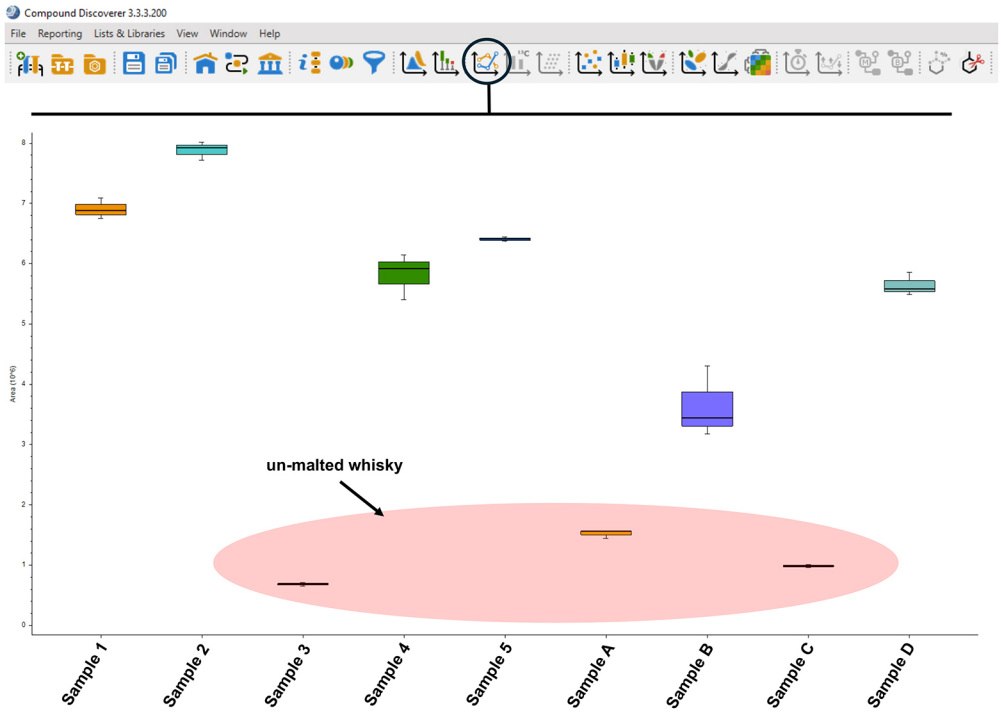

Figure 3. Boxplot of furfural response (area) across all samples. Boxplot distribution is based on triplicate analysis with the median represented by the blank line, and the outer box edges represent the 25th and 75th percentile. Highlighted samples represent the furfural signature for un-malted whiskies.

Using the trend chart function within Compound Discover software (Figure 3), comparison of furfural concentration can be made easily across all samples. Samples 3, A, and C displayed significantly lower levels of furfural compared to the other samples, indicating these whiskies were produced from un-malted cereals. This divergence from the other whiskies is also captured in the PCA loading plot (Figure 1), suggesting that malted vs. un-malted whiskies is a significant contributor to the variance observed.

Whisky age/regional differences vs. adulteration

Further investigation of the PCA loading plot (Figure 1) shows that age is an important explanatory factor. For samples in which age was provided, the largest variance is observed between sample 2 (15 years) and sample 5 (10 years) along the second principal component, while sample 1 and 4 (both 12-year-old whiskies) were closely associated with one another. Using the differential analysis functionality present within Compound Discoverer software, differences in the chemical profile between sample 2 and sample 5 can be visualized using a volcano plot (Figure 4). A key class of compounds causing distinction between these two samples is the carboxylic esters, which are found to be dominant in sample 2. Ester content will increase during the aging process due to continued reaction with carboxylic acids extracted from wooden casks and the original distillate. Thus, it is not surprising that the content of these is higher in the older whiskies. It is interesting to note that the high ester content will provide a stronger fruit flavor and floral aroma, which is a characteristic signature of whiskies produced in the Highland region of Scotland.

Only a few compounds were observed to dominate in sample 5 compared to sample 2. These were identified as isoamyl acetate, naphthalene, and phenylethyl acetate. The presence of naphthalene is not desirable as it can give an unwanted musty flavor and is likely a result of the whisky being stored under poor conditions (i.e., moldy barrels). However, previous studies have also identified drying of malt barley using peat fires as a source of PAHs.[3] This imparts a unique smoky flavor due to the presence of phenolic and cresol compounds. This is supported by findings of *p*-cresol within sample 5, which was significantly higher compared to all other whiskies investigated (Appendix, Figure A2).

Isoamyl acetate and phenyl acetate are produced naturally within whisky, providing a fruity/floral aroma. However, the amounts found within sample 5 were significantly higher compared to that found in sample 2 (Figure 4) as well as all other whiskies investigated. Phenylethyl acetate is known to be used as a flavoring/aroma to help imitate properties of whisky.[4] High contents of these compounds may suggest they have been intentionally added to mask unwanted flavor/aroma from naphthalene, causing suspicion towards the authenticity of sample 5.

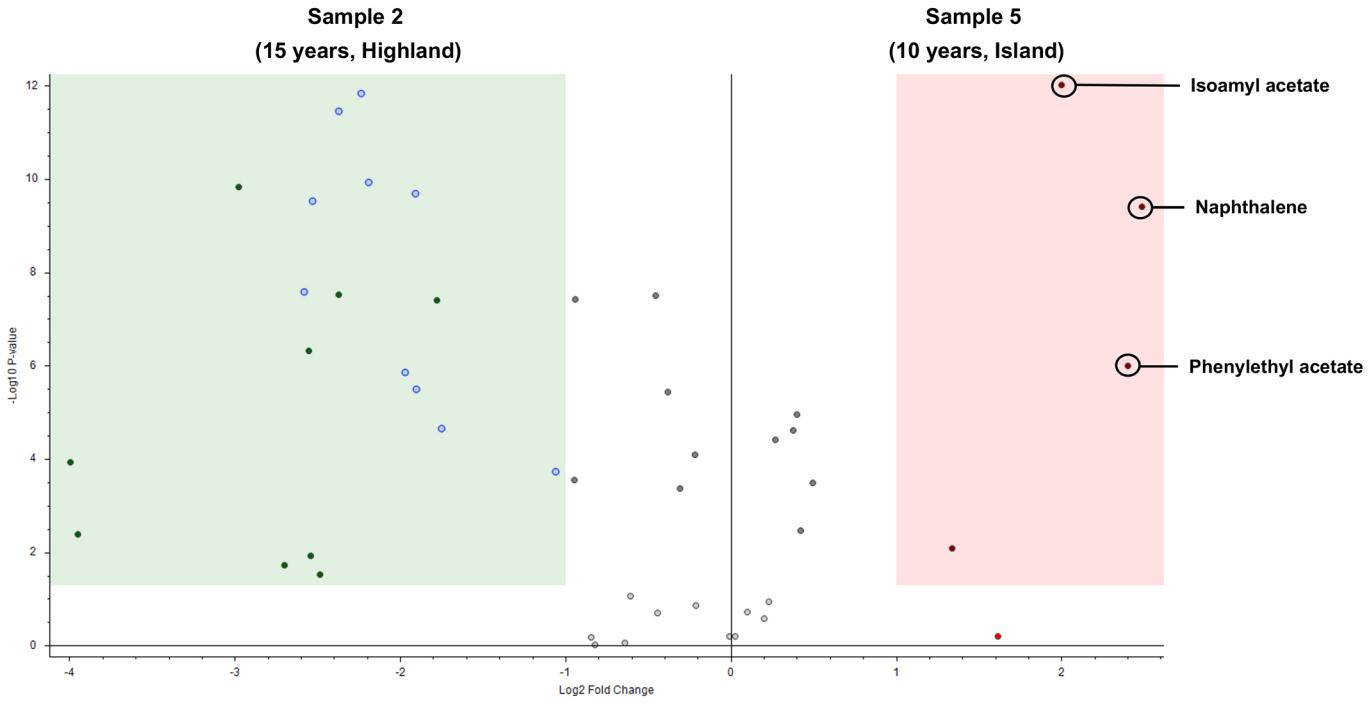

Figure 4. Volcano plot of chemical profile detected in sample 2 and sample 5 after background correction. Results are filtered to compounds with search index (SI) values greater than 700. Compounds highlighted in blue represent esters detected and identified. The X axis represents the difference in response observed by a factor of 2-fold change. The Y axis represents the *p*-value based on the statistical analysis between the two sample groups. Shaded regions represent compounds whose response differs greater than 2-fold and are significantly different at the 95% confidence interval ($p \leq 0.05$).

Samples 1 and 2 both originate from the Highland region in Scotland but exhibited very different chemical profiles (Figure 1). Ethyl dodecanoate has been identified as a useful tracer to help distinguish between genuine and fraudulent whiskies.[4] Although its presence is found in both, lower concentrations were observed in fraudulent whiskies. Whiskies originating from the Highland region are known for their fruity and floral aroma, a characteristic driven by the esters, such as ethyl dodecanoate, produced through the aging process. However, levels of ethyl dodecanoate in sample 1 are significantly lower compared to those observed in sample 2 (Figure 5A). The higher levels found in sample 2 may reflect the longer maturation process (15 vs. 12 years).

Benzaldehyde is another compound naturally found in whiskies. Its presence is attributed to migration from the wooden casks during the maturation process. Although it occurs naturally, it can also be added intentionally to modify the flavor of whisky. One would expect higher benzaldehyde concentration with longer maturation period within the cask. However, levels of benzaldehyde were comparable between the 12-year-old (sample 1) and the 15-year-old (sample 2) whiskies from the Highland region (Figure 5B).

Conclusion

The complex and versatile chemical profile existing among the various whisky types causes challenges in identifying genuine and fraudulent whiskies. Targeted analytical approaches can be applied but cannot provide a complete picture of the chemical composition, potentially missing fraudulent clues. The combination of HS-SPME Arrow with Orbitrap Exploris GC HRAM capabilities provides rapid analysis of both targeted and untargeted compounds giving the following advantages:

- Time savings with minimal sample preparation and online extraction using the TriPlus RSH SMART robotic autosampler
- Full scan acquisition at high mass resolution (i.e., 120 000 at *m/z* 200) providing targeted quantitative analysis with non-targeted chemical profile determination
- Dedicated Flavor and Fragrances HRAM library enabling accurate identification at sub ppm mass accuracy
- Mass spectral deconvolution combined with statistical tools for sample differentiation all combined within Compound Discover software

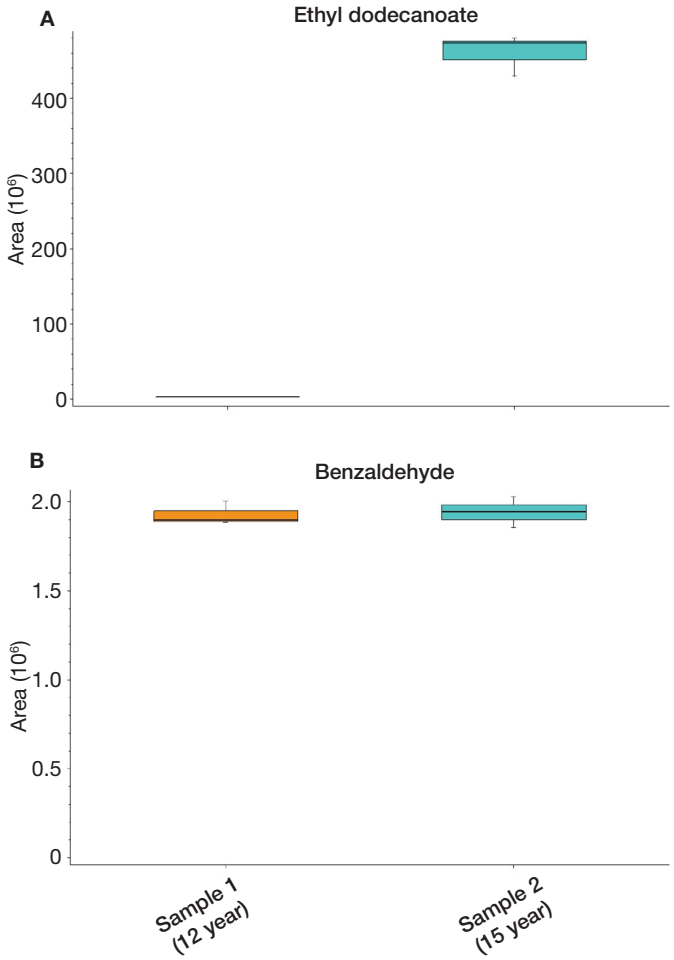

Figure 5. Boxplot of (A) ethyl dodecanotate and (B) benzaldehyde concentration in whisky samples 1 and 2 from the Spreyside region

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Appendix

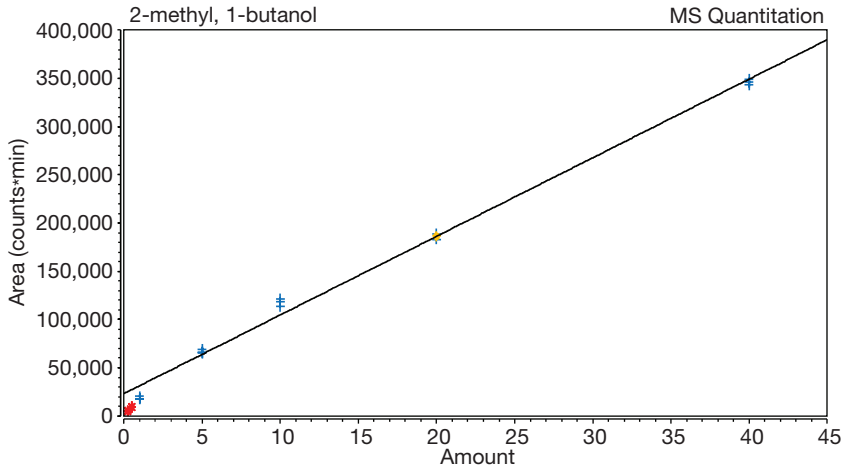

Figure A1. Calibration curve (0.25–40 mL^{-1}) for 2-methyl-1-butanol identified in whisky samples

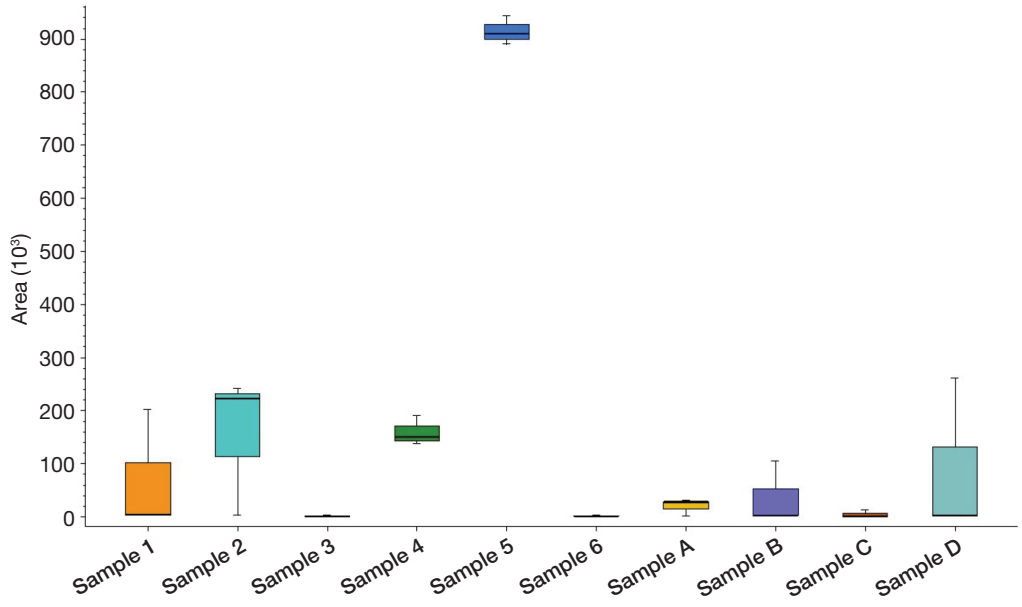

Figure A2. Boxplot of area response of *p*-cresol identified in whisky samples (samples 1-5, A-D) and French Brandy (sample 6)

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