

Liquid Chromatography

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Analysis of Phenols in Whisky by HPLC with FL Detection

Introduction

One of the world's most popular spirits is whisky and it comes in many different classes and types. The character and flavor of these differing types vary widely due to their varied

chemical composition. Whisky contains a multitude of compounds, which can be influenced by the variety of grain, the distillation process, and the wood used in the barrels for the aging process.¹

Phenolic compounds contribute bitterness and smokiness to a whisky's flavor.² They are more distinct in whiskies produced in Scottish distilleries where barley is dried using peat fires. During the drying process, the phenolic compounds in the peat smoke are absorbed by the barley and the flavors are later transferred to the whisky during the malting process. Additionally, during the maturation of the whisky in charred barrels, phenolic compounds may be produced in the spirit.¹

The most important flavor-contributing phenolic compounds in whisky are phenol, cresols, xylenol and guaiacol. Cresols, particularly m-cresol, are compounds responsible for the somewhat medicinal aroma in both Scotch whiskies and adhesive bandages.¹ Guaiacol imparts a slight smoky aroma and eugenol, more commonly found in cloves, is found in many whiskies and is partly responsible for their spicy aroma.^{1, 3}

This application focuses on the HPLC separation and quantitation of ten phenols in three store-bought Scotch whiskies. Method conditions and performance data, including linearity and repeatability are presented.

Experimental

Hardware/Software

For all chromatographic separations, a PerkinElmer Altus™ HPLC System was used, including the A-10 Solvent and Sample Manager, A-10 column heater, integrated vacuum degasser and an A-10 fluorescence (FL) detector. All instrument control, analysis and data processing was performed using the Waters® Empower® 3 Chromatography Data Software (CDS) platform.

Method Parameters

The HPLC method parameters are shown in Table 1.

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade and filtered via 0.45-µm filters.

The phenolic standard set was obtained from Sigma Aldrich®, Inc. (Milwaukee, WI), consisting of phenol, o-cresol, m-cresol, p-cresol, guaiacol, 4-ethylguaiacol, 4-ethylphenol, 2,5-xylenol, 2-ethylphenol and eugenol. A stock 100-ppm standard was prepared using methanol as diluent. The lower level standards

Table 1. HPLC Method Parameters.

HPLC Conditions						
Column:	PerkinElmer Brownlee™ SPP 2.7 mm C18 3.0 X 100 mm (Part# N9308410)					
Mobile Phase:	Solvent A: Water Solvent B: Acetonitrile Solvent program:					
	Time (min)	Flow Rate (mL/min)	%A	%B	%C	%D
	1 Initial	0.5	75.0	25.0	0.0	0.0
	2 10.0	0.5	60.0	40.0	0.0	0.0
	3 10.1	0.5	75.0	25.0	0.0	0.0
Analysis Time	10 min; 6-minute injection delay time between injections					
Flow Rate:	0.5 mL/min. (3500 psi)					
Oven Temp.:	25 °C					
Detection (FL):	Excitation wavelength: 272 nm; Emission wavelength: 298 nm					
Injection Volume:	10 µL					
Sampling (Data) Rate:	5 pts./sec					

were then serially prepared from this stock solution.

The whisky samples were purchased at a local store. They were labeled Whisky1, Whisky2, and Whisky3. Each whisky sample was then diluted 1:1 with methanol.

Prior to injection, all calibrants and samples were filtered through 0.45-µm filters to remove small particles.

Results and Discussion

Figure 1 shows the chromatographic separation of the 100-ppb phenolic standard using the conditions described above. The analysis time was under 10 minutes. Eight of the phenols were well resolved, while m-cresol and p-cresol co-eluted at 4.28 min.

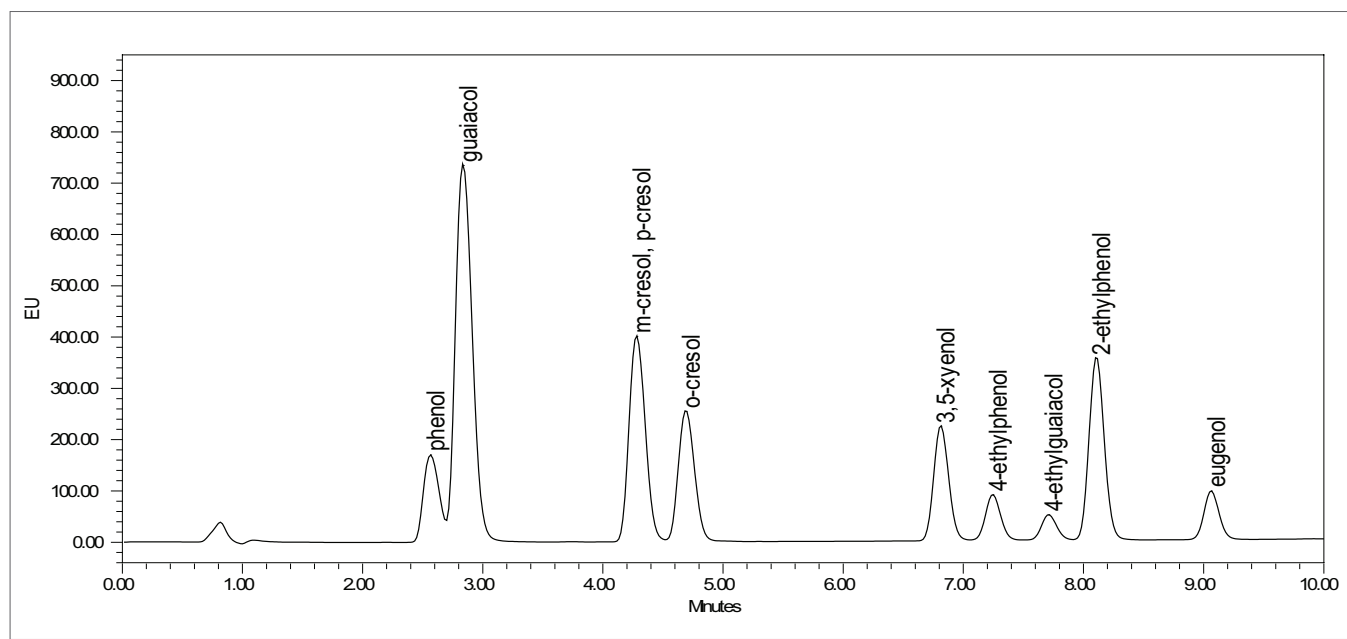


Figure 1. Chromatogram of the 100-ppb phenolic standard.

Figure 2 shows the overlay of 10 replicate 100-ppb phenolic standard injections, demonstrating exceptional reproducibility. The retention time (RT) %RSD for eugenol was 0.11%.

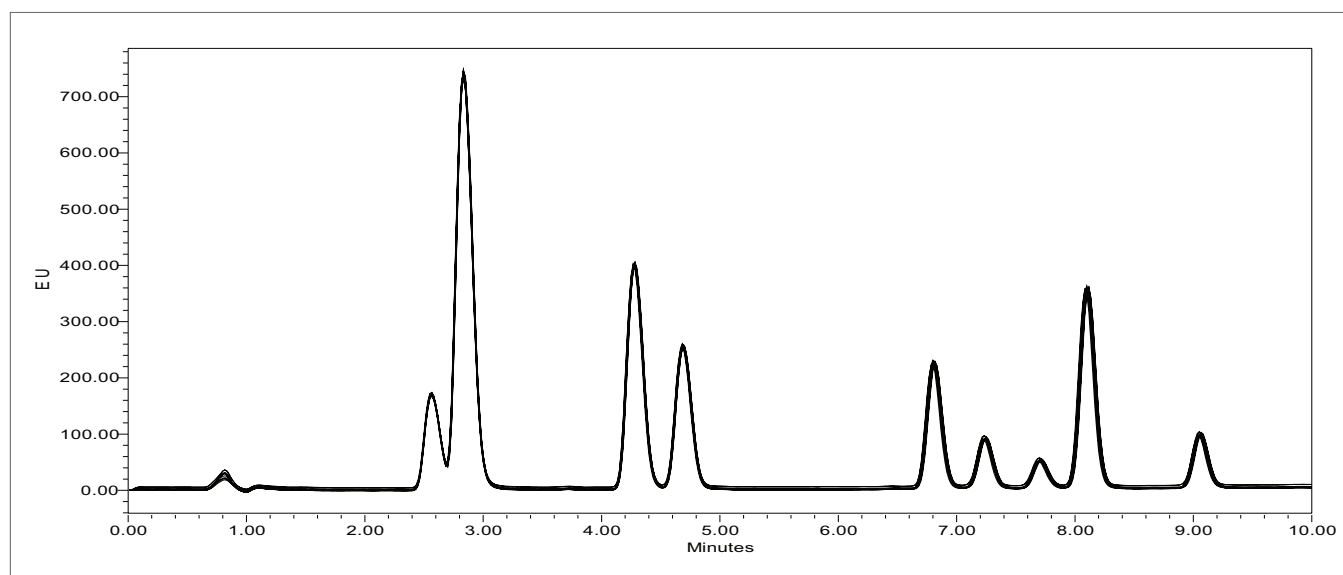


Figure 2. Overlay of 10 replicates of the 100-ppb phenolic standard.

From a serial dilution series of the phenolic standard mix, ranging in concentration from 5 to 100 ppb, a 5-level calibration set was generated. Figure 3 shows the calibration results for four representative phenols. All nine phenols followed a linear (1st order) fit and had R^2 coefficients > 0.999 (n = 3 at each level).

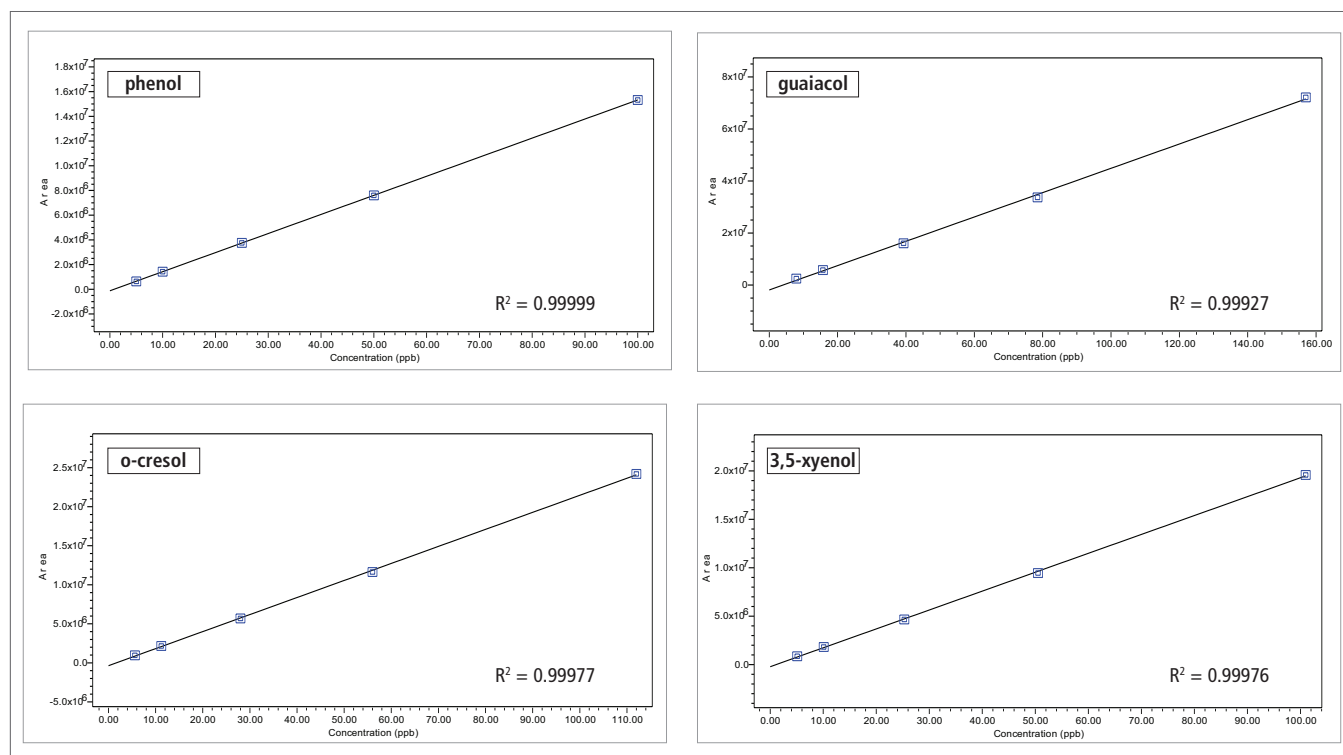


Figure 3. Four representative linearity results of the 5-level calibration set of the phenolic standard mix.

Using the same chromatographic conditions, three whisky samples were analyzed: Whisky1, Whisky2, and Whisky3. The results are shown in Figure 4. Comparing the chromatograms of these whisky samples with the 100-ppb phenolic standard, it can be observed that the samples contained a majority of the phenols.

For Whisky1, there was no peak corresponding to the RT of m-cresol and p-cresol, though an unknown component eluted

nearby at 4.20 min. For Whisky2 and Whisky3, while a peak corresponding to m-cresol and p-cresol was detected, as these two phenols coeluted, individual quantitation was not possible. Also, for all three whisky samples, the peak at 7.38 min, corresponding to 4-ethylphenol, appeared as part of a doublet and, therefore, could not be quantitated. The unknown peak in the doublet is likely attributable to the sample matrix.

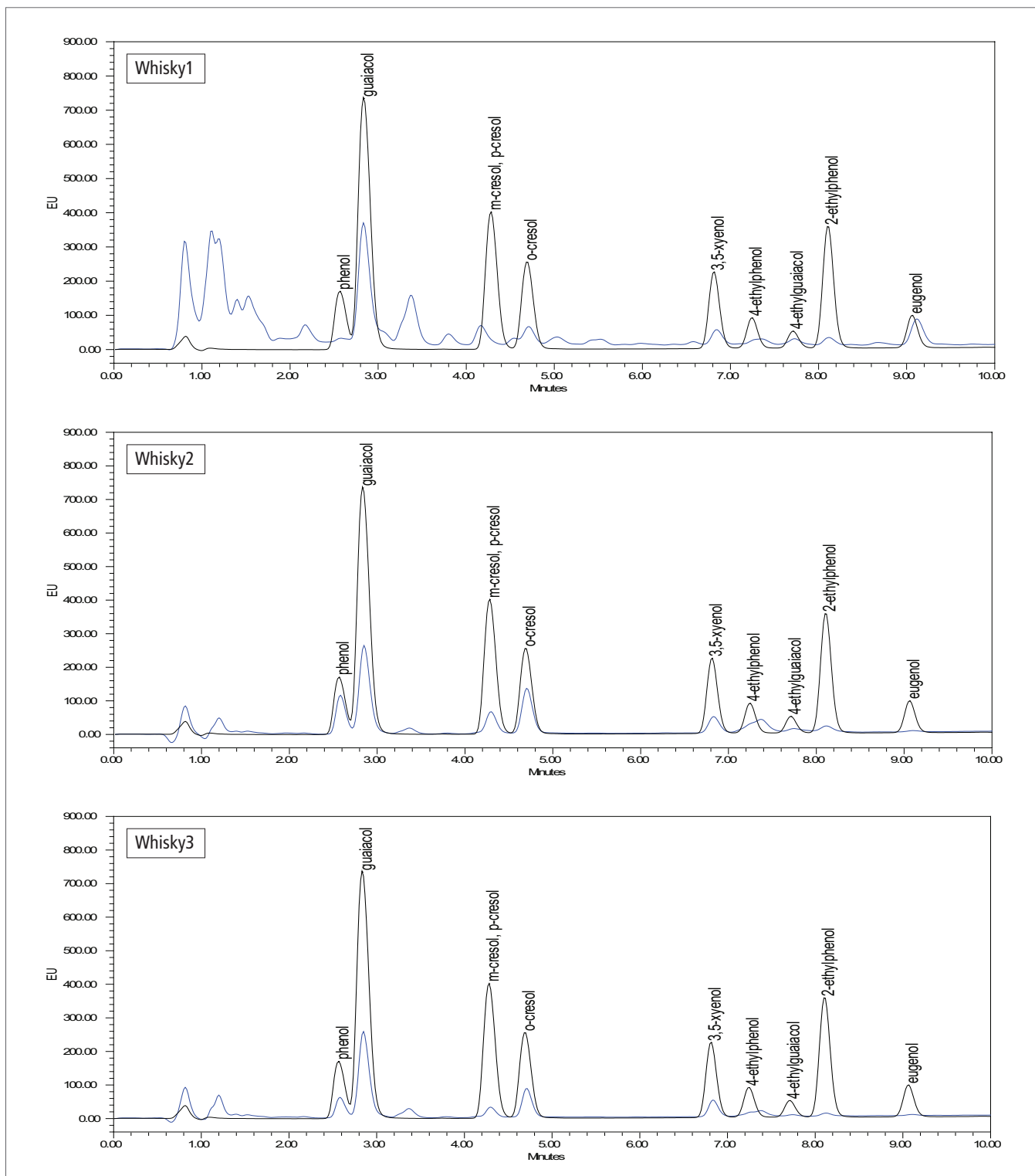


Figure 4. Chromatograms of Whisky1, Whisky2, and Whisky3 (blue) overlaid with 100-ppb phenolic std. (black).

Considering the above and based on the standard calibration plots, the quantitative results for each whisky sample are shown in Table 2. This was based on the average of three replicates per injection. Compared to one another, each whisky sample had differing concentrations of each of the phenols. Overall, the results show Whisky1 had significantly higher eugenol concentration compared to Whisky2 and Whisky3, and more than double the total amount of phenol of Whisky3.

Table 2. Quantitative Results for Whisky Samples.

Phenols	Whisky1 (ppb)	Whisky2 (ppb)	Whisky3 (ppb)
phenol	16	120	63
guaiacol	158	106	103
m-cresol	ND*	NA**	NA**
p-cresol	ND*	NA**	NA**
o-cresol	47	113	67
3,5-xenol	43	46	41
4-ethylphenol	NA***	NA***	NA***
4-ethylguaiacol	112	69	25
2-ethylphenol	21	20	9
eugenol	325	79	21
Total Phenols	722	553	329

* Not detected

** Not available: m-cresol and p-cresol coeluted

*** Not available: 4-ethylphenol eluted as part of a doublet

Conclusion

This work has demonstrated the applicability of this method for the effective analysis of phenols using a PerkinElmer Altus HPLC System with FL detection. The results exhibited very good retention time repeatability as well as excellent linearity over the tested concentration ranges. All the phenolic compounds were well separated in under ten minutes, except for m-cresol and p-cresol, which are typically difficult to resolve from one another.

The phenolic compounds in the three whisky samples were easily detectable and, apart from the two co-eluters and 4-ethylphenol, easily quantitated.

References

1. [Online]. <http://www.compoundchem.com/2015/03/31/whisky/>. [Accessed 20 May 2015].
2. M. Lehtonen. Chromatographia. 16, 201, 1982.
3. [Online]. <http://whiskyscience.blogspot.com/2011/201/peat.html>. [Accessed 18 May 2015].