UHPLC of Polyphenols in Wine

Judy Berry, John W. Henderson Jr., William Long Agilent Technologies 2850 Centerville Rd. Wilmington Del. USA 19808

2440-5P

Pittsburgh Conference , Orlando, Florida, USA 2010

Agilent Technologies

Abstract

Generating high peak capacity is necessary for the analysis of complex samples in order to reduce the number of overlapping peaks. 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 micron 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 100mm to 200mm. An additional 15% improvement in peak capacity was achieved by increasing the column length an extra 100mm to 300mm. The data show that higher quality separations can be achieved using longer column lengths and is demonstrated by the analysis of polyphenols in wine. The Agilent 1290 Infinity LC system was used because the increased column length resulted not only in a significant improvement in resolution, but also system pressures in the 600-1000 bar range.

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 that gives the necessary peak capacity so that accurate identification and quantitation are achieved. This work shows the influence of column length on peak capacity for gradient analysis of complex samples.

Peak Capacity

Peak capacity is defined as the number of peaks that can be theoretically separated within a gradient time. Complex samples such as wine can contain many overlapping peaks. The best way to decrease the number of co-eluting peaks is to increase the peak capacity. Peak capacity is more important than selectivity when separating numerous peaks of interest within a complex sample. 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.

Conditional Peak Capacity

$$n_c = \frac{t_{R,n} - t_{R,1}}{W}$$

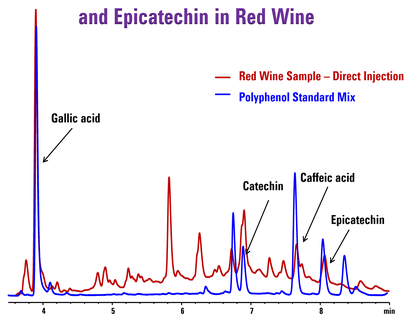
$t_{R,n}$ and $t_{R,1}$: Retention times of the last and first eluting peaks.

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

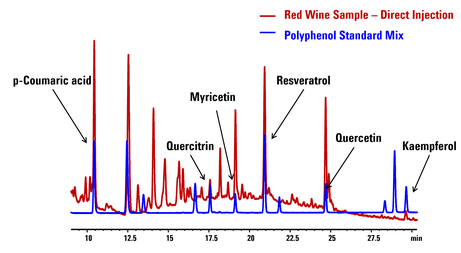
$W_{1/2}$ is the average peak width at half height.

Identification of Polyphenols in Red Wine with an RRHD SB-C18 2.1 x 150mm 1.8 µm Column

Identification of Gallic acid, Catechin, Caffeic acid and Epicatechin in Red Wine



Identification of p-Coumaric acid, Quercitrin, Myricetin, Resveratrol, Quercetin and Kaempferol in Red Wine



A red wine sample was analyzed on a 2.1 x150mm RRHD SB-C18 column and several polyphenols were identified using UV diode array detection with retention time matching of standards. Increasing the column length increases the resolution and peak capacity to decrease the number of overlapping peaks. Mass spectral identification is also possible with formic acid present in the mobile phase.

Method Parameters

Columns:	2.1 x 100mm ZORBAX RRHD SB-C18 1.8 µm p/n 858700-902 2.1 x 150mm ZORBAX RRHD SB-C18 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:	30ºC
Detection:	UV Diode Array at 280nm and 325nm
Injection Volume:	1.0 uL for standards for 100mm column 1.5 uL for standards for 150mm column 2.0 uL for standards for 200mm column 3.0 uL for standards for 300mm column 3.0 uL for wine sample on 150mm column
Standard Mix/Sample	The standard mix was made by dilution of standard stock solutions with water to the 10-60ppm range. Most of the standard stock solutions were aqueous, but some were methanol or methanol/H_2O due to solubility issues. Wine samples were directly injected after filtration using a 0.45 µm syringe filter.
UHPLC:	Agilent Infinity 1290 system

Gradient for 100mm Column Length

Time(min)	%B	Time(min)	%B	
0	0	27	100	Clean-Up of Column
3.5	15	29	100	
7.1	15	30	0	Re-Equilibration
25	40	35	0	
26	40			

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

Gradient for 150mm Column Length

Time(min)	%B	Time(min)	%B	
0	0	40.5	100	Clean-Up of Column
5.25	15	43.5	100	
10.65	15	45	0	Re-Equilibration
37.5	40	57	0	
39	40			

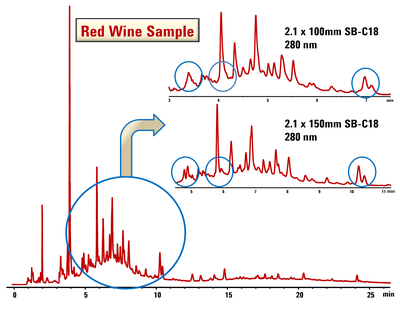
Gradient for 200mm Column Length

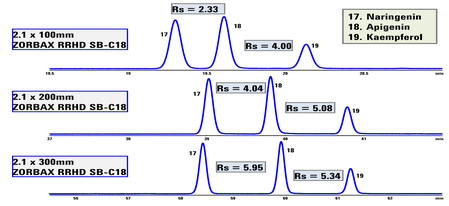
Time(min)	%B	Time(min)	%B	
0	0	54	100	Clean-Up of Column
7.0	15	58	100	
14.2	15	60	0	Re-Equilibration
50	40	76	0	
52	40			

Gradient for 300mm Column Length

Time(min)	%B	Time(min)	%B	
0	0	81	100	Clean-Up of Column
10.5	15	87	100	
21.3	15	90	0	Re-Equilibration
75.0	40	114	0	
78.0	40			

Enhanced Resolution with Longer Column Lengths

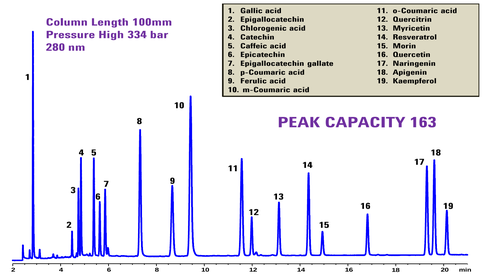




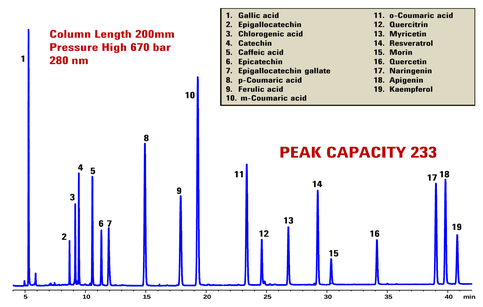
A noticeable increase in resolution and peak detail is observed for the 4 – 11 minute segment of the red wine chromatogram to the left for the 150mm column as compared to the 100mm column. The red-circled areas are just three examples of several where resolution has improved with the 150mm SB-C18 column. The above chromatograms show significant increases in resolution and peak capacity with column length for three polyphenols.

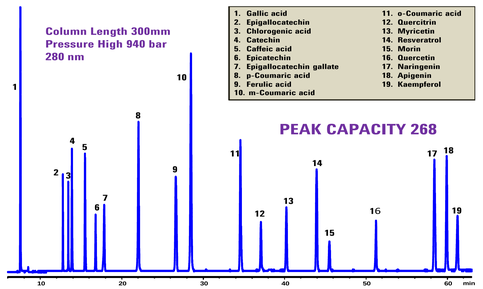
Increase in Peak Capacity with Column Length

Generating high peak capacity is necessary for the analysis of complex samples because this is the best way to reduce the number of overlapping peaks. Increasing the column length clearly gives larger peak capacity numbers, however it also causes the back pressure to rise. Two 100mm columns were connected in series to get a 200mm column length and two 150mm columns were connected in series to achieve a 300 mm column length.

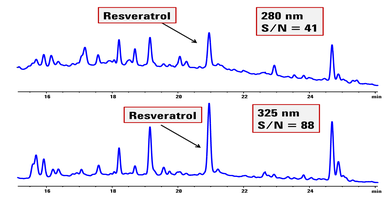


1. Gallic acid
2. Epigallocatechin
3. Chlorogenic acid
4. Catechin
5. Caffeic acid
6. Epicatechin
7. Epigallocatechin gallate
8. p-Coumaric acid
9. Ferulic acid
10. m-Coumaric acid
11. o-Coumaric acid
12. Quercitrin
13. Myricetin
14. Resveratrol
15. Morin
16. Quercetin
17. Naringenin
18. Apigenin
19. Kaempferol

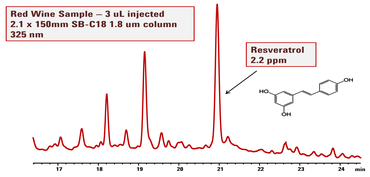




Optimum Wavelength Increases S/N for Resveratrol



The signal–to-noise ratio for Resveratrol is more than doubled using 325 nm as compared to 280nm. The choice of wavelength can improve the detection of Resveratrol by maximizing sensitivity and minimizing interferences. Also, the use of a smaller 2.1mm diameter column further enhances sensitivity. A red wine sample was found to contain 2.2 ppm of Resveratrol using this method. The presence of formic acid in the mobile phase can also aid in mass spectral detection.



Conclusion

The high efficiency of Agilent ZORBAX RRHD SB-C18 sub 2-micron 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 allows challenging separations to 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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