Analytical Quantification of Phenolic Compounds in Barrel-Aged Alcoholic Beverages as a Control Measurement in the Manufacturing Process

Application Note

Food Testing and Agriculture

Author

Karen Lorena López
Agilent Technologies, Inc.
Mexico City, Mexico

Abstract

This Application Note describes both HPLC and UHPLC methods to simultaneously separate and identify seven tannin compounds as aging markers in barrel-aged alcoholic beverages. The proposed methodology was applied to four different matrixes: whisky, brandy, rum, and tequila, to demonstrate a correlation between aging time and total tannin concentration level. Such a methodology could easily lead to an authenticity verification method to be implemented at the manufacturing process as a quality control measurement, preventing frauds or adulteration.

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Introduction

Different high-alcohol beverages can be obtained after aging in barrels. In the majority of cases, the beverages are defined with sensory attributes such as sharpness or bitterness. Therefore, aging in oak barrels is essential to give these beverages the sensory characteristics that consumers like. The interactions among components from wood and beverage result from different reactions such as polymerization, esterification, acetalization, hydrolysis, and oxidation; the initial product modifies its chemical composition and sensory characteristics such as visual aspects (color and limpidity), taste, and flavor. The identification and quantification of phenols is crucial due to their influence on the chemical composition, sensory characteristics of the resulting aged beverage, and as aging markers on their truthfulness as a nonadulterated drink.

The term tannin is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups to form strong complexes with various macromolecules. The astringency from tannins is what causes the dry and bitter feeling in the mouth following the consumption of unripe fruit or oak-aged alcoholic beverages. Likewise, the presence of tannins with time plays an important role in the aging of alcoholic beverages.

In aged alcoholic beverages such as wine, brandy, and tequila, the presence of low molecular weight phenolic compounds such as gallic acid, vanillin, syringaldehyde, sinapinaldehyde, coniferaldehyde, syringic acid, ferulic acid, esculetin, scopoletin, and furanic compounds has been reported.

During whisky, rum, brandy, and tequila aging, complex chemical changes occur in the oak casks. Wood constituents are extracted into the alcoholic matrix, providing it color and a characteristic taste. The aging step in tequila, for example, is one of the most susceptible to adulteration, as oak extracts or caramel coloring may be added to nonaged tequila during production to simulate the color of tequilas that have been aged in oak barrels. The occurrence of these compounds in alcoholic beverages is the result of oak lignin degradation and oxidation processes. This is considered as evidence that the alcoholic beverage has been aged authentically in an oak cask. By identifying the compounds that these alcoholic beverages acquire during aging, it may be possible to authenticate this production stage, and to elucidate sensory characteristics.

Experimental

Instrumentation

The Agilent 1290 Infinity II LC used for the experiments was comprised of the following modules:

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- Agilent 1290 Infinity II Multisampler with Sampler Cooler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector FS (G7117A) with 60 mm Max-Light cartridge cell (G4212-60007)

Software

Agilent OpenLAB CDS EZChrom Edition Version. A.04.07

Columns

- Agilent ZORBAX Eclipse Plus C18 4.6 × 150 mm, 3.5 µm (p/n 959963-902)
- Agilent ZORBAX Eclipse Plus C18 3.0 × 50 mm, 1.8 µm (p/n 959757-302)

Chemicals

All solvents were LC grade. Methanol and acetonitrile were purchased from J.T. Baker, US. Ultrapure water was LC grade purchased from J.T. Baker, US. Formic acid was from Agilent (p/n G2453-85060).

Samples and sample preparation

Seven tannin compounds were obtained as individual standards from Sigma-Aldrich: vainillic acid, caffeic acid, vainillin, *p*-coumaric acid, syringaldehyde, scopoletin, and synapaldehyde. Each tannin compound was mixed to reach a final concentration of 0.02 µg/mL, and labeled as the standard mix stock solution, from which four 1:10 serial dilutions were performed to finally obtain a four-level calibration curve. Alcoholic drink samples were directly placed into a vial for HPLC injection. Four different matrixes from a wide variety of brands and ageing time were submitted for comparison: whisky, rum, brandy, and tequila.

Chromatographic conditions

The separation was achieved using a ternary linear elution gradient with (A) 0.1 % formic acid in water (v/v), (B) methanol, and (C) acetonitrile. Table 1 shows the chromatographic conditions for the HPLC gradient, and Table 2 shows the chromatographic conditions after method optimization to UHPLC.

Table 1. Chromatographic conditions, HPLC gradient.

Parameter	Value			
Long gradient				
Mobile phases	A) 0.1 % Formic acid in water B) Methanol C) Acetonitrile			
Ternary gradient	Time (min)	%A	%B	%C
	0.00	94.00	3.00	3.00
	1.00	94.00	3.00	3.00
	25.00	84.00	8.00	8.00
Stop time	40 minutes			
Post time	4 minutes			
Flow rate	1 mL/min			
Injection volume	1 µL			
Sample temperature	19 °C			
Column temperature	35 °C			
DAD	290 nm/4 nm, Ref.: 360 nm/100 nm			
Peak width	> 0.1 minutes (2 second response time) (2.5 Hz)			

Table 2. Chromatographic conditions, UHPLC gradient.

Parameter	Value			
Short gradient				
Mobile phases	A) 0.1 % Formic acid in water B) Methanol C) Acetonitrile			
Ternary gradient	Time (min)	%A	%B	%C
	0.00	94.00	3.00	3.00
	0.25	94.00	3.00	3.00
	7.00	84.00	8.00	8.00
Stop time	9 minutes			
Post time	2 minutes			
Flow rate	0.850 mL/min			
Injection volume	1 µL			
Sample temperature	19 °C			
Column temperature	35 °C			
DAD	290 nm/4 nm, Ref.: 360 nm/100 nm			
Peak width	> 0.1 minutes (2 second response time) (2.5 Hz)			

Results and Discussion

A mix of seven tannin standards was separated using an HPLC ternary gradient. The relative standard deviation (RSD) of retention time (RT) and area were evaluated over seven subsequent runs. Figure 1 shows the results of the separation. The analysis showed excellent precision of retention time and area. The RSD for RT was < 0.3 for all analyzed phenol standards. The area RSD was < 1.5 for all tannin standards.

To optimize the gradient duration, the method was transferred to a UHPLC sub-2 µm column (Agilent ZORBAX Eclipse Plus-C18, 3.0 × 50 mm, 1.8 µm). With this column, it was possible to reduce the gradient time down to approximately nine minutes while maintaining excellent, even partly improved, resolution. Figure 2 displays the UHPLC analysis together with the precision results for retention time and area. The RSD for RT was < 0.160 for all analyzed tannin standards. The area RSD was < 0.934 for all tannin standards.

The standard mix solution was diluted 1:10 from 0.2 µg/mL down to 0.02 µg/mL to obtain the calibration curve. Table 3 shows the results of the evaluation for the UHPLC method, which was the method defined to perform the complete analysis to optimize resources.

Four different alcoholic beverages were analyzed. The tannin (phenolic) content was quantified by an external standard method. Data analysis and comparison to elucidate the tannin behavior was carried out per each matrix.

Rum shows a low tannin concentration level in the early years of aging; total concentrations of < 2.0 µg/mL were obtained for rum samples with less than 10 years age. However, two different 30-year aged rum samples were analyzed, and resulted in high concentrations, with 9.828 and 12.357 µg/mL.

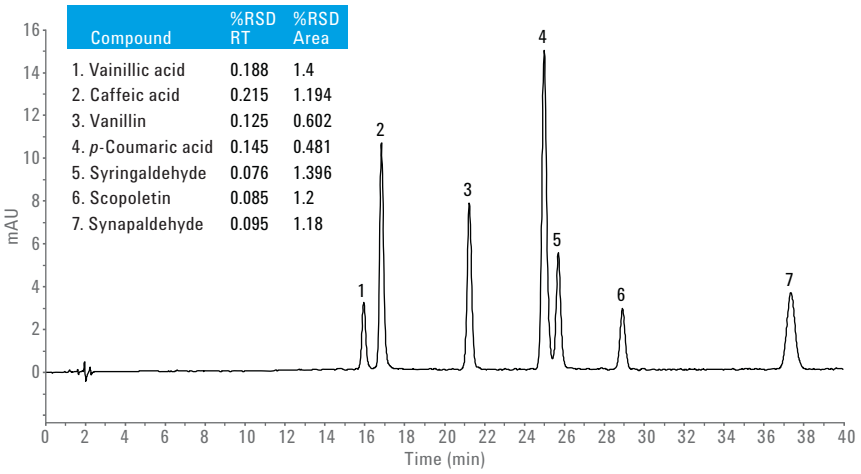

Figure 1. Chromatogram obtained by HPLC gradient for the standard mix sample where the seven tannin compounds are separated.

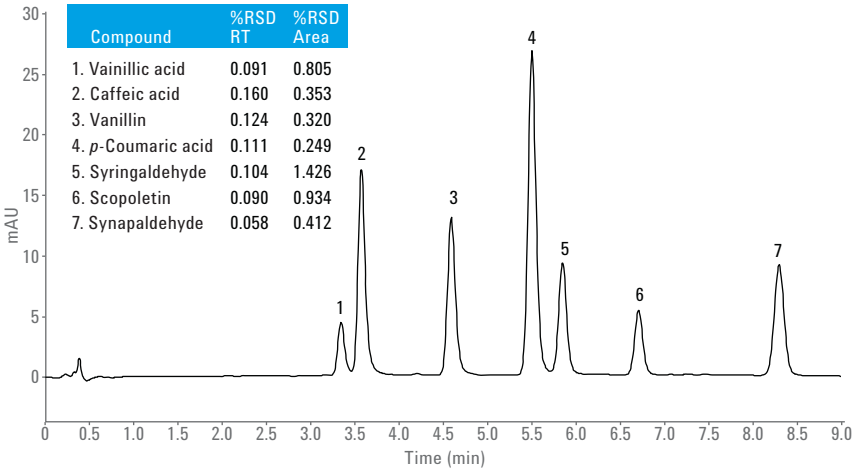

Figure 2. Chromatogram obtained by an optimized UHPLC gradient for the standard mix sample where the seven tannin compounds are separated.

Table 3. Linearity results for UHPLC conditions.

Tannin compound	R^2 UHPLC
Vanillic acid	0.99906
Caffeic acid	0.99944
Vanillin	0.99941
p-Coumaric acid	0.99950
Syringaldehyde	0.99941
Scopoletin	0.99882
Synapaldehyde	0.99936

Similar results were obtained for brandy samples, which did not present high tannin concentration until reaching more than 10-years aging. Two samples from the same brandy manufacturer (submitted to the same aging conditions and procedure) were analyzed: 10 years and 20 years; the tannin quantification analysis showed an increment (10x) between both samples: 1.002 µg/mL for 10 years and 11.873 µg/mL for 20 years.

Tequila samples, however, are classified according to the percentage of sugars used during the fermentation process, but can also be classified according to aging time in oak casks. In this way, the silver variety or tequila *blanco* is the name for unaged tequila; aged tequila or tequila *reposado* is tequila that has been aged for at least two months in oak casks; and extra-aged tequila or tequila *añejo* is tequila that has been aged for at least one year in oak casks.

From this perspective, the three varieties of tequila were analyzed, showing a correlated behavior according to aging time. Tannin quantification results for tequila *blanco* are discarded due to quantification level less than the LOD. However, tequila *reposado* resulted in concentrations > 0.5 µg/mL, while results for extra-aged tequila *añejo* showed a significant increase, with tannin concentration levels between 9.0 and 15.0 µg/mL, depending on the oak cask. It was not the purpose of this study to establish any analytical tests for verifying the authenticity of tequila type or age. Nevertheless, it has recently detected the unethical practices of adding oak extracts or caramel coloring to tequila blanco to produce fake aged tequilas, for which they can increase price.

Finally, different aged whisky samples were submitted to analysis. In this particular matrix, it was imperative to know the barrel origin and characteristics to elucidate proper conclusions, since the aging time is not the only variable to consider as part of the whisky characterization. For example, different brands of blended 12-years aged whisky were analyzed, resulting in a total tannin quantification range from 1.7 to 8.7 µg/mL. In addition, barrel-type investigation and the fact that these were blended whisky samples, made it impossible to find a direct relation between quantification and aging time. Blended whisky refers to a mix of distillates submitted to different aging procedures.

However, a series of 12, 15, 18, and 30 years-aged single-malt whisky samples from the same manufacturer were submitted for analysis. Each one of the samples came from the same barrel type (American type, previously filled with sherry), so in this case it was possible to consider the aging time as the only variable while forming conclusions. Additional information was obtained to consider a sensorial analysis comparison according to tannin concentration. Total quantification results were as follows:

- 12-years aged – 5.325 µg/mL
- 15-years aged – 9.339 µg/mL
- 18-years aged – 10.586 µg/mL
- 30-years aged – 12.588 µg/mL

It is possible that a correlation between total tannin concentration and aging time can be defined according to this data.

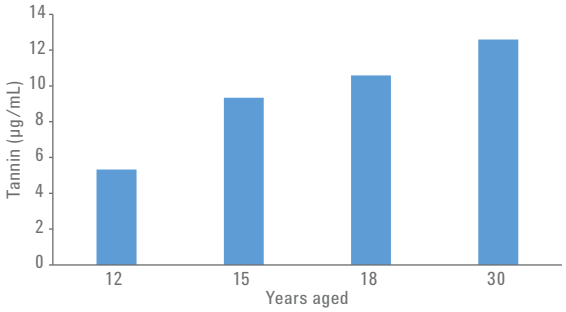

Figure 3. Results for the calculated total phenolic content in four whisky samples – same manufacturer, aging process, and barrel type.

Additionally, two special edition whisky samples from the same manufacturer were analyzed, 19-year aged and 21-year aged, resulting in a 5.297 µg/mL total tannin concentration. It is clearly a lower response than the 18-year aged sample analyzed. When information from the barrel type was retrieved, it was possible to identify a variation, since the barrel used in the aging process of the special edition samples were previously filled with Caribbean rum rather than sherry. This might explain the behavior, and makes it impossible to perform a comparison.

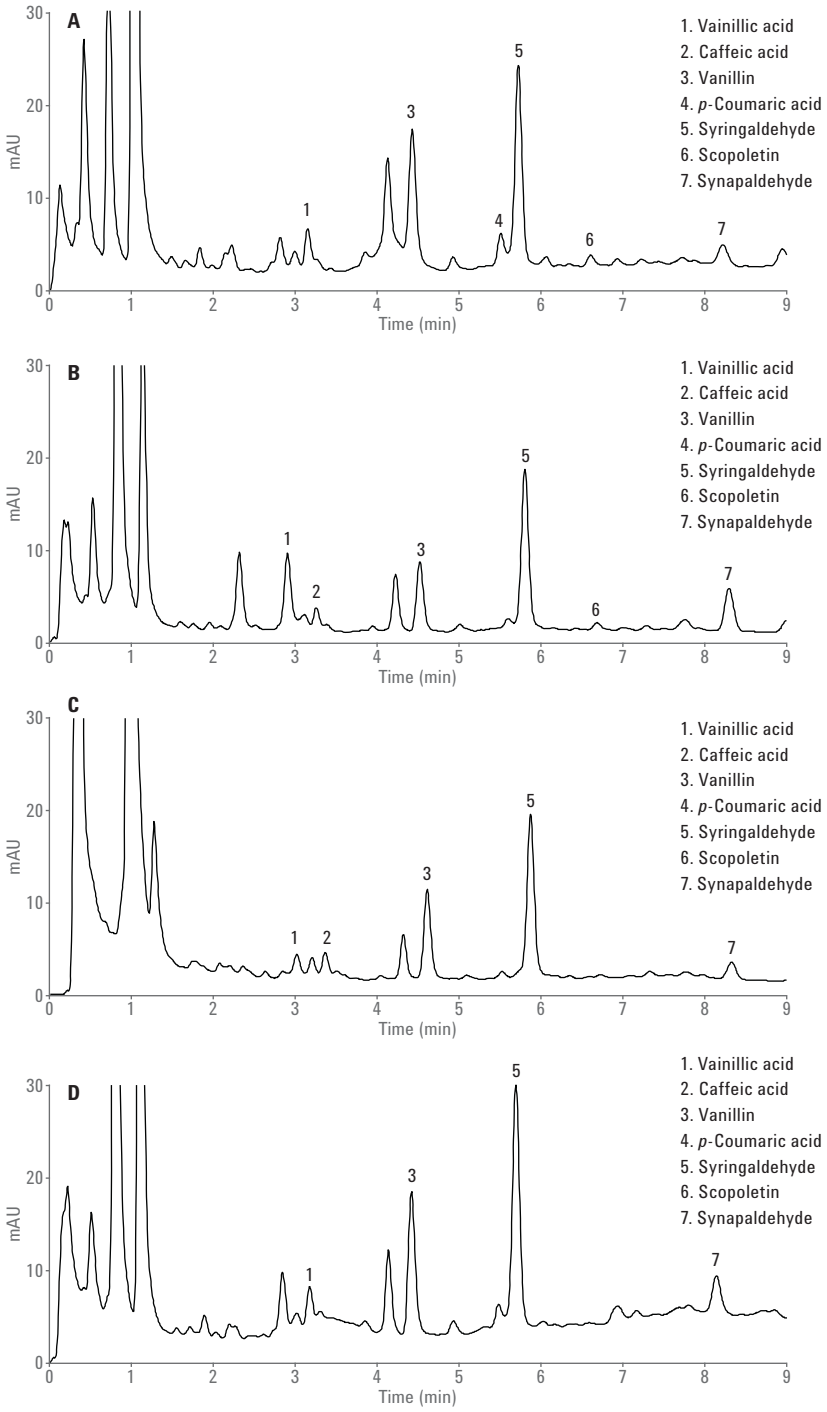

Figure 4. Chromatograms of four different barrel-aged alcoholic beverages with high tannin content: A) whisky 30-year aged, B) tequila añejo, C) rum 30-year aged, and D) brandy 20-year aged.

Conclusion

An analytical methodology for the analysis of tannin (phenolic) compounds in four different alcoholic beverages was developed for both HPLC and UHPLC using ternary gradients with the Agilent 1290 Infinity II LC, which can easily support both conventional and RRHD columns up to 1,300 bar. Both methods revealed excellent retention time and area precision, as well as excellent linearity for the analysis of tannin standard compounds. The UHPLC method resulted in considerably shorter run times while maintaining the same, or even improved, results. In addition, it was possible to save solvent consumption by reducing the diameter of the column from 4.6 to 3 mm. For further separation and characterization analysis, the Agilent 1290 Infinity II 2D-LC solution can be considered in addition to the inclusion of additional individual standards.

A total of 18 samples from rum, brandy, tequila, and whisky were analyzed using the UHPLC method. Each matrix showed particular quantification results according to other variable considerations, such as barrel type.

The correlation found between aging time and total tannin concentration level by this method could lead to an authenticity verification method to be implemented at the manufacturing process as a quality control measurement. This test could be used to uncover frauds in tequila, an alcoholic drink that is one of the most susceptible to adulteration. Monitoring abnormal concentrations of tannin compounds in known alcoholic sample chromatographic runs might be a starting point when searching for evidence of inconsistencies in the production processes of alcoholic beverages.

References

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