Chemometric Profiling of Whiskey Using the 5977A GC/MSD

Application Note

Food Testing & Agriculture

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Abstract

Nontargeted compound analysis and statistical tools were used in combination with the high sensitivity of the Agilent 5977A Series GC/MSD with Extractor EI Source to generate compound profiles that were used to differentiate five brands of whiskey.

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Introduction

Gas chromatography/mass spectrometry (GC/MS) is widely used in food analysis for applications such as R&D, quality control, and quality assurance. Advances in GC/MS performance have enabled reliable detection of the myriad of trace compounds common to most natural products. Although human sensory tests (smell and taste) are still an essential part of flavor quality control, GC/MS is able to provide increasingly valuable details about changes and differences in the concentration profiles of major and trace components without the limitation of human sensors.

Chemometrics can be used to solve both descriptive and predictive problems. In descriptive applications, properties of chemical systems are modeled with the intent of learning the underlying relationships and structure of the system. In predictive applications, properties of chemical systems are modeled to predict new properties or behavior of interest. GC/MS is often used to derive the data used in both descriptive and predictive chemometrics. In the predictive mode, this technique has been used to predict whether olive oil will pass the extra virgin sensory test [1], distinguishing wine [2] varieties, and whether shochu is contaminated during the manufacturing process [3]. In the descriptive mode, this technique can be used to distinguish closely related food products, such as different brands of whiskey.

While these chemometric analyses are often performed using very powerful MS instrumentation, lower cost single quadrupole mass detectors can also provide useful information. This application note demonstrates the use of sophisticated statistical analysis of data generated by the 5977A GC/MSD to distinguish differences between five different brands of whiskey. The 5977A GC/MSD, in combination with the Agilent 7890B GC, is an ideal platform for sensitive and sophisticated statistical profiling of food products such as whiskeys, and automated solid phase micro-extraction (SPME) on the PAL Automated Sample Injector enables very sensitive headspace sampling of the whiskey aromas. Mass Profiler Professional (MPP) Software enables classification of the composition of complex samples such as whiskey using a range of statistical tools.

This study used nontargeted compound analysis and statistical tools such as one-way analysis of variance (ANOVA), principal component analysis (PCA) and hierarchical cluster analysis (HCA) to identify differences between the various brands of whiskeys. Data and statistical analyses were performed using NIST AMDIS (Automated Mass Spectral Deconvolution and Identification System), Agilent MassHunter ID Browser and Mass Profiler Professional software. This approach enabled classification of the whiskeys into four groups based on the relative concentrations of 46 different entities.

Experimental

Samples

Five different whiskeys were obtained commercially in the US, and they are described in Table 1.

Table 1. Whiskey Samples Used in the Study

Sample	Description	Subjective aroma
Popular brand (PB)	Most popular whiskey in the market	A soft, thin entry to an off-dry
Competitor (A)	Described as premium whiskey	Similar to PB
Competitor (B)	Popular knock-off whiskey	Sweet with light caramel and vanilla flavors. Stronger aroma than PB
Competitor (C)	Claims to be even higher quality than PB	Sweet aroma, slightly stronger than PB
Competitor (D)	Claims to be a deep flavor whiskey	Honey, butter, and a hint of dark fruit (plums, raisins). Stronger aroma than PB

Instruments

This study was performed on an Agilent 7890B GC equipped with automated solid phase micro-extraction (SPME) on the PAL Automated Sample Injector and coupled to the single quadrupole Agilent 5977A GC/MSD with Extractor EI Source. The instrument conditions are listed in Tables 2 and 3.

Sample preparation

The volatile odor and flavor components from each sample type were collected using headspace SPME. Each 5 mL whiskey sample was transferred to a 10 mL headspace vial. A 50 µm × 2 cm DVB/CAR/PDMS was exposed to the headspace of the sample at 60 °C for 10 minutes with agitation. Volatile compounds absorbed on the SPME fiber were thermally desorbed at 240 °C for 1 minute into an injection port.

Table 2. PAL Automated Sample Injector SPME Conditions

Sample volume	5 mL of whiskey in a 10 mL vial
Syringe	2 cm Fiber 50/30 µm DVB/CAR/PDMS
Pre-incubation time	60 seconds
Incubation temperature	60 °C
Pre-incubation agitator speed	500 rpm
Agitator time	On at 0 seconds, off at 2 seconds
Vial needle penetration	11 mm
Vial fiber exposure	22 mm
Extraction time	600 seconds
Desorb to	Split/splitless inlet
Injection needle penetration	32 mm
Injection fiber exposure	22 mm
Desorption time	60 seconds

Table 3. GC and Mass Spectrometer Conditions

GC run conditions	
Analytical column	HP INNOWAX (25 m × 0.20 mm, 0.40 µm) (p/n 19091N-202)
Injection method	SPME (50/30 µm DVB/CAR/PDMS)
Inlet temperature	Isothermal at 260 °C
Injection mode	Split, 50:1 ratio
Oven temperatures	1.5 minutes hold at 40 °C 40 °C to 240 °C at 30 °C/min Hold at 240 °C for 3 minutes
Column flow	1.1 mL/min constant flow
Carrier gas	Helium
Transfer line temp	255 °C
GC run time	16 minutes
MS conditions	
Ionization mode	EI, 70 eV
Ion source temperature	230 °C
Quadrupole temperature	150 °C
Acquisition mode	Scan (50–550 amu), normal mode
A/D sample	4
EM setting gain	1.0
Threshold	150
Trace ion detection	On
Tuning	etune.u and atune.u

Data processing and statistical analysis

Entity extraction from the GC/MS data was done using AMDIS on the Agilent MSD Productivity ChemStation (F.01.00). The .ELU files from AMDIS were imported into Mass Profiler Professional (MPP) for differential analysis. MPP 12.1 was used for data filtering and statistical analysis, and compound identification was performed using the NIST 11 MS Library and Agilent MassHunter ID Browser. The settings used for these software packages are shown in Table 4.

Table 4. Data Processing and Statistical Analysis Software Settings

Deconvolution (AMDIS 2.67)	
Component width	12
Omit *m/z*	0 (TIC), 207, 267
Adjacent peak subtraction	Two
Resolution	Medium
Sensitivity	Low
Shape requirement	Medium
Entity creation (Mass Profiler Professional 12.1)	
Compound quality score	> 20
Minimum abundance	> 1,000
Ions	> 3
RT tolerance	< 0.10
Match factor	> 0.3
Normalization type	None
Compound identification (NIST MS Library and Agilent MassHunter ID Browser)	
MS library	NIST 11
Match factor	> 50, Best hit

Results and Discussion

Detection of trace compounds, Etune versus Atune

The 5977A GC/MSD features a unique Extractor EI Source and its Etune tuning protocol, which increase MSD sensitivity in order to achieve lower detection limits and improve the identification of trace-level compounds. The Atune algorithm from previous generations of the Agilent MSD is still available for use with the Extractor EI Source. Both tuning protocols were used in the detection of trace compounds from the aromas of the whiskey samples, in order to compare their relative efficiencies for this application.

Analyzing samples using Etune and Atune generated a combined list of 142 entities from four replicate injections. A comparison of the relative intensities revealed that 48 of the 142 entities with a fold change ≥ 2 between the two tuning protocols passed the t-test at a probability p-value < 5%, as shown in red in the volcano plot in Figure 1. All 48 exhibited higher intensities with Etune, versus Atune. In fact, four entities found using Etune were not detected using Atune, under the same AMDIS parameters (Figure 2).

Profiling of whiskey aroma compounds

In order to fully characterize the compounds constituting the aroma of the five whiskey samples, GC/MS analysis was conducted in triplicate on all five whiskey samples. The detected entities were then filtered using a coefficient of variation (CV) filter of noise reduction of 75%, resulting in 74 entities common to the five whiskey samples. These were then divided into two groups (Figure 3), those with relative peak intensities <1,000,000 (low and medium abundance), and those with peak intensities ≥ 1,000,000 (high abundance).

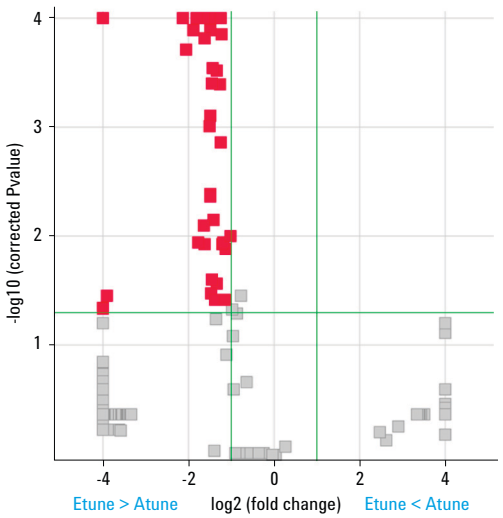


Figure 1. Volcano plot of fold-change comparison between compounds detected with Atune versus Etune in sample D. The green lines show the cutoff values for fold-change (≥ 2) and probability p-value (< 5%), and the entities meeting the criteria are shown in red. Note that all the compounds that met these criteria gave at least two-fold higher intensities with Etune, versus Atune.

Retention time	Mass	Compound	Atune	Etune
6.522	401	401.0 at 6.52155	0	27,685
11.415	**88**	**Pentadecanoic acid, ethyl ester**	**0**	**25,542**
7.86	475	475.0 at 7.86025	0	18,527
8.646	143	143.0 at 8.646299	0	19,100

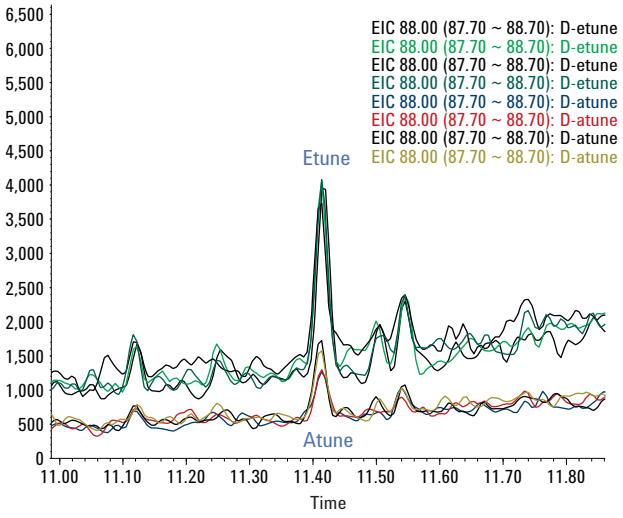


Figure 2. Four compounds were detected in four replicate analyses of sample D using Etune (upper chart) that were not seen when using Atune and the same AMDIS integration threshold. The lower extracted ion chromatograms (EIC) show the 88u peak (pentadecanoic acid, ethyl ester) in the four replicates, using Etune and Atune.

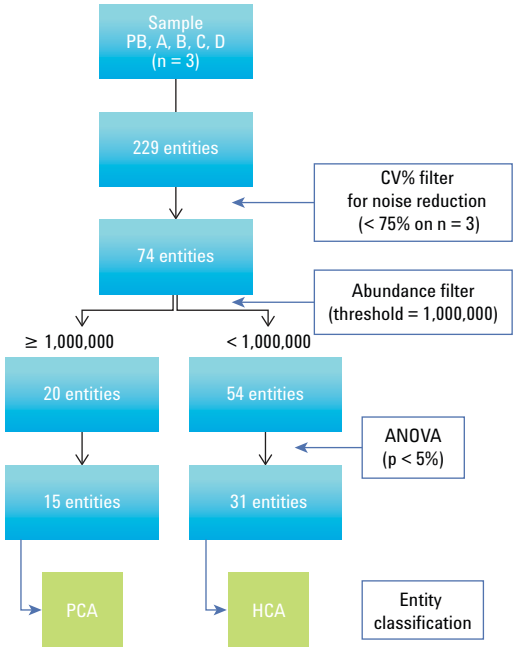


Figure 3. The workflow for chemometric profiling of the whiskey samples, culminating in classification of the relevant compounds by principal component analysis (PCA) or hierarchical cluster analysis (HCA).

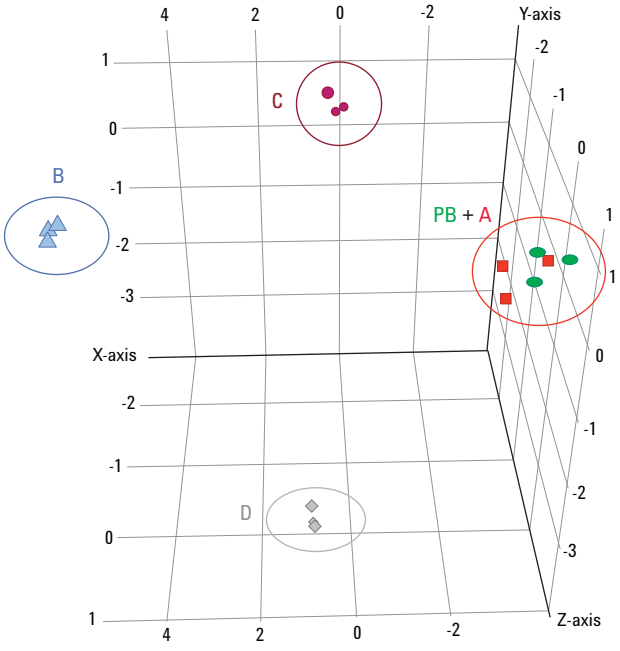
High abundance entities

Twenty compounds with peak intensities ≥ 1,000,000 were identified using Mass Profiler Professional (MPP). For comparisons of the differences in the tuning, both Atune and Etune were again used. Filtering these using one-way analysis of variance (ANOVA) with a p-value < 5% resulted in 15 compounds of statistical significance (Figure 4). Principal component analysis (PCA) of these 15 resulted in four groups of distinguishable samples, PB+A, B, C, and D (Figure 5).

Retention Time	Mass	Compound	[PB]	[A]	[B]	[C]	[D]
2.590	61.0	Trimethylsilylmethanol	3759228	4107974	3257738	6529116	8362442
4.515	74.0	2-Amino-2-methyl-1,3-propanediol	3863118	3302027	1622565	2550368	1281017
4.772	70.0	1-Butanol, 3-methyl-, acetate	3286931	3442918	1255734	584678	384032
5.724	88.0	Hexanoic acid, ethyl ester	855511	840595	2161864	991785	1130796
7.246	88.0	Octanoic acid, ethyl ester	6435439	7319735	35215980	7083341	16339178
7.590	96.0	3-Furaldehyde	3406372	3151482	1785684	1587822	3265564
7.937	88.0	Nonanoic acid, ethyl ester	619252	708628	1281332	674696	716060
8.593	88.0	Decanoic acid, ethyl ester	9160368	10945965	91484704	15420839	43557596
8.721	70.0	Octanoic acid, 3-methylbutyl ester	99822	132939	1301459	366510	160848
8.767	110.0	Ethyl trans-4-decenoate	528019	555572	1170285	717243	240995
9.803	88.0	Dodecanoic acid, ethyl ester	1428005	1770075	31862326	3835626	14279053
9.912	70.0	(-)-1-Methylbutyl decanoate	35299	45598	1048773	303444	187453
10.348	91.0	Phenylethyl Alcohol	1451716	1323375	2749470	2825832	1035489
10.901	88.0	Tetradecanoic acid, ethyl ester	183026	188006	3107707	483733	1213085
11.906	88.0	Hexadecanoic acid, ethyl ester	343726	300003	2559052	741498	1686823

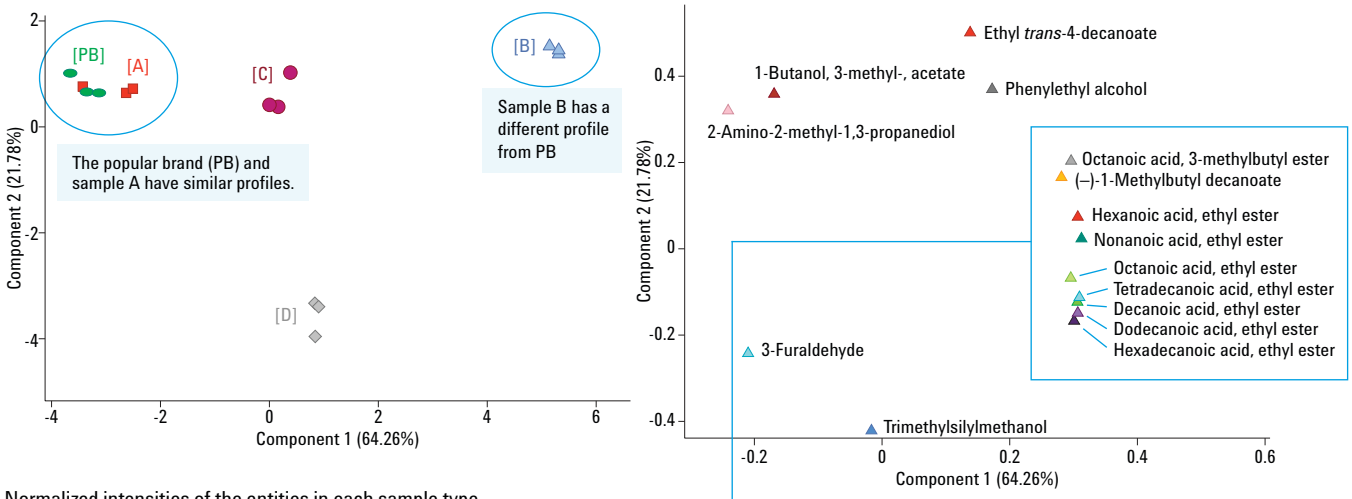
Figure 4. Fifteen high abundance compounds identified using MPP and filtered using ANOVA with a p-value < 5% to assure statistical significance.

In Component 1, sample B has a high positive score in the PCA Score Plot and sample A and PB have negative scores (Figure 5). The entities are located in the PCA Loading Plot (Figure 6) according to the loading of Components 1 and 2. In the PCA Loading Plot, the entities that are unique to sample B are placed on the positive loading of Component 1. By comparing the PCA Score Plot (Figure 6A) and the Loading Plot (Figure 6B), we can identify unique entities that differentiate the various whiskey samples. The table in Figure 6 shows the example of the entities that are unique to sample B, and these are the entities that provide sample B with a high score for Component 1, which is the x-axis in the PCA Loading Plot (Figure 5). Also, the relative peak intensities of the components of each group are characteristic of that group (Figure 6).



Component	Negative (–)	Neutral (near zero)	Positive (+)
1 (64%) X-axis	A, PB	C, D	B
2 (22%) Y-axis	D	–	A, B, C, PB
3 (11%) Z-axis	C	–	A, B, D, PB

Figure 5. PCA analysis of the 15 significantly relevant compounds in the high abundance group results in four distinctive groups of compounds that differentiate all of the samples except A and B.



Normalized intensities of the entities in each sample type

Retention time	Mass	Compound	[A]	[B]	[C]	[D]	[PB]
8.593	88	Decanoic acid, ethyl ester	10,945,965	91,484,704	15,420,839	43,557,596	9,160,368
7.246	88	Octanoic acid, ethyl ester	7,319,735	35,215,980	7,083,341	16,339,178	6,435,439
9.803	88	Dodecanoic acid, ethyl ester	1,770,075	31,862,326	3,835,627	14,279,053	1,428,005
10.901	88	Tetradecanoic acid, ethyl ester	188,006	3,107,707	483,733	1,213,085	183,026
11.906	88	Hexadecanoic acid, ethyl ester	300,003	2,559,052	741,498	1,686,823	343,726
5.724	88	Hexanoic acid, ethyl ester	840,595	2,161,864	991,785	1,130,796	855,511
8.721	70	Octanoic acid, 3-methylbutyl ester	132,939	1,301,459	366,510	160,848	99,822
7.937	88	Nonanoic acid, ethyl ester	708,628	1,281,332	674,696	716,060	619,252
9.912	70	(–)-1-Methylbutyl decanoate	45,598	1,048,773	303,444	187,453	35,299

Figure 6. PCA scores illustrate the separation of the four sample groups (upper score plots), and the relative normalized intensities of the components of each group are characteristic for that group (lower table). Some of the components in the PCA Loading Plot are overlapped because they have similar profiles. Red: very high intensity; Orange: high intensity; Yellow: moderate intensity; Green: low intensity.

Low and medium abundance entities

Fifty-four entities with peak intensities <1,000,000 were detected using Etune and identified using Mass Profiler Professional (MPP). Filtering these using ANOVA with a p-value <5% resulted in 31 compounds of statistical significance. Hierarchical Cluster Analysis (HCA) of these 31 again grouped the samples into four groups, PB+A, B, C, and D (Figure 7). In turn, the 31 compounds were classified by HCA according to the similarity of the normalized intensity profiles into eight clusters, which are shown in detail in Figure 8.

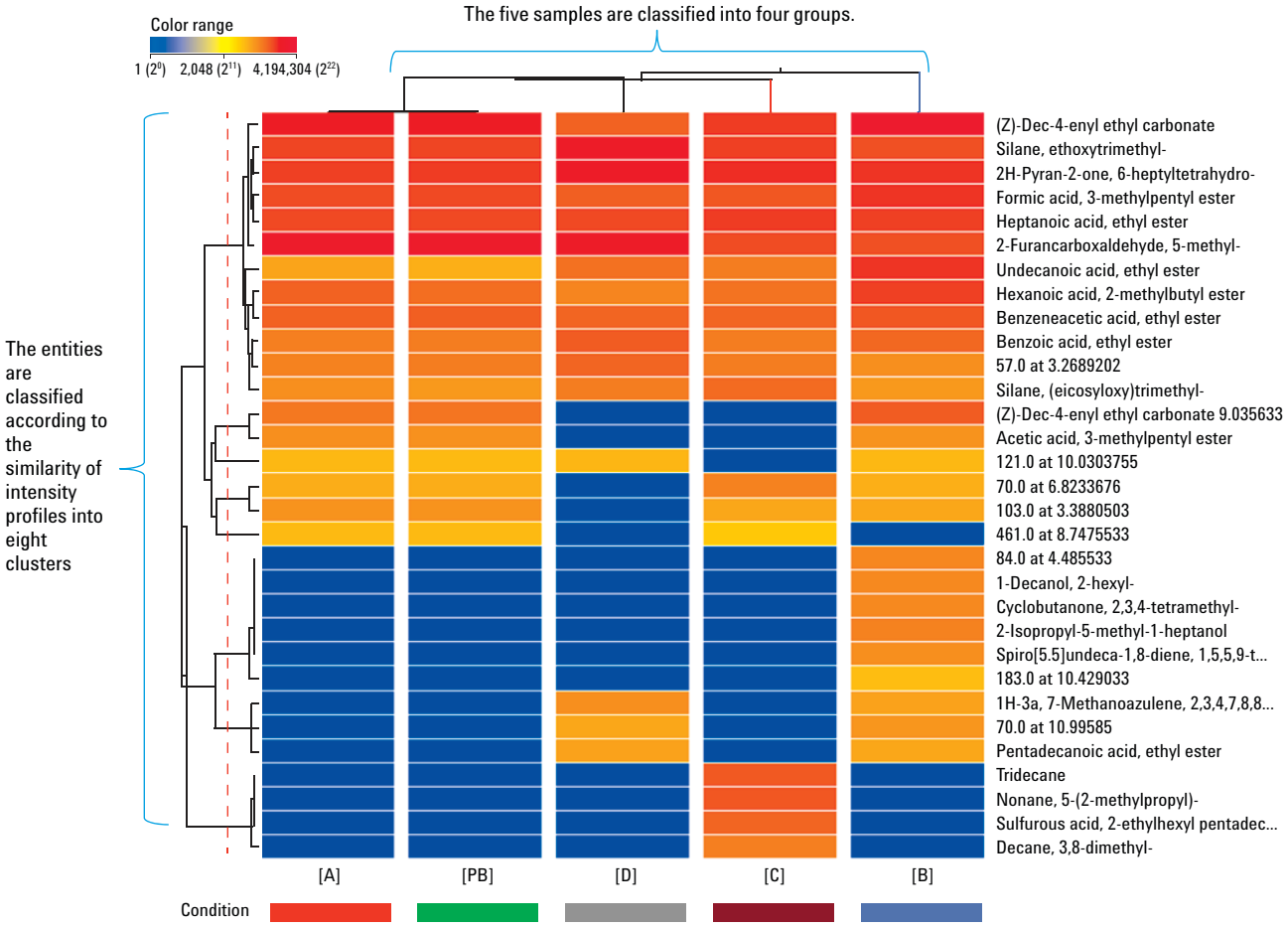


Figure 7. Classification of the samples and entities using those entities with normalized intensities < 1,000,000 and HCA. The samples were classified into four groups, and the entities were grouped into eight clusters according to profile similarity.

Of the 12 entities present in all five samples (Cluster 0), one was unidentified and another two were system blanks. The remaining nine were classified into three groups of distinguishable samples by entity intensity: PB+A, C+D, and B (Figure 9). The normalized intensities ranged from 14,000 to 500,000.

Samples B and D both contained three entities in Cluster 6 (Figure 10). One of these was pentadecanoic acid ethyl ester, which had been identified earlier in Sample D in the comparison of the Atune and Etune protocols. This compound was only found using Etune.

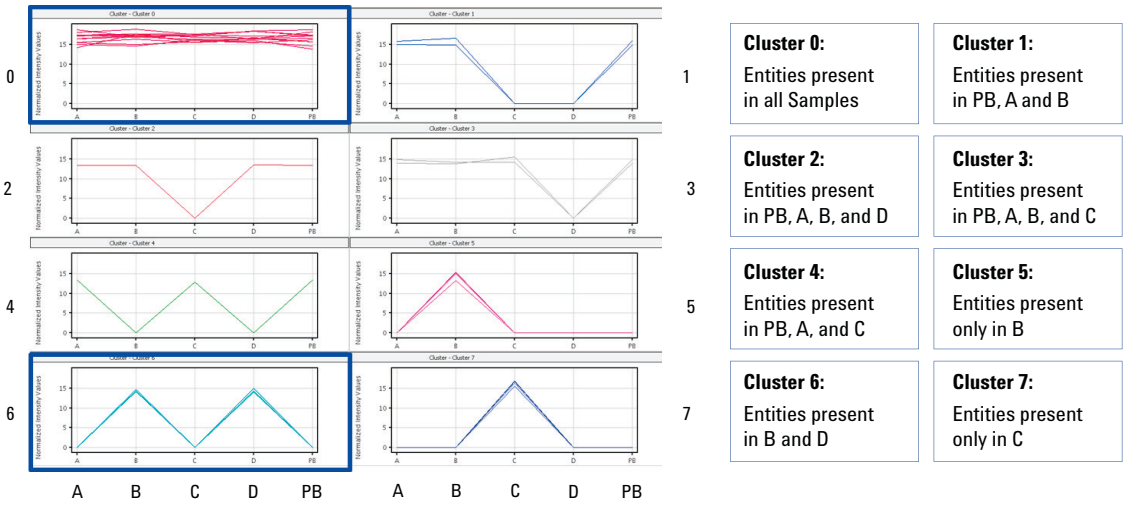


Figure 8. The low and medium abundance entities were classified into eight clusters, and the intensities of the entities in each cluster (y axis) were plotted against each sample group (x axis).

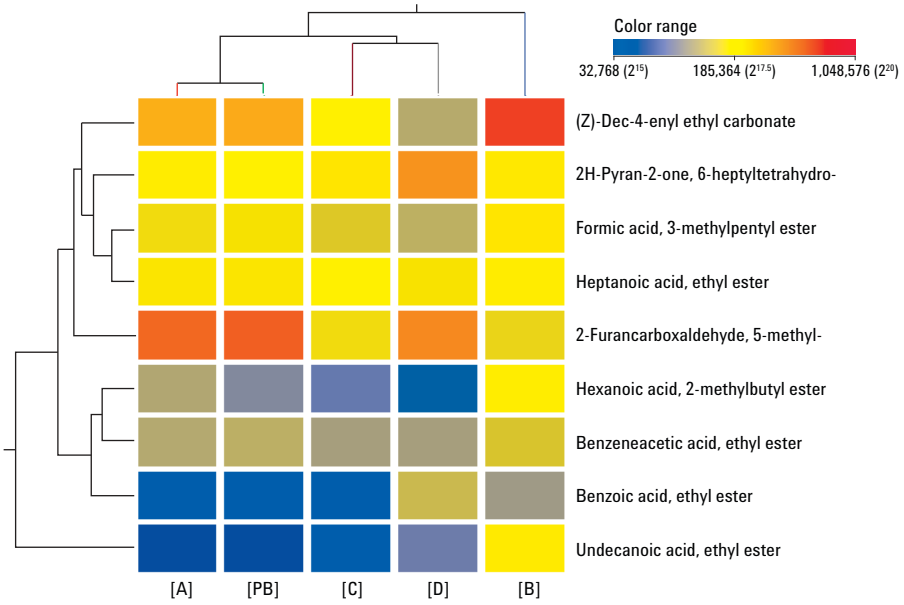
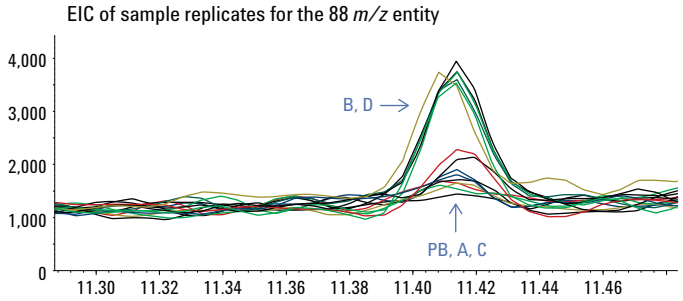


Figure 9. HCA analysis of the nine compounds identified as present in all five samples, resulting in three groups of samples classified by Cluster 0 compound intensity patterns: PB+A, C+D, and B.



Cluster 6 entities

Retention time	Mass	Compound	[A]	[B]	[C]	[D]	[PB]
8.383	119	1H-3a, 7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	0	20,890	0	33,060	0
10.996	70	70.0 at10.99585	0	28,591	0	17,961	0
11.415	**88**	**Pentadecanoic acid, ethyl ester**	**0**	**18,581**	**0**	**21,067**	**0**

Figure 10. Identities and normalized intensities of the Cluster 6 entities, as well as extracted ion chromatograms (EICs) for the replicate analyses of the five samples for the 88u entity (pentadecanoic acid, ethyl ester), showing its presence only in samples B and D.

Conclusions

This approach successfully generated chemometric profiles that resulted in the classification of the five whiskey samples into four groups, and could additionally be used to analyze for unintended contamination [3], optimization of product storage conditions, and determination of sample deterioration over time.

References

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3. T. Serino, "Detecting Contamination in Shochu Using the Agilent GC/MSD, Mass Profiler Professional, and Sample Class Prediction Models" Agilent Technologies Application Note 5991-0975EN.

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