UHPLC/MS Profiling of Nonvolatiles in Whiskeys Using the Agilent 6530 Accurate-Mass Q-TOF LC/MS and Mass Profiler Professional

Application Note

Food

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Abstract

Coupling UHPLC with accurate-mass Q-TOF mass spectrometry, combined with multivariate statistical analysis, has been demonstrated to be a promising tool for profiling whiskey. Using this technique, sets of compounds were identified that enabled discrimination among whiskey types, bourbon producers, and whiskey age.

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Introduction

Given the high value of the American whiskey industry to the local economies where it is produced, as well as to the US economy, producers are vulnerable to counterfeiting and other types of fraud. Process optimization is also a key driver for whiskey producers. An in-depth understanding of the chemical composition of whiskeys and the factors that can change it can help promote the efficient production of high quality product, and protect it from fraudulent practices.

A variety of analytical approaches has been used to study whiskey composition. Whiskey analysis methods should be rapid and avoid complex sample preparation, while enabling the separation and identification of large numbers of compounds. Ultra high pressure liquid chromatography/mass spectrometry (UHPLC/MS) using a quadrupole time-of-flight (Q-TOF) MS can enable such methods.

This application note describes a published study that used a nontargeted UHPLC/Q-TOF MS approach to evaluate the nonvolatile composition of a set of 63 bourbon, rye, Tennessee, and other American whiskeys [1]. This approach, when combined with multivariate statistical analysis using Mass Profiler Professional (MPP), enables optimal detection of compounds that differentiate multiple whiskey types. The Agilent 6530 Q-TOF LC/MS provides the mass accuracy required to determine chemical formulas, and the tandem mass spectrometry (MS/MS) capability to elucidate the structures of many compounds of interest. Using this approach, sets of compounds (≤ 40 compounds per set) were identified that provided differentiation among whiskey types, bourbon producers, and age of the whiskey. The method requires no sample preparation, and generates chromatographic separations in just 12 minutes, making it suitable for process optimization and quality control.

Experimental

Whiskeys

The 63 commercial whiskey samples analyzed in this study included 37 bourbons, 13 rye whiskeys, six Tennessee whiskeys, and seven other American whiskeys. Analysis was performed on the neat whiskeys, at their bottling proof. UHPLC/MS analysis was performed in triplicate and random order, and each sample was subjected to untargeted MS for profiling and Auto MS/MS analysis for marker compound identification.

Reagents and standards

Reagents and reference standards were obtained and used as described [1].

Instruments

This profiling approach was developed using an Agilent 1290 Infinity LC System coupled to an Agilent 6530 Q-TOF LC/MS. The HPLC and MS run conditions are shown in Table 1.

Table 1. Instrument Run Conditions

LC conditions			
Column	Agilent ZORBAX Eclipse Plus RRHD C18, 5 cm × 2.1 mm, 1.8 µm (p/n 959757-902)		
Column temperature	60 °C		
Injection volume	5 µL		
Mobile phase	A) 0.1 % Acetic acid in water B) 20 % A/80 % Methanol		
Flow rate	0.6 mL/min		
Gradient	Time (min)	% A	% B
	0.00	97	3
	1.00	97	3
	Linear gradient		
	9.00	0	100
	10.00	0	100
	11.00	97	3
	12.00	97	3
Total run time	12 minutes		
Q-TOF MS conditions			
Ionization mode	ESI, negative ion		
Acquisition mode	Profile and centroid		
Acquisition rate	3 spectra/sec		
Mass range	*m/z* 75–1,500		
N_2 Drying gas temperature	350 °C		
N_2 Drying gas flow	10 L/min		
Nebulizer pressure	45 psig		
Sheath gas temperature	400 °C		
Sheath gas flow	11 L/min		
Capillary voltage	3,000 V		
Nozzle voltage	1,000 V		
Q-TOF Auto MS/MS conditions			
Acquisition rate	3 spectra/sec each for MS and MS/MS		
Mass range	*m/z* 75–1,500 for single MS, *m/z* 50–1,450 for MS/MS		
Octopole 1 RF voltage	750 V		
Skimmer voltage	65 V		
Fragmentor voltage	175 V		
Collision energy	20 eV		
Isolation width	0.3 Da to the left of the precursor ion and 3.7 Da to the right of the precursor ion (medium)		
Precursor selection	2 at maximum abundance, threshold 200 cps		
Active exclusion on	After 2 spectra and 0.1 minutes		

Data analysis

Agilent MassHunter Qualitative Analysis Software, version 6.00, was used for the initial processing of the LC/MS data. Compounds were revealed using the Molecular Feature Extractor (MFE) tool in the software. Agilent Mass Profiler Professional (MPP) Software version 12.6.1 was used to align mass and retention time data across the samples within the set, and to perform the statistical analyses required to profile the samples, including principal component analysis (PCA). The Agilent Metlin Metabolite Personal Compound Database and Library (PCDL) version 5.0 was used to identify compounds based on the MS/MS spectra.

Results and Discussion

Identifying a set of compounds that can differentiate whiskey types

All whiskey samples were analyzed in triplicate. Using MFE to identify peaks and MPP to align masses and retention times revealed a set of approximately 7,600 compounds present in all whiskey samples. Screening out compounds that were not present in all of the replicates for a given sample reduced this number to 3,100. The next screening step eliminated compounds that were not present in all of the samples in at least one whiskey type, leaving 266 compounds. Selecting only those compounds with a minimum peak abundance level $\geq 1 \times 10^6$ counts reduced the set to 43 compounds. Conducting an analysis of variance (ANOVA) to limit the set to those compounds that varied at the $p < 0.05$ level across the sample set produced a final set of 40 compounds that were used for subsequent statistical analyses.

Comparison of bourbon, Tennessee, and rye whiskeys

A PCA was performed to differentiate bourbons, ryes, and Tennessee whiskeys. Since the American blended and craft whiskeys were well-differentiated from the other whiskeys, they were removed from this sample set (Figure 1). Most of the Tennessee whiskeys were separated from the bourbon and rye whiskeys, but a few were not, preventing the complete discrimination of Tennessee whiskeys from the other types. Some separation of rye whiskeys from bourbon whiskeys could be seen, and those rye whiskeys that were well-separated from bourbon whiskeys were primarily from smaller producers that focus on rye whiskey. Conversely, the rye whiskeys that were not well-separated from the bourbon whiskeys were from producers that also make bourbon whiskeys.

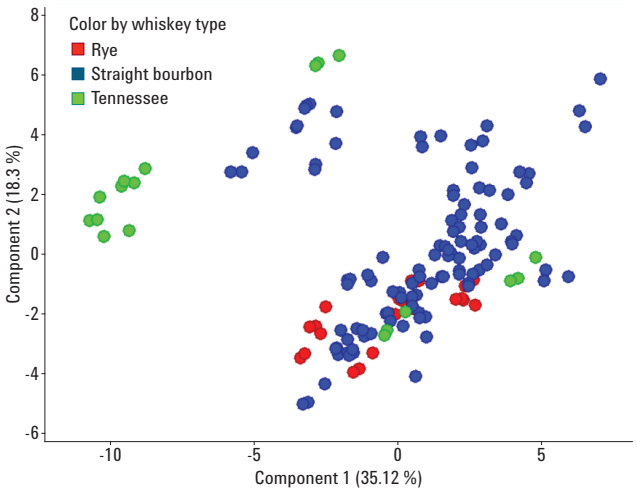

Figure 1. Two-dimensional (2D) PCA plot, generated by MPP, of 60 bourbon, rye, and Tennessee whiskeys, demonstrating partial differentiation of Tennessee whiskeys and rye whiskeys.

Comparison of bourbons from different producers

To use compounds best suited for characterization of the whiskeys of major bourbon producers, a set of 35 whiskeys from these producers was reanalyzed using MPP. These samples were filtered in the same manner as the original total set of samples. A 2D PCA plot demonstrated that only the bourbon whiskeys from Producer 4 were well differentiated from the other five major producers represented in the graph (Figure 2).

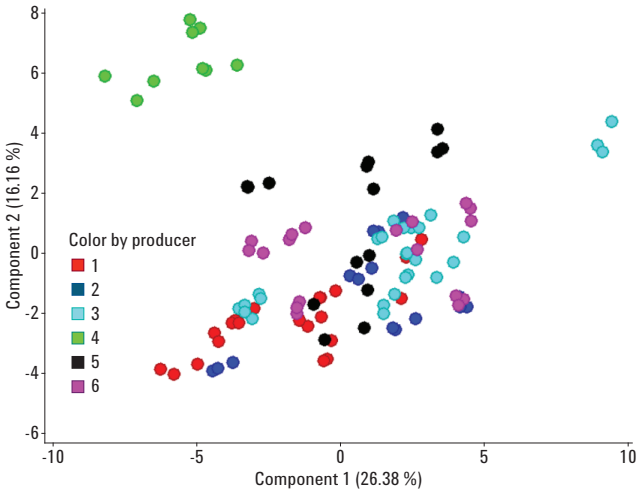

Figure 2. MPP-generated 2D PCA plot of 35 whiskeys from six bourbon producers, illustrating good separation of Producer 4 from the other whiskeys.

Differentiation of whiskeys by age

Whiskeys (33) from the major producers were placed into three categories by age: barreled four years or less at bottling, barreled between four and eight years, and barreled eight years or longer at bottling. Figure 3 shows the results of the PCA, using the averages of the three replicates for each whiskey. While the whiskeys of intermediate age (4 to 8 years) could not be totally differentiated from the oldest whiskeys, the youngest whiskeys (< 4 years) were differentiated from the others. The lack of total differentiation by age may be due in part to the difficulty of obtaining accurate age information on the various products. In particular, some of the whiskeys > 8 years may be blends of whiskeys of various ages.

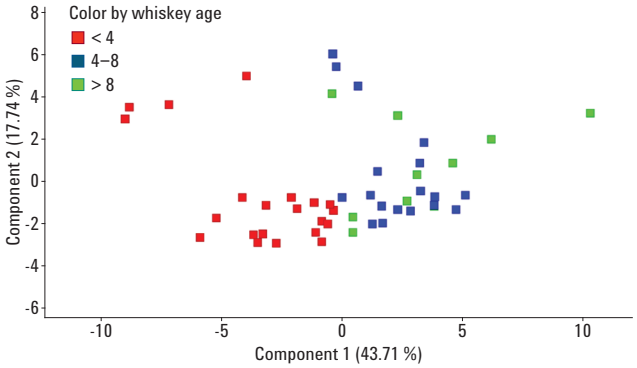

Figure 3. MPP-generated 2D PCA plot of whiskeys of various ages, indicating the ability to differentiate the less than 4 year old whiskeys from those older than 4 years.

Table 2 shows compounds associated with whiskeys of varying ages. Identification was made by performing an MS/MS library search of the Auto MS/MS spectra from the whiskey samples against the Metlin PCDL and comparison of MS/MS spectra with spectra in the literature [2]. Not surprisingly, wood-related compounds were associated with each of the age categories. The youngest whiskeys were characterized by short chain (C8–C12) lipids and fatty acids, and longer chain (C18, C20) fatty acids were associated with the oldest whiskeys.

Table 2. Compounds Associated with Whiskey Aging

Compound	Accurate mass	RT (min)	Basis for ID
Shorter aged whiskeys			
Octanoic acid	144.1097	7.30	Metlin MS/MS*
Decanoic acid	172.1459	8.56	Metlin MS/MS
Coniferaldehyde	178.0502	4.08	Metlin MS/MS
Diferulic acid	386.094	3.54	Metlin MS/MS
Longer aged whiskeys			
Syringaldehyde	182.0548	3.53	Metlin MS/MS
Ellagic acid	302.0035	4.52	Metlin + standard
Octadecanoic acid, dihydroxy- or hydro-peroxy	346.2327	7.25	Metlin MS/MS
Dodecanoic acid	200.1791	5.39	MacNamara†
Vanillin	152.0473	3.14	MacNamara
Unknown	518.3223	8.02	MacNamara

* The Agilent Metlin Personal Compound Data Base and Library (PCDL)

† See reference 2.

Conclusions

A nontargeted approach using UHPLC on the Agilent Infinity 1290 LC System coupled with high-resolution, high mass accuracy mass spectrometry on the Agilent 6530 Q-TOF LC/MS is effective for the profiling of bourbon, rye, Tennessee, and other American whiskey types. Multivariate statistical analysis using MPP enables differentiation among different whiskey types and characterization of the whiskeys from different producers and of varying ages. This suggests that this approach can be useful for the confirmation of authenticity of whiskeys of various types. The use of MS/MS analysis and spectral libraries including the Agilent Metlin PCDL facilitates the identification of the compounds that can be used for differentiation. These compounds could also be used as markers for routine monitoring in quality control programs.

References

1. T. S. Collins, J. Zweigenbaum, S. Ebeler. "Profiling of novolatiles in whiskeys using ultra high pressure liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF MS)." *Food Chem*. **163**, 186-96 (2014).
2. K. MacNamara, D. Dabrowska, M. Baden, N. Helle. "Advances in the ageing chemistry of distilled spirits distilled in oak barrels." *LC/GC* **14**, 6–22 (2011).

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