Profiling Flavonoids in Whiskey and Related Spirits with HPLC UV-MS Using a C18 Column

Best Poster Award Participant

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Introduction

The flavor and sensory characteristics of distilled spirits are strongly influenced by phenolic and aromatic compounds derived from grain composition, fermentation, and oak barrel aging. These molecules serve as chemical markers of production method and spirit style, making chromatographic profiling a useful tool for comparative analysis across whiskey categories.

In this work, reversed-phase HPLC was used to analyze 19 commercially available distilled spirits, including bourbons, American whiskeys, Japanese whiskeys, and Canadian blended whiskeys, using a YMC Triart C18 stationary phase. An initial screening targeted seven flavor-active phenolic compounds associated with oak aging: vanillic acid, vanillin, syringaldehyde, *p*-coumaric acid, scopoletin, sinapaldehyde, and ellagic acid. Two compounds were not observed in authentic spirit samples and were excluded from further evaluation.

Chromatographic fingerprints and relative phenolic distributions were compared across spirit types to assess compositional trends and method reproducibility. The results demonstrate the suitability of reversed-phase HPLC for profiling flavor-relevant phenolics in complex distilled spirit matrices and highlight the applicability of Triart C18 columns for comparative whiskey analysis.

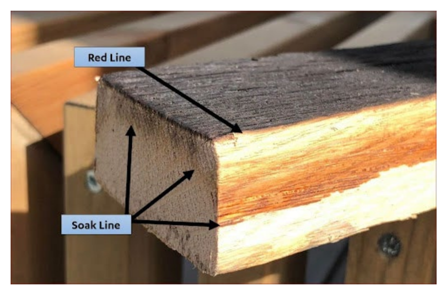
HPLC Running Conditions

Column:	Triart 3 µm C18
Dimensions:	100 x 4.6 mm
Part No.:	TA12S03-1046WT
Mobile Phase:	A: 20mM Ammonium Formate pH 3.0 B: 50:50 Acetonitrile: Absolute Ethanol

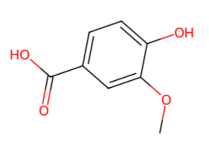
Gradient: Time (min)	% B
0.0	2
3.0	2
15.0	30
23.0	42
26.0	54

Flow Rate:	1.0 mL/min
Injection Volume:	10 µL
Temperature:	25 °C
LC System:	Agilent 1290 Binary
Detection:	UV @ 290nm

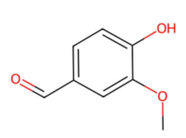
Phenolic Compounds of Interest



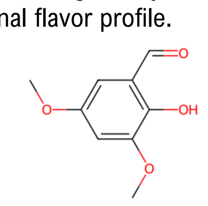
Barrel charring drives much of whiskey's phenolic character by thermally transforming the oak. The char layer forms carbonized material at the surface, while the underlying soak line marks where the spirit penetrates the wood during aging. Heat-induced lignin degradation generates phenolic compounds such as vanillin and related aldehydes, which are extracted into the whiskey over time. The char layer also acts as a natural filter and reaction zone, contributing smoky, vanilla, caramel, and spice notes that define the whiskey's final flavor profile.



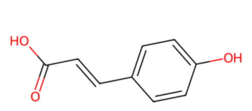
Vanillic Acid



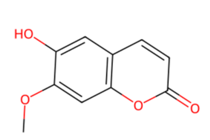
Vanillin



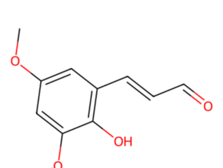
Syringaldehyde



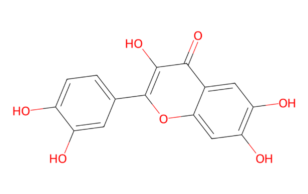
p- Coumaric Acid



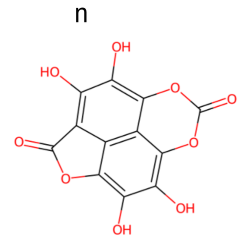
Scopoletin



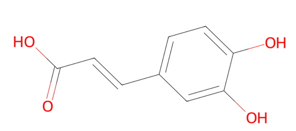
Sinapaldehyde



Quercetin

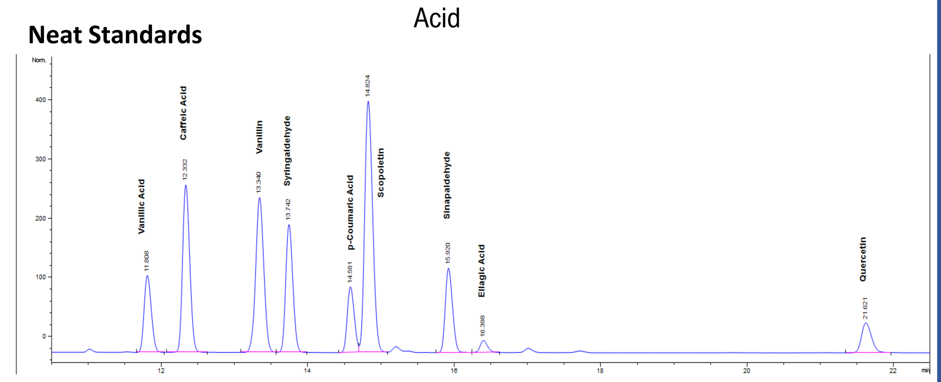


Ellagic Acid



Caffeic Acid

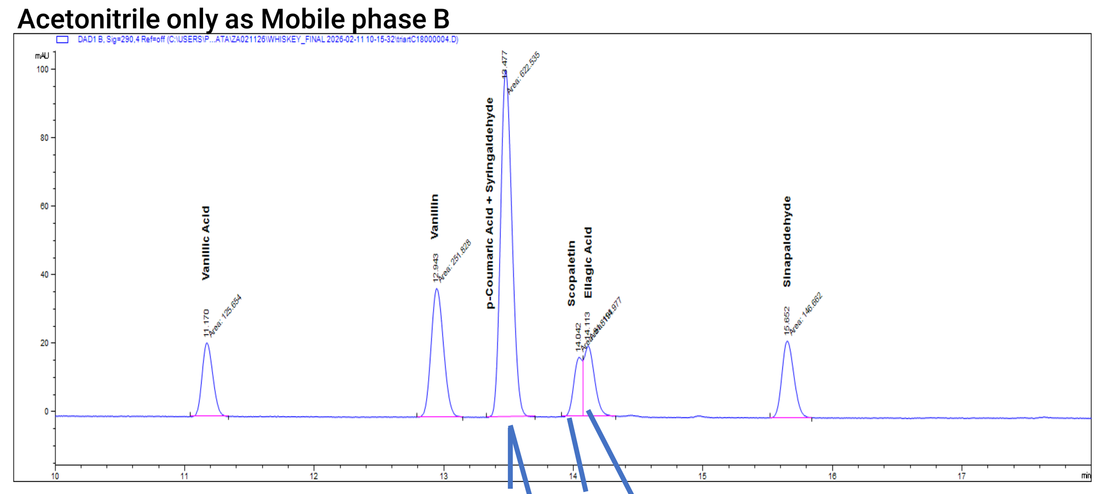
Neat Standards



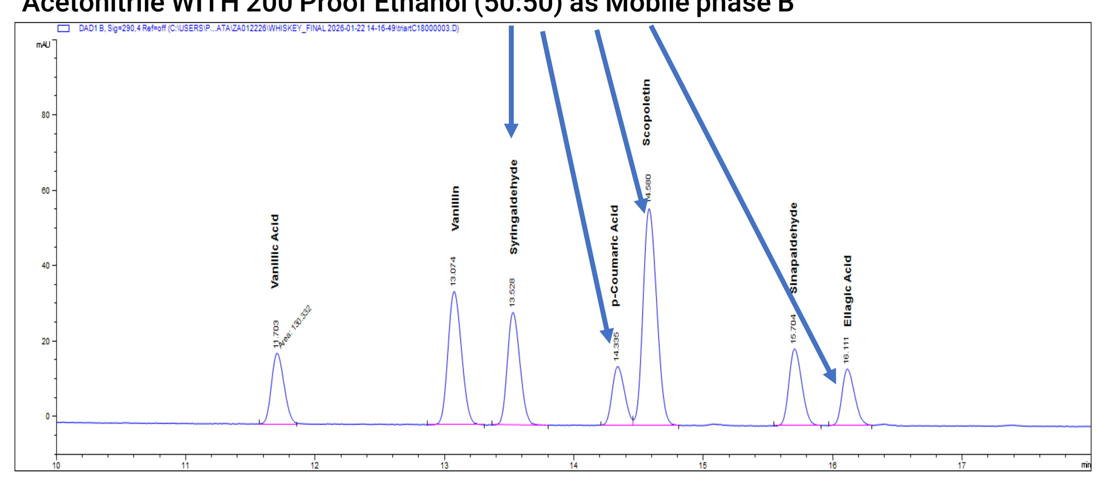
Nine phenolic standards commonly associated with whiskey maturation were analyzed to establish retention times and confirm peak identity. In commercial whiskey samples, seven of these markers were consistently observed and used to generate comparative phenolic fingerprints.

Acetonitrile vs. Acetonitrile:Absolute Ethanol

Acetonitrile only as Mobile phase B



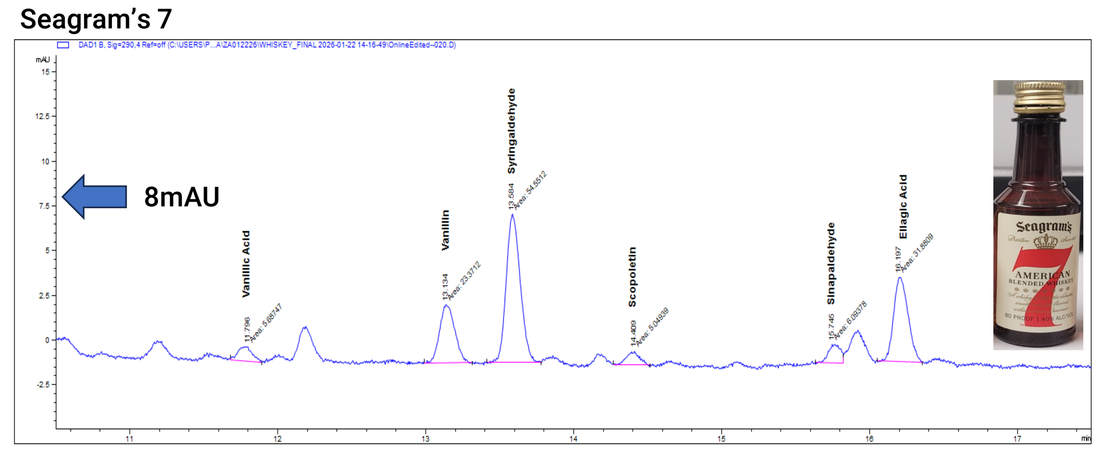
Acetonitrile WITH 200 Proof Ethanol (50:50) as Mobile phase B



When acetonitrile was used as the sole strong solvent, the Triart C18 stationary phase did not provide sufficient selectivity to resolve closely eluting phenolic compounds. Replacing the strong solvent with a 50:50 Absolute ethanol:Acetonitrile mixture altered solvent polarity and hydrogen-bonding interactions, enhancing differential interactions between phenyl-containing analytes and the hybrid silica C18 surface. The presence of ethanol modulated π–π and secondary interactions, resulting in improved selectivity and increased resolving power for structurally similar phenolic species.

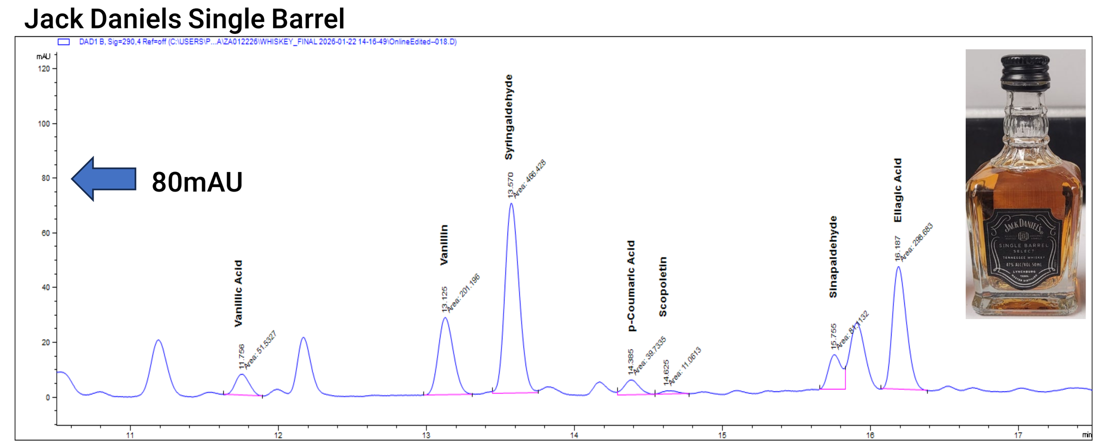
Different interesting Whiskey samples

Seagram's 7



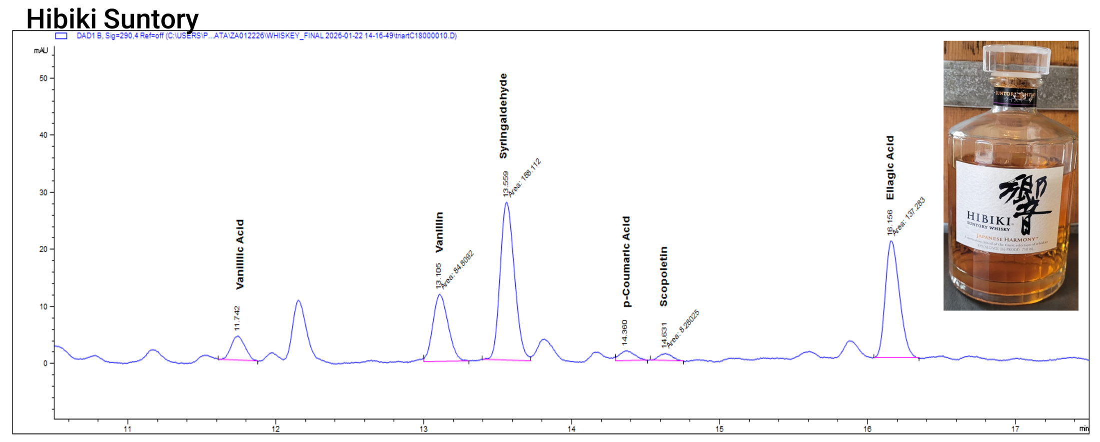
Seagram's 7 exhibited the **lowest total phenolic content** among the analyzed whiskies, reflecting minimal extraction of oak-derived phenolic compounds. This is consistent with its classification as a blended whiskey, which typically relies on refill or previously used barrels rather than newly charred oak, resulting in reduced availability of lignin- and tannin-derived phenolics.

Jack Daniels Single Barrel



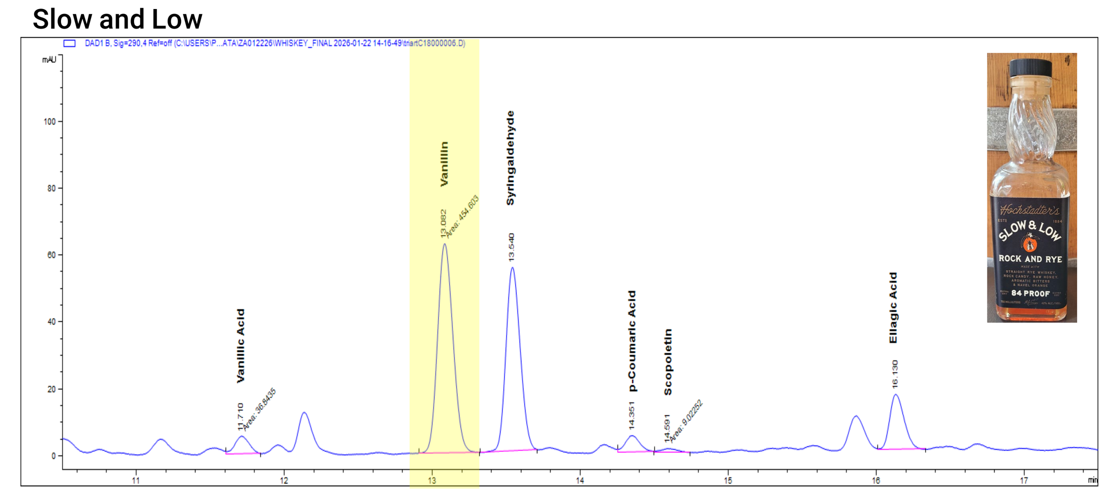
Jack Daniels Single Barrel exhibited the **highest total phenolic content** among all samples, indicating extensive extraction of oak-derived compounds during maturation. This elevated phenolic load is consistent with the use of newly charred American oak barrels, which promote the release of lignin-derived aldehydes and hydrolyzable tannins. The single-barrel format further amplifies this effect by preserving barrel-to-barrel variability and often representing more intensely extracted casks. **Nearly 10x higher phenolic concentration!**

Hibiki Suntory



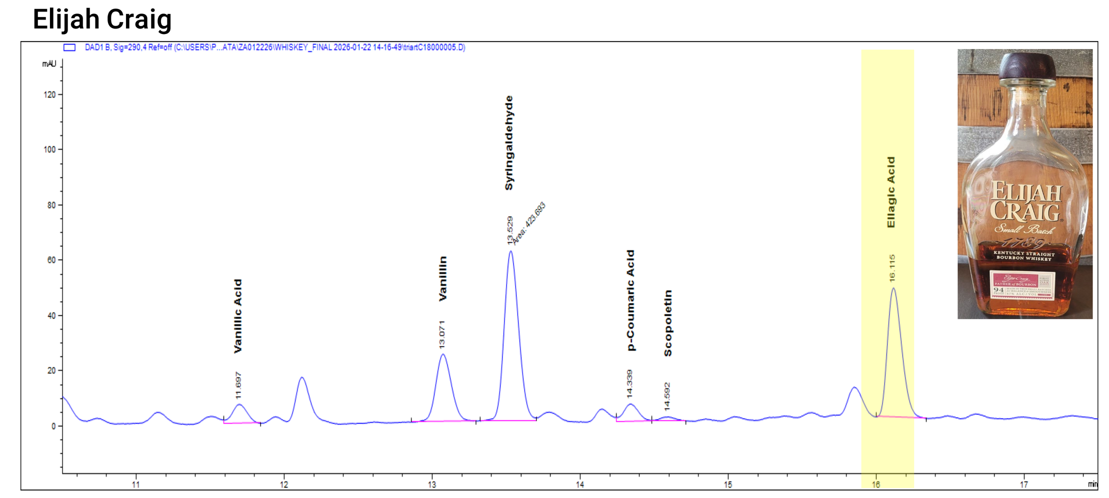
Hibiki Suntory exhibited a moderate but diverse phenolic profile, reflecting **balanced extraction** rather than dominance by a single compound class. This is consistent with its use of multiple cask types—including American oak, sherry casks, and Japanese mizunara oak—which collectively contribute to phenolic diversity without the intensity seen in heavily charred, single–oak maturation programs.

Slow and Low



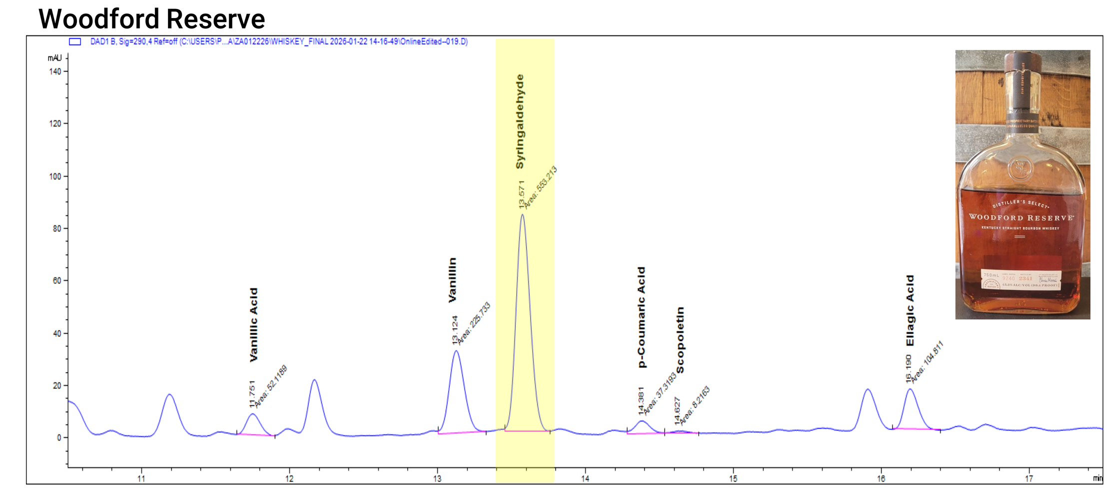
Slow & Low showed the **highest vanillin** concentration among the whiskies analyzed. As a blended rye–bourbon, this likely reflects targeted barrel selection or blending choices that emphasize vanilla-forward, lignin-derived flavor contributions.

Elijah Craig



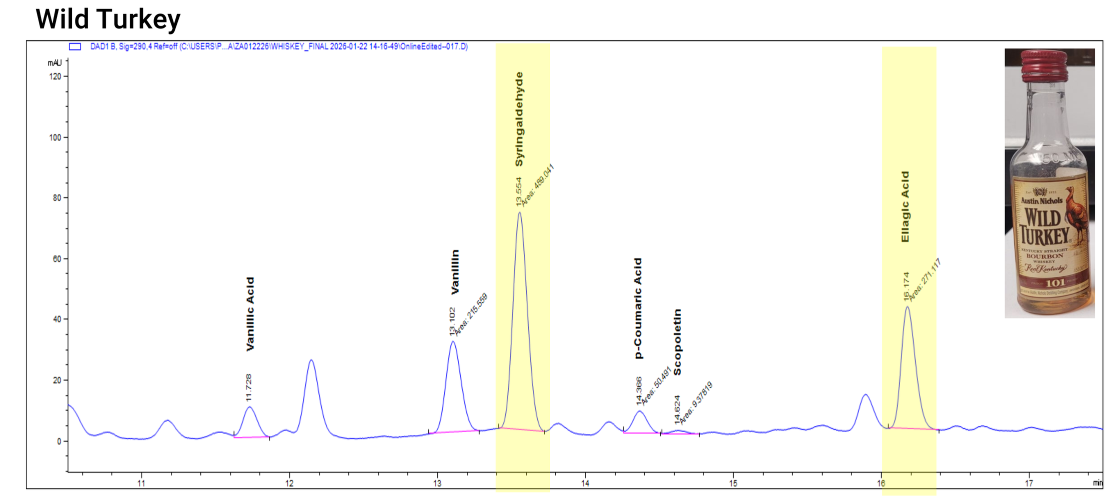
Elijah Craig exhibited the **highest ellagic acid** concentration among the analyzed whiskies, indicating substantial extraction of oak-derived tannins. This is consistent with maturation in new charred American oak barrels, which are rich in ellagitannins that hydrolyze during aging to form ellagic acid.

Woodford Reserve



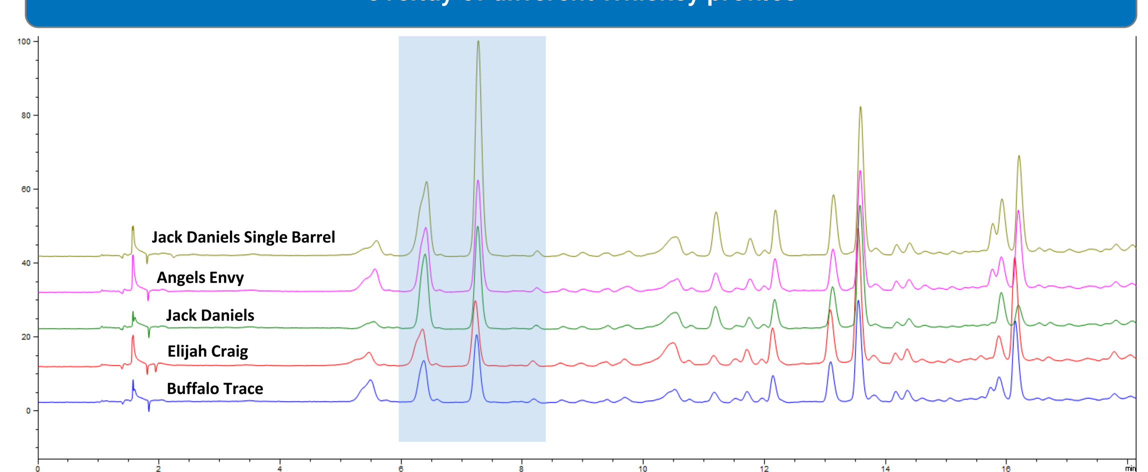
Woodford Reserve showed the **highest syringaldehyde** concentration among the samples, highlighting pronounced lignin-derived extraction during aging. As a bourbon matured in new charred American oak, this profile is consistent with extensive thermal degradation of lignin during barrel charring, leading to elevated aromatic aldehydes.

Wild Turkey



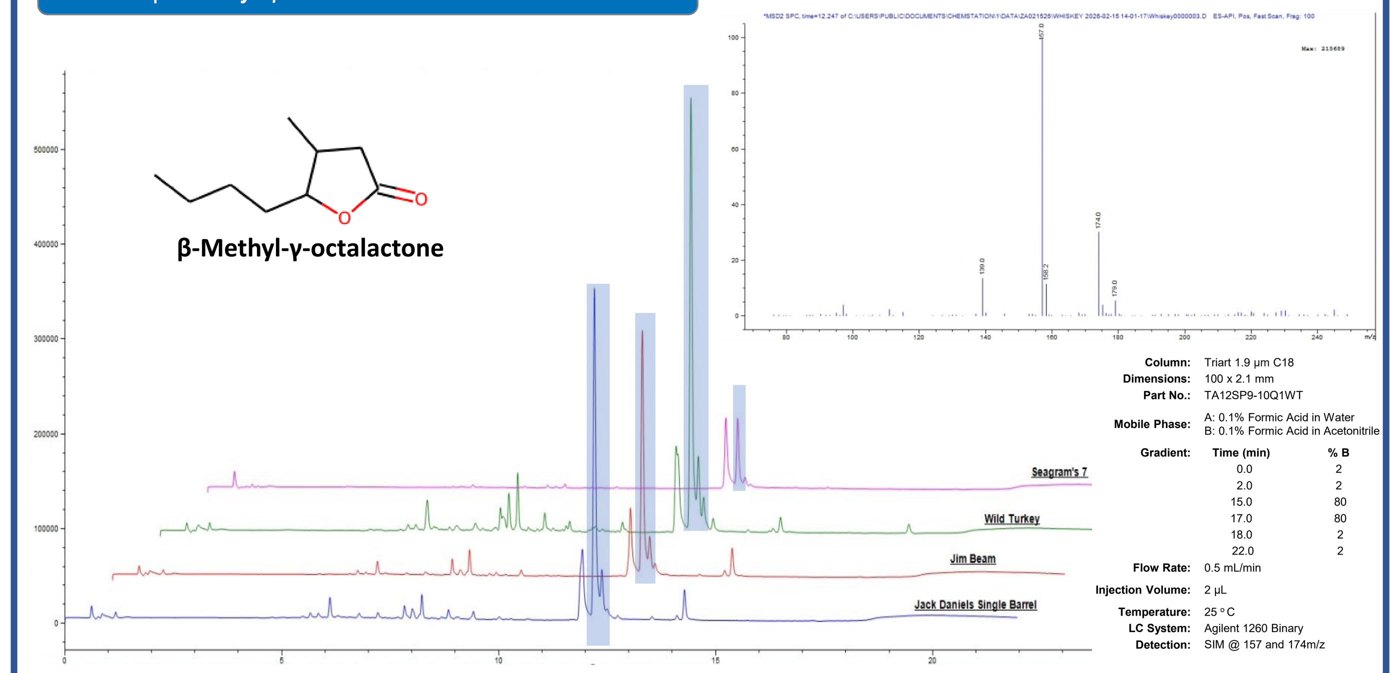
Compared to Woodford Reserve, which is strongly characterized by syringaldehyde-driven lignin extraction, Wild Turkey exhibits a broader oak-derived profile by pairing similarly high **syringaldehyde** levels with elevated **ellagic acid**, reflecting a more tannin-forward and structurally intense maturation character.

Overlay of different Whiskey profiles



The overlaid chromatograms show clear differences in phenolic composition between the whiskey brands, reflecting their unique flavor profiles. Variations in peak intensity indicate differing levels of oak-derived compounds that contribute to the characteristic taste and aroma of each whiskey. There are other smaller esters and lactones that elute between 6 and 8mins that provide fruity, maple and floral notes.

β-Methyl-γ-octalactone LC/MS Data



Column:	Triart 1.9 µm C18
Dimensions:	100 x 2.1 mm
Part No.:	TA12SP9-10Q1WT
Mobile Phase:	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Acetonitrile

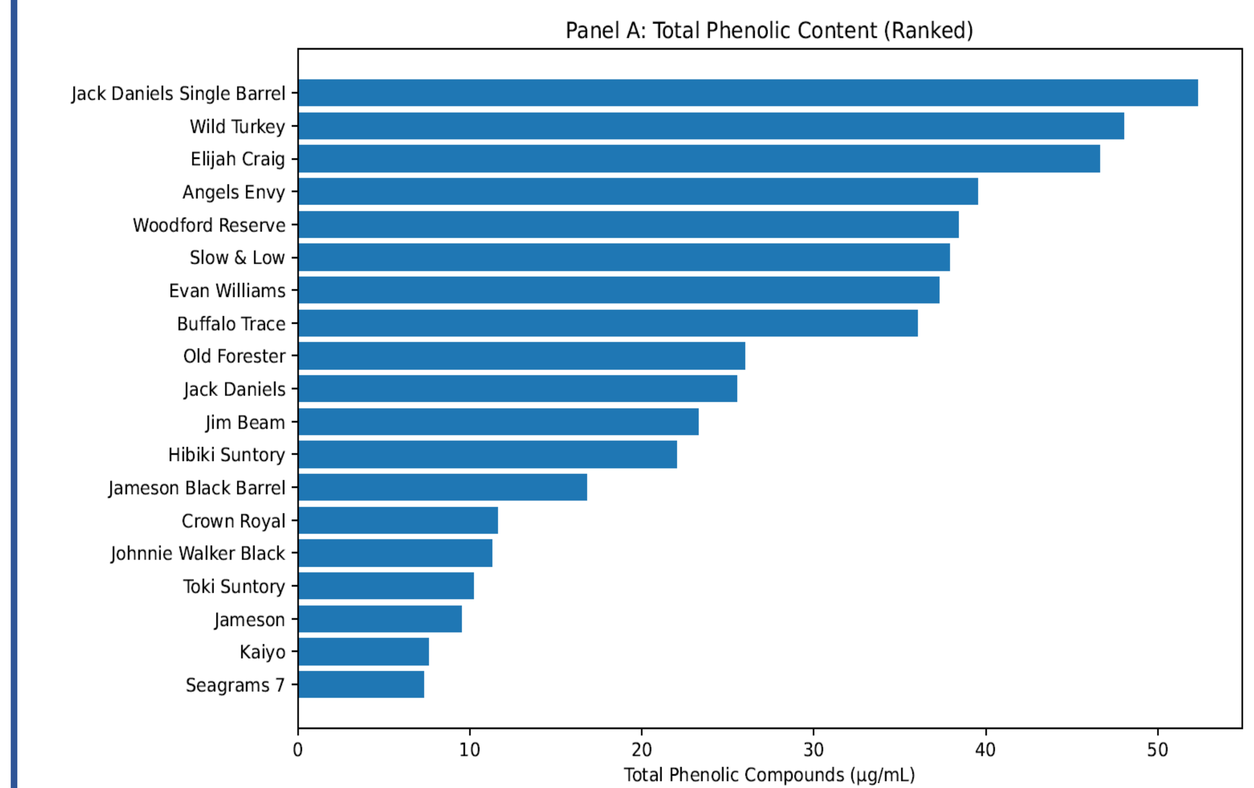
Gradient: Time (min)	% B
0.0	2
2.0	2
15.0	80
17.0	80
18.0	2
22.0	2

Flow Rate:	0.5 mL/min
Injection Volume:	2 µL
Temperature:	25 °C
LC System:	Agilent 1260 Binary
Detection:	SIM @ 157 and 174m/z

β-Methyl-γ-octalactone (often referred to as the "whiskey lactone") is a key flavor contributor derived primarily from oak during barrel aging and is strongly associated with coconut, woody, and sweet aromatic notes that define many bourbon and American whiskey profiles. Higher concentrations typically reflect greater oak extraction, new charred barrel use, and longer or more aggressive maturation conditions, while lower levels may indicate lighter barrel impact, reuse of cooperage, or blending practices. Because this compound has a weak UV chromophore and is difficult to detect using conventional LC-UV methods, LC/MS was used to achieve sensitive and selective detection in our workflow. Among the four whiskeys evaluated, **Wild Turkey showed the highest concentration of β-Methyl-γ-octalactone**, followed by Jack Daniel's Single Barrel, then Jim Beam, while **Seagram's 7 exhibited the lowest level**, consistent with its lighter barrel influence and blended production style.

Results and Discussion

Panel A: Total Phenolic Content (Ranked)



Concentration µg/mL	Seagrams 7	Kaiyo	Jameson	Toki Suntory	Johnnie Walker Black	Crown Royal	Jameson Black Barrel	Hibiki Suntory	Jim Bean	Jack Daniels	Old Forester	Buffalo Trace	Evan Williams	Slow & Low	Woodford Reserve	Angels Envy	Elijah Craig	Wild Turkey	Jack Daniels Single Barrel
Vanillic Acid	0.2	0.5	0.4	1.0	0.8	0.6	0.9	1.6	1.1	1.9	2.1	c	1.7	2.0	2.9	2.2	2.8	3.8	2.9
Vanillin	0.7	0.9	1.1	1.5	1.3	1.5	2.1	2.5	2.5	3.8	4.6	3.7	3.4	12.9	6.4	3.9	5.2	6.2	5.8
Syringaldehyde	2.9	2.8	2.9	4.4	3.8	4.3	5.2	7.6	9.4	13.7	16.0	11.7	10.7	14.0	20.5	13.3	15.9	18.2	17.4
p-Coumaric Acid			0.1		0.1	0.1	0.1	0.1	0.4	0.4	0.3	0.5	0.5	0.5	0.6	0.3	0.7	0.8	0.6
Scopoletin	0.1	0.1	0.2	0.1	0.5	0.2	1.2	0.5	0.6	0.3	0.3	0.5	0.7	0.5	0.5	0.8	0.7	0.5	0.7
Sinapaldehyde	0.7		0.6				0.7		0.5	0.8	0.7	1.9	2.0			3.1			4.5
Ellagic Acid	2.7	3.3	4.2	3.2	4.8	4.9	6.6	9.7	8.8	4.6	2.0	15.8	18.3	8.0	7.5	15.9	21.3	18.5	20.4
Total Phenolic Compounds	**7.3**	**7.6**	**9.5**	**10.2**	**11.3**	**11.5**	**16.7**	**22.0**	**23.3**	**25.5**	**25.9**	**36.1**	**37.4**	**37.9**	**38.4**	**39.5**	**46.6**	**48.1**	**52.2**

Panel A presents the total concentration of seven oak-derived phenolic marker compounds measured across the analyzed whiskey samples. The bar chart ranks each whiskey by its total phenolic content (µg/mL), highlighting clear differences in the extent of phenolic extraction among brands and styles. American bourbons and single-barrel expressions such as Jack Daniels Single Barrel, Wild Turkey, and Elijah Craig exhibit the highest total phenolic levels, consistent with extensive interaction with new charred American oak during maturation. In contrast, blended whiskies and several Japanese expressions show lower overall phenolic concentrations, reflecting more moderate oak extraction and differing barrel management practices.

The table beneath the chart provides the individual concentrations of each phenolic compound—including vanillic acid, vanillin, syringaldehyde, p-coumaric acid, scopoletin, sinapaldehyde, and ellagic acid—for each whiskey sample. Together, the ranked totals and compound-level values allow direct comparison of both overall phenolic abundance and the specific compound contributions that shape each whiskey's chemical fingerprint.

Conclusion

This work demonstrates that a YMC Triart C18 platform integrating LC/UV and LC/MS detection provides a comprehensive workflow for whiskey characterization. LC/UV enabled robust and reproducible quantitation of seven targeted oak-derived phenolic markers—vanillic acid, vanillin, syringaldehyde, p-coumaric acid, scopolamine, sinapaldehyde, and ellagic acid—while LC/MS expanded coverage to include β-methyl-γ-octalactone (whiskey lactone), a key flavor compound with weak UV absorbance. Importantly, the use of absolute ethanol in the organic mobile phase significantly improved chromatographic selectivity compared to acetonitrile alone, reducing co-elution and enhancing resolution of structurally related phenolic aldehydes. This highlights how solvent–stationary phase interactions on Triart C18 can be leveraged to optimize separations in complex spirit matrices.

The resulting data revealed that different whiskeys exhibit distinct and reproducible chemical "fingerprints" driven by phenolic aldehyde and oak-extraction chemistry. Bourbons and single-barrel expressions showed elevated lignin-derived aldehydes and ellagitannin-related compounds consistent with new charred American oak, whereas blended and Japanese whiskeys displayed lower overall phenolic intensity and broader compositional diversity. Together, this workflow demonstrates the value of Triart C18 for high-resolution phenolic and flavor marker profiling, enabling differentiation of whiskey styles and supporting applications in quality control, authenticity assessment, and maturation research.

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