UHPLC of Polyphenols in Red Wine

Application Note

Food

Authors

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Abstract

Generating high peak capacity is necessary in order to reduce the number of overlapping peaks in complex sample analyses. Greater peak capacity and resolution can be easily obtained for gradient analysis of complex samples such as wine, by using the higher efficiency of sub-2-µm particles in longer column lengths. This was confirmed by a 43% increase in peak capacity for the analysis of 19 polyphenol standards when the column length of a narrow bore Agilent ZORBAX Rapid Resolution High Definition (RRHD) StableBond SB-C18 column was increased from 100 mm to 200 mm. An additional 15% improvement in peak capacity was achieved by increasing the column length an extra 100 mm to 300 mm. The data show that higher quality separations can be achieved using longer column lengths, as demonstrated by the analysis of polyphenols in red wine. The Agilent 1290 Infinity LC system was used because the increased column length resulted in a significant improvement in resolution, and system pressures in the 600–1000 bar range.

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Introduction

Red wine is a very complex mixture and a rich source of polyphenols; a class of compounds that has gained considerable interest due to research suggesting their many health-related benefits. In addition, polyphenols are quality attributes of wine and contribute to color and sensory properties such as flavor and astringency. Given the importance of polyphenols and the complexity of wine samples, a method of analysis is required to give the necessary peak capacity for accurate identification and quantitation. This work shows the influence of column length on peak capacity for gradient analysis of complex samples.

Experimental

The polyphenols were purchased from Sigma Aldrich and include gallic acid (1), epigallocatechin (2), chlorogenic acid (3), catechin (4), caffeic acid (5), epicatechin (6), epigallocatechin gallate (7), p-coumaric acid (8), ferulic acid (9), m-coumaric acid (10), o-coumaric acid (11), quercitrin (12), myricetin (13), resveratrol (14), morin (15), quercetin (16), naringenin (17), apigenin (18), kaempferol (19). The standard mix was made by diluting standard stock solutions with water to the 10–60 ppm range. Most of the standard stock solutions were aqueous, but some of the polyphenols were made in methanol or methanol and H_2O due to solubility issues. They include: quercitrin (50/50 MeOH/H_2O), myricetin (MeOH), morin (50/50 MeOH/H_2O), quercetin (70/30 MeOH/H_2O), naringenin (50/50 MeOH/H_2O), apigenin (MeOH), and kaempferol (75/25 MeOH/H_2O). Red wine samples were directly injected after filtration using a 0.45-µm syringe filter.

Table 1. Experimental Conditions

Columns:	Agilent ZORBAX RRHD SB-C18 2.1 mm × 100 mm, 1.8 µm p/n 858700-902 Agilent ZORBAX RRHD SB-C18 2.1 mm × 150 mm, 1.8 µm p/n 859700-902
Mobile phase:	Solvent A: water (0.1% formic acid) Solvent B: acetonitrile (0.1% formic acid)
Flow rate:	0.3 mL/min
Column temperature:	30 °C
Detection:	UV Diode Array at 280 nm and 325 nm
Injection volume:	1.0 uL for standards for 100 mm column 1.5 uL for standards for 150 mm column 2.0 uL for standards for 200 mm column 3.0 uL for standards for 300 mm column 3.0 uL for wine sample on 150 mm column
UHPLC:	Agilent 1290 Infinity LC system

Gradient for 100 mm Column Length

Time (min)	%B	Time (min)	%B	
0	0	27	100	Column Cleanup
3.5	5	29	100	
7.1	15	30	0	Re-equilibration
25	40	35	0	
26	40			

The method for the 100 mm column length is easily transferred to longer column lengths by increasing the gradient times and sample injection volumes proportionally with column length.

Gradient for 150 mm Column Length

Time (min)	%B	Time (min)	%B	
0	0	40.5	100	Column Cleanup
5.25	15	43.5	100	
10.65	15	45	0	Re-equilibration
37.5	40	52.5	0	
39	40			

Gradient for 200 mm Column Length

Time (min)	%B	Time (min)	%B	
0	0	54	100	Column Cleanup
7.0	15	58	100	
14.2	15	60	0	Re-equilibration
50	40	70	0	
52	40			

Gradient for 300 mm Column Length

Time (min)	%B	Time (min)	%B	
0	0	81	100	Column Cleanup
10.5	15	87	100	
21.3	15	90	0	Re-equilibration
75.0	40	105	0	
78.0	40			

Results and Discussion

Peak capacity is defined as the number of peaks that can be theoretically separated within a gradient time. Complex samples such as red wine can contain many overlapping peaks. The best way to decrease the number of co-eluting peaks is to increase the peak capacity. Increasing column length is one way to increase peak capacity. The following equation was used to calculate the conditional peak capacity (n_c), which is directly related to the average peak resolution and computed from experimental data:

$$\text{Conditional peak capacity} = n_c = \frac{t_{R,n} - t_{R,1}}{W}$$

$t_{R,n}$ and $t_{R,1}$: Retention times of the last and first eluting peaks.

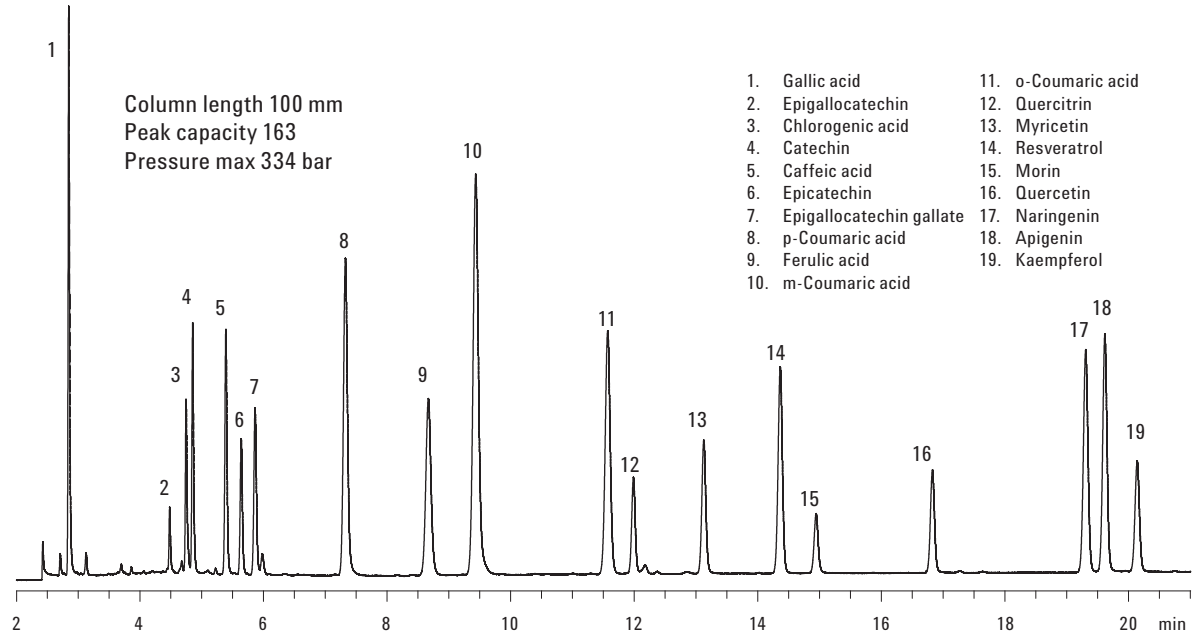
$$W: \frac{\overline{W_{1/2}}}{2.35} \times 4 \text{ (Average } 4\sigma \text{ peak width)}$$

$W_{1/2}$ is the average peak width at half height.

This equation was used in all peak capacity calculations. The effect of column length on peak capacity was evaluated by calculating peak capacity on a 100 mm column, 200 mm column and 300 mm column using a group of 19 polyphenol standards that may be present in red wine. Two 100 mm columns were connected in series to achieve a 200 mm column length, and two 150 mm columns were connected in series to achieve a 300 mm column length.

The chromatograms obtained for the group of 19 polyphenols on the three different column lengths and their corresponding peak capacities are shown in Figures 1 and 2. The peak capacity increased 43% when column length changed from 100 mm to 200 mm using the same flow rate of 0.3 mL/min and increasing the gradient length proportionally with column length. A further 15% increase in peak capacity was achieved when column length changed from 200 mm to 300 mm. The 19 polyphenols were separated in 20 minutes with the 100 mm column and a maximum pressure of 334 bar. Increasing the column length not only increased the peak capacity but also changed the run time to 41 minutes for the 200 mm column length and 62 minutes for the 300 mm column length. The maximum pressure of the HPLC system also increased to 670 bar (200 mm column length) and 940 bar (300 mm column length). The choice of column configuration depends on the instrumentation available to the analyst, which in turn determines the pressure limits of the method. The 200 mm and 300 mm methods would require the use of an UHPLC system like the Agilent 1290 Infinity LC system because the pressure maximum is above 600 bar. Other factors that influence the choice of column configuration for a given analysis include the complexity of the sample which affects the number of overlapping peaks, as well as the compounds of interest.

Efficient Separation of Nineteen Polyphenols on an Agilent ZORBAX RRHD SB-C18 2.1 mm × 100 mm, 1.8 µm Column

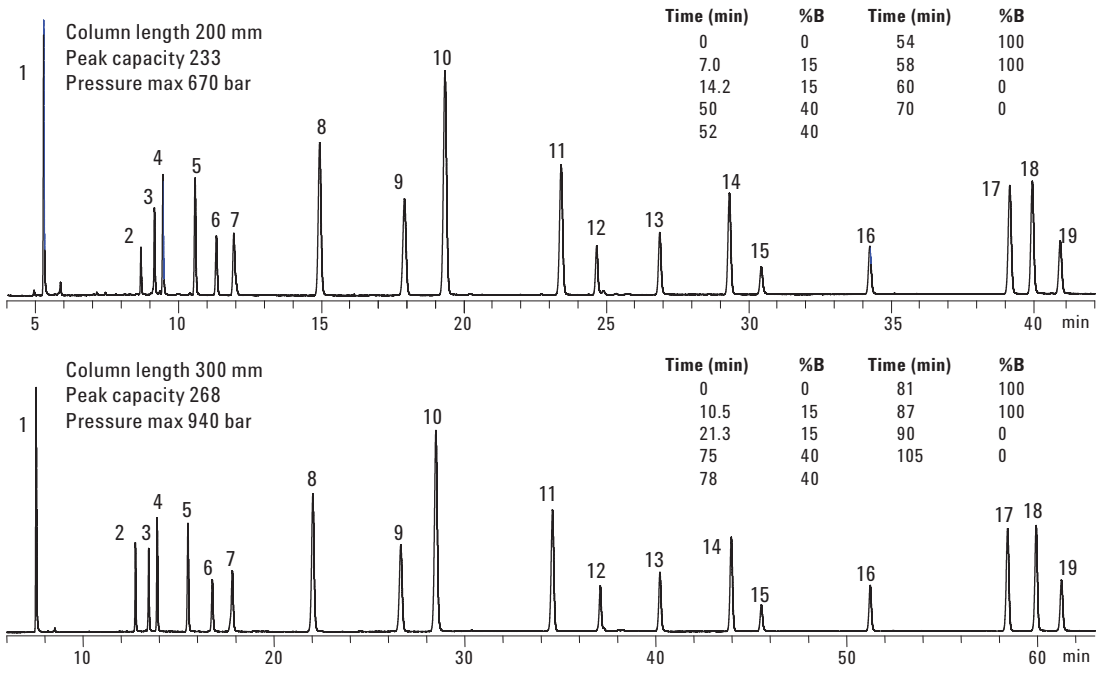


Column:	Agilent ZORBAX RRHD SB-C18 2.1 mm × 100 mm, 1.8 µm p/n 858700-902
Mobile phase:	Solvent A: water (0.1% formic acid) Solvent B: acetonitrile (0.1% formic acid)
Flow rate:	0.3 mL/min
Column temperature:	30 °C
Detection:	UV Diode Array at 280 nm
Injection volume:	1.0 uL for standards for 100 mm column
UHPLC:	Agilent 1290 Infinity LC system

Time (min)	%B	Time (min)	%B	
0	0	27	100	Column Cleanup
3.5	5	29	100	
7.1	15	30	0	Re-equilibration
25	40	35	0	
26	40			

Figure 1. Chromatogram and experimental conditions for the separation of nineteen polyphenols on an Agilent ZORBAX RRHD SB-C18 100 mm column.

Significant Increase in Peak Capacity with Column Length Simplifies Analysis of Complex Red Wine Samples



Columns: 200 mm - Two Agilent ZORBAX RRHD SB-C18
2.1 mm × 100 mm, 1.8 µm columns p/n 858700-902
300mm - Two Agilent ZORBAX RRHD SB-C18
2.1 mm × 150 mm, 1.8 µm colulmns p/n 859700-902

Injection Volume: 2.0 µL for 200 mm column
3.0 µL for 300 mm column

See Figure 1 for identification of compounds and additional experimental conditions.

Figure 2. Chromatograms of 19 polyphenol standards using an Agilent ZORBAX RRHD SB-C18 1.8 µm columns in lengths of 200 mm and 300 mm.

A red wine sample was analyzed using direct injection on a RRHD SB-C18 2.1 mm × 150 mm column and several polyphenols were identified using UV diode array detection with retention time matching of standards as shown in Figure 3. Increasing the column length from just 100 mm to 150 mm resulted in a 34% increase in peak capacity with a minimal increase in analysis time. The polyphenols that were identified in the red wine sample include: gallic acid, catechin, caffeic acid, epicatechin (top chromatogram) p-coumaric acid, quercitrin, myricetin, resveratrol, quercetin and kaempferol (bottom chromatogram). As discussed previously, if more peak capacity is needed for a particular wine sample it can be gained simply by adding column length and increasing the gradient time proportionally. Mass spectral identification is also possible with formic acid present in the mobile phase.

Resveratrol is one of the more well known polyphenols because much has been written regarding its positive health benefits. As seen in Figure 3, 2.2 ppm of Resveratrol was identified in a red wine sample using external standard calibration. It was determined that the signal-to-noise ratio for Resveratrol is more than doubled using 325 nm compared to 280 nm as shown in Figure 4. The choice of wavelength can improve the detection of Resveratrol by maximizing sensitivity and minimizing interferences. The use of a smaller 2.1 mm diameter column further enhances sensitivity.

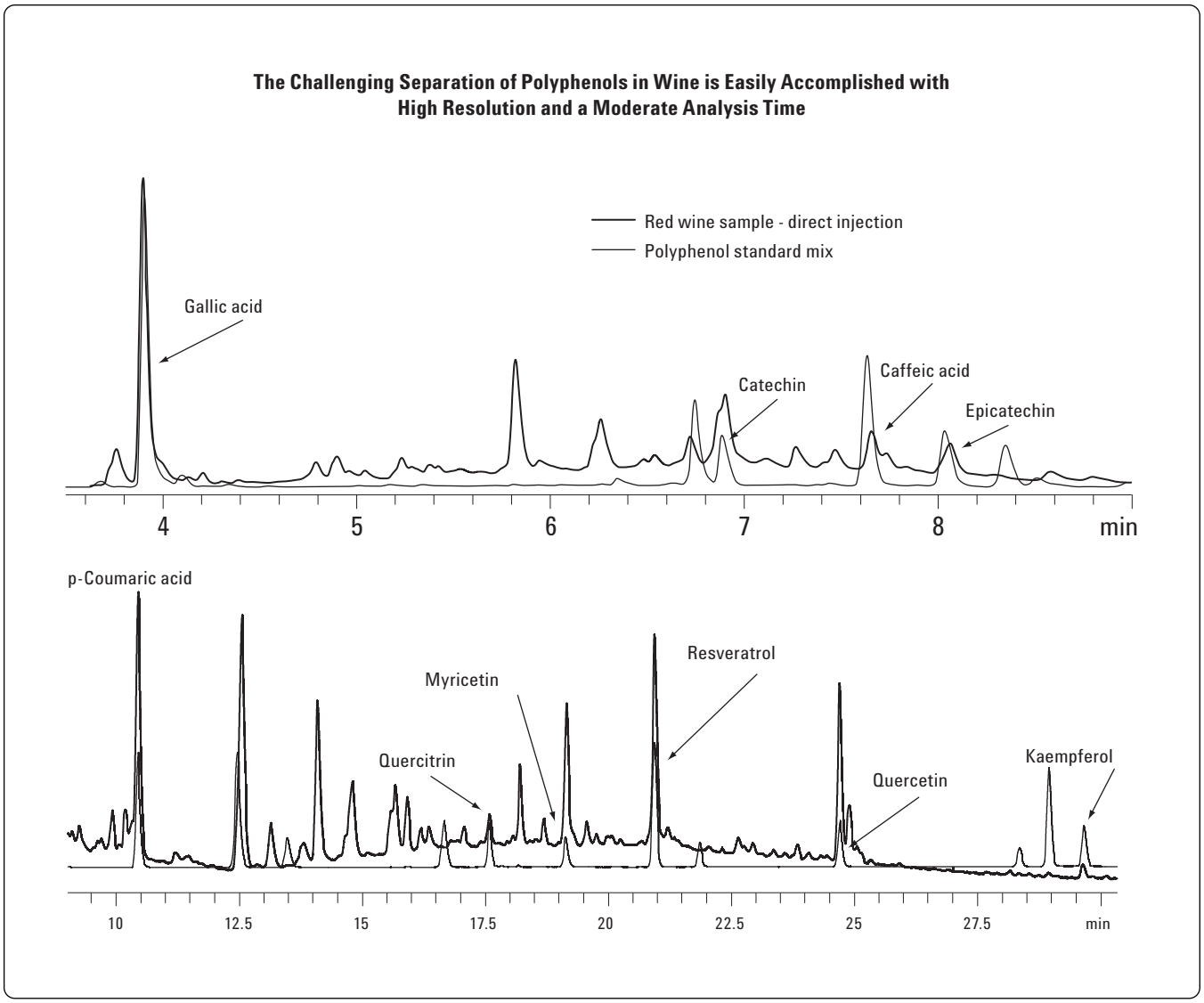


Figure 3. Identification of polyphenols in red wine on an Agilent ZORBAX SB-C18 RRHD 2.1 mm × 150 mm, 1.8 µm column using retention time matching of standards.

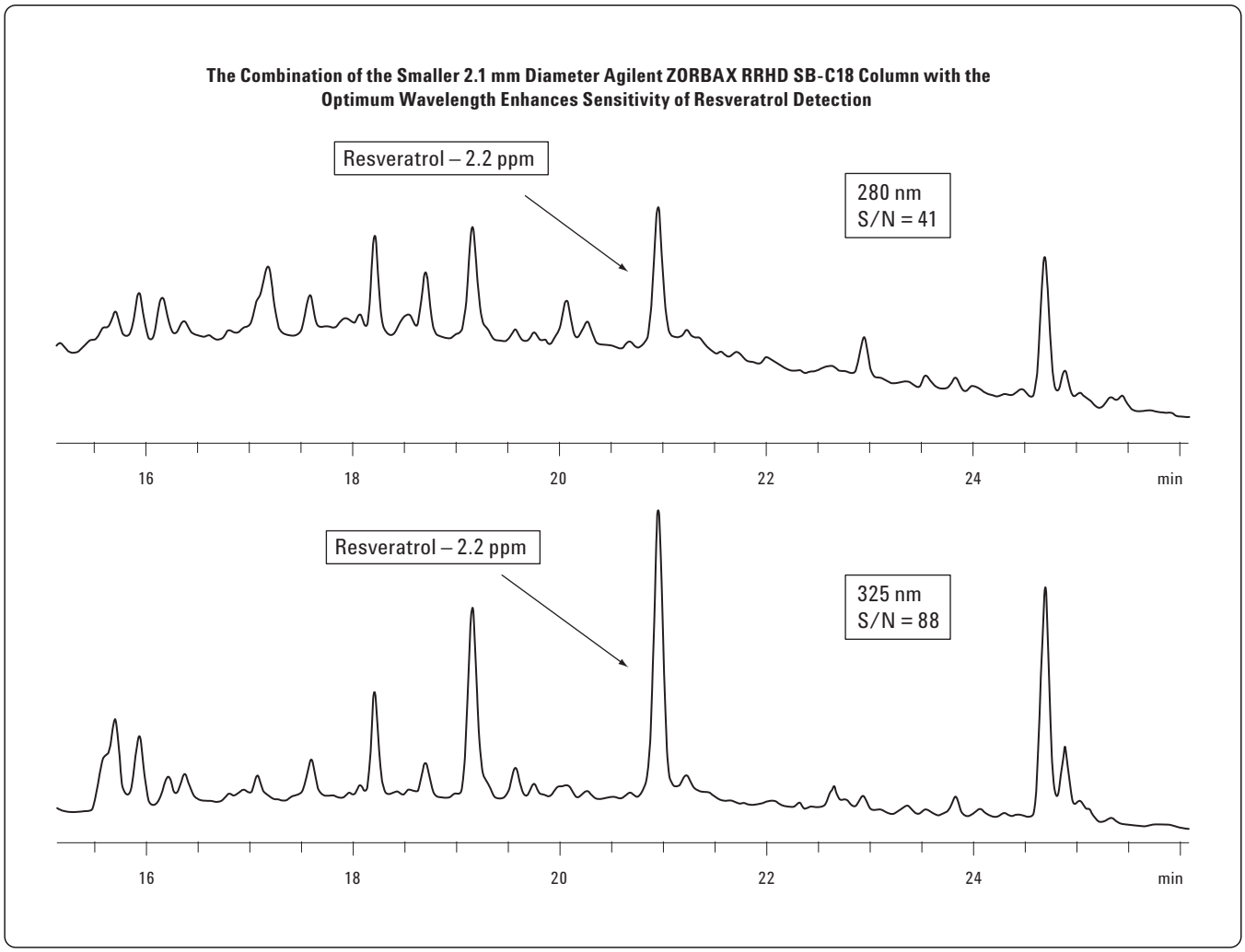


Figure 4. Choice of optimum wavelength enhances signal-to-noise ratio for Resveratrol peak identification on an Agilent ZORBAX RRHD SB-C18 2.1 mm × 150 mm, 1.8 µm column.

Conclusion

The high efficiency of Agilent ZORBAX RRHD SB-C18 sub-2-µm columns combined with extra column length maximizes the peak capacity of the analysis of very complex samples such as Polyphenols in a red wine sample. The benefit of the longer column length is that challenging separations can be accomplished with high resolution and moderate analysis times. The higher separation power of the Agilent Infinity 1290 UHPLC system with long columns packed with smaller particles provides the analyst a high degree of confidence in the accuracy of the analytical results.

References

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