Easy Method Transfer and Improved Performance with Agilent Poroshell 120 4 µm Columns

Application Note

Food Testing and Agriculture

Author

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Abstract

A method for separating nine phenol compounds originally developed on a 4.6 × 100 mm, 5 µm column was transferred to Agilent Poroshell 120 EC-C18, 4.6 × 100 mm columns with 4 µm and 2.7 µm particles. The performance of these columns was compared to the performance of a similarly sized column with 1.8 µm totally porous particles. Gradient and flow rate were scaled, maintaining a constant retention index to determine the optimal flow rate for each column. By switching to a Poroshell 120 EC-C18 4 µm column and optimizing the gradient, the peak capacity increased from 50 to 67. Simple guidelines for transferring a method are provided. The pressure of the 4 µm method is below 200 bar and can be easily transferred to any HPLC system.

Introduction

Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or pathogens. In the last decade, there has been much interest in the potential health benefits of dietary plant polyphenols as antioxidants. Epidemiological studies and associated meta-analyses strongly suggest that long term consumption of diets rich in plant polyphenols offer protection against the development of cancer, cardiovascular disease, diabetes, osteoporosis, and neurodegenerative disease [1-3].

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In food, polyphenols can contribute to bitterness, astringency, color, flavor, odor, and oxidative stability. All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid [4].

Many phenolic-flavonoids found in plants exhibit antipyretic, analgesic, anti-inflammatory, and antioxidant properties. Such compounds include ellagic acid, catechol, gallic acid, quercetin, resorcinol, tannic acid, vanillin, salicylic acid, acetyl salicylic acid, and benzoic acid [5].

Environmental phenols are generated by industrial processes. These phenols include antioxidants used in plastics, pesticides, combustion of coal, petroleum and wood, and the manufacture of phenolic resins. In many cases, phenols are generated by natural processes. However, when these compounds are discharged to ground water, they can devastate many aquatic organisms. The analytical determination of phenolic compounds is, therefore, important because of the toxicity of these compounds and their widespread use [6,7].

For many years, the 5 µm HPLC column has been the technology standard. Many investigators were willing to sacrifice good efficiency and robustness for high pressure, unfamiliar instrumentation, and possible column clogging. Agilent Poroshell 120 4 µm columns can improve separating power over methods using similarly sized 5 µm columns. Poroshell 120 4 µm columns generate only slightly more pressure than 5 µm columns, and can be easily used on the same instrument. In addition, they use the same 2 µm frits found on 5 µm columns, making them robust against column clogging, and requiring no additional sample preparation.

In this work, a gradient method was transferred from a 4.6 × 100 mm, 5 µm column to a Poroshell 120, 4.6 × 100 mm, 4 µm column, or a Poroshell 120 2.7 µm column. Gradients were scaled to determine the flow rate at optimum peak capacity. Finally, the pressures of the experiments were compared, showing how Poroshell 120 columns can be easily used on any LC.

Experimental

An Agilent 1260 Infinity LC was used throughout this study. A G1312B Binary Pump SL was set up with mobile phase A (0.1 % formic acid in water) and mobile phase B (0.1 % formic acid in acetonitrile). The gradient is shown in Table 1, with time segments proportionally scaled relative to the flow rate for a constant retention index throughout the experiment. The pump was configured with the pulse damper and mixing column removed.

Table 1. Gradient program used with 4.6 × 100 mm columns.

% B	Time (min)						
5	4	2	1.33	1	0.8	0.67	0.34
40	34	17	11.33	8.5	6.8	5.67	2.84
40	40	20	13.33	10	8	6.67	3.34
5	42	21	14	10.5	8.4	7	3.5
5	50	25	16.67	12.5	10	8.34	4.17
Flow rate (mL/min)	0.5	1	1.5	2	2.5	3	3.5

A G1367C Automatic Liquid Sampler was used, with 20 µL injection volumes. The G1316B Thermostatted Column Compartment was set to 35 °C. A G4212A Diode Array Detector was set to 270,4 nm with a reference wavelength of 360,100 nm, incorporating a G4212-60008 flow cell, with 10 mm path and 1 µL capacity. Agilent Open Lab software version 1.05C was used to control the HPLC and process the data.

Columns

- Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 4 µm (p/n 695975-902)
- Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-902)
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 1.8 µm (p/n 959964-902)
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm (p/n 959996-902)

The compounds of interest are shown in Figure 1, with their respective structures. Compounds were dissolved in water at 1 mg/mL. Equal aliquots were combined to produce a mixed sample, which was diluted 1/10 in water. Thiourea was used as a void volume marker in all samples to determine t_0.

Thiourea, hydroquinone, resorcinol, phenol, 4-nitrophenol, *p*-cresol, *o*-cresol, 2,3 dimethylphenol, 2,5 dimethylphenol, 1-naphthol, and formic acid were purchased from Sigma-Aldrich, Corp. Acetonitrile was purchased from Honeywell. Water was 18 MΩ Milli-Q, made on site.

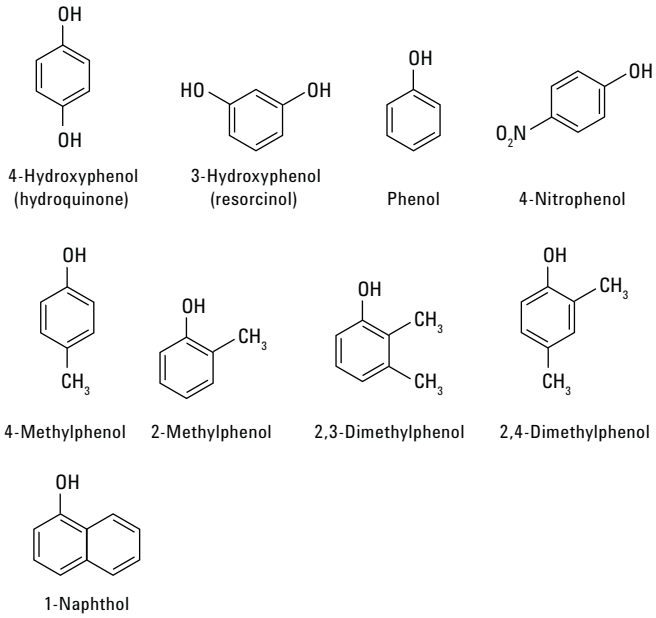

Figure 1. Compounds of interest.

Results and Discussion

The work by Coman and Moldovan [6] showed an excellent separation and quantitation scheme for phenols commonly identified in drinking and surface water. The objective in our work was to chromatographically improve the method, either by increasing peak capacity of the analysis, or by substantially shortening the chromatographic run time. The use of formic acid instead of acetic acid lowers the mobile phase pH. In addition, including formic acid in both the aqueous and organic mobile phases results in more level baselines. However, the k* value must be maintained when varying these column conditions so as not to change selectivity while gaining peak capacity. As shown in a previous note [7], the initial gradient was scaled, keeping column volumes constant and preserving method selectivity. In this case, the flow rate was varied between 0.5 and 3.5 mL/min at 0.5 mL/min intervals. Using Equation 1 as a guideline, the conditions listed in Table 1 were developed. These conditions were calculated manually and were the basis of the chromatographic programs used for the 100 mm columns. As can be seen, all steps in the program were proportionately shortened as the flow rate increased.

$$k^* = (t_g F)/(d/2)2L(\Delta\%B) \qquad \textbf{Equation 1}$$

Where:
t_g is the gradient time
F is the flow rate
L is the column length
d is the column diameter
Δ%B is the change in organic content across the gradient segment

A sample chromatogram is shown in Figure 2, including 1.8 µm and 5 µm totally porous ZORBAX Eclipse Plus C18, and 4 µm and 2.7 µm Poroshell 120 EC-C18. As can be seen, the elution order and relative spacing was the same for all four columns. However, the retention for the two superficially porous columns (4 and 2.7 µm) was slightly less than for the two totally porous columns (5 and 1.8 µm). This method was easily transferred between these columns.

Figure 3 demonstrates the relationship between pressure and particle size of the four columns. Larger particles generated lower pressure. Both the 5 µm column and the 4 µm Poroshell 120 column were under 200 bar using the conditions shown in Figure 2 (1.5 mL/min). Most methods with the 4 µm Poroshell 120 EC-C18 column will be run at this flow rate.

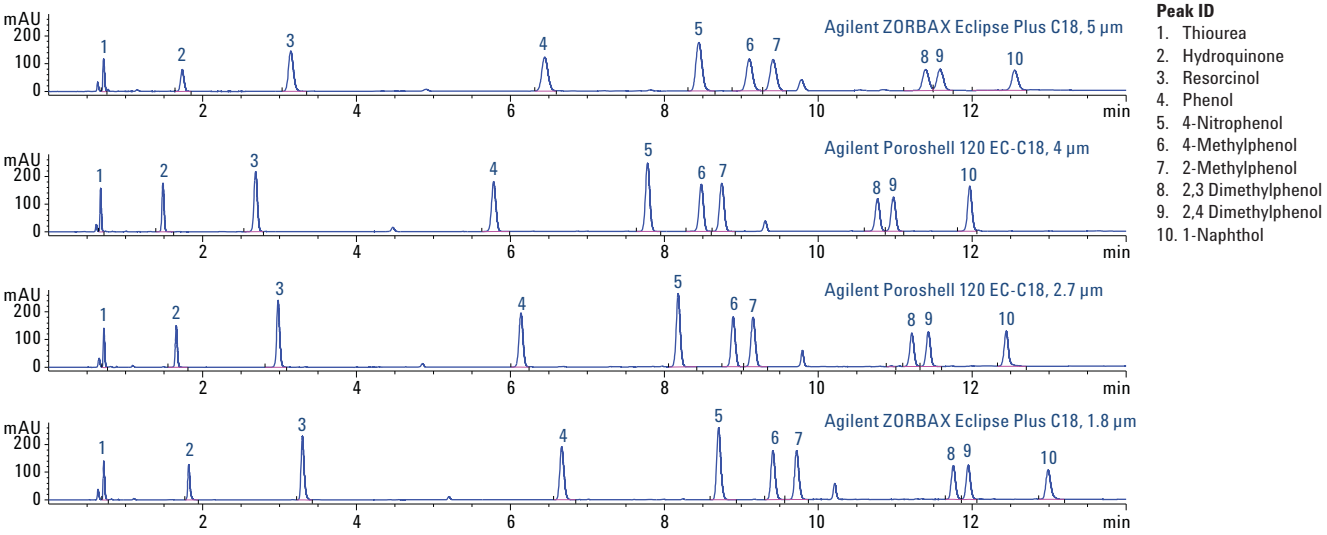

Figure 2. Overlay of chromatograms at 1.5 mL/min.

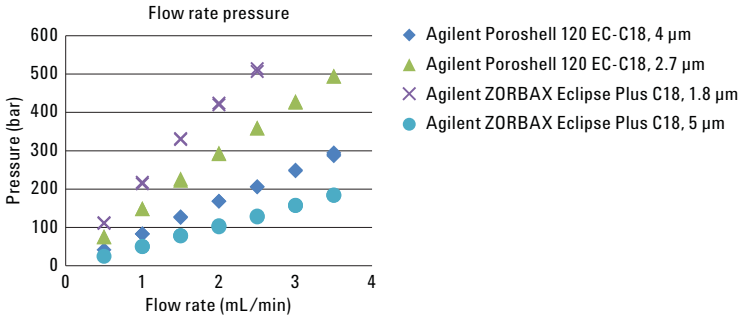

Figure 3. Pressure versus flow rate for different columns.

Conditional peak capacity nc = $(t_{R,n} - t_{R,1})/w$ **Equation 2**

Where:
$t_{R,n}$ and $t_{R,1}$ are retention times of the last and first eluting peaks
w is the 4σ peak width, = (W½ /2.35) × 4
W½ is peak width at half height

Peak capacity for each of the chromatograms is shown in Figure 4. The highest peak capacity was found for the 1.8 µm ZORBAX Eclipse Plus C18 column at 2.5 mL/min. It is possible that the peak capacity would have been higher at faster flow rates. However, this would have exceeded the system pressure limit of 600 bar. The 100 mm Poroshell 120 EC-C18, 2.7 µm column generated the next highest peak capacity, between 2 and 3 mL/min.

As can be seen in Figure 4, at lower flow rates the 2.7 µm Poroshell 120 and the 1.8 µm ZORBAX Eclipse Plus C18 had nearly identical peak capacities. The Poroshell 120 EC-C18, 100 mm, 4 µm column generated the next highest peak capacity, between 1 and 2 mL/min, a flow rate that is within the starting range of most chromatographers using 4.6 mm id columns. The 5 µm column has an optimal peak capacity between 1 and 1.5 mL/min. In general, with totally porous columns of the same dimensions, larger particle columns yield lower peak capacities at lower optimal flow rates.

Figures 5A and 5B show how resolution of two peak pairs changed on each column. Peak pairs 6/7 are 4-methyl phenol and 2-methyl phenol, Peak pairs 8/9 are 2,3-dimethylphenol and 2,4-dimethylphenol. These peak pairs of highly related compounds represent the type of problem most method development chemists face daily. As shown in Figure 5B, optimal peak resolutions were 2.4 at 2.5 mL/min for the 2.7 µm Poroshell 120, 2 at 2.5 mL/min for the 1.8 µm column, 1.9 at 1.5 mL/min for the 4 µm Poroshell 120, and 1.2 at 1.5 mL/min for the 5 µm column. As with peak capacity, larger particle columns yield lower optimal peak resolution. In addition, the optimal peak resolution of larger particles is found at lower flow rates.

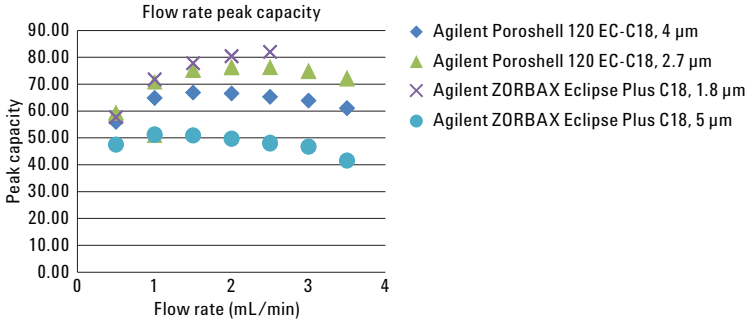

Figure 4. Optimization of peak capacity between 0.5 and 3.5 mL/min.

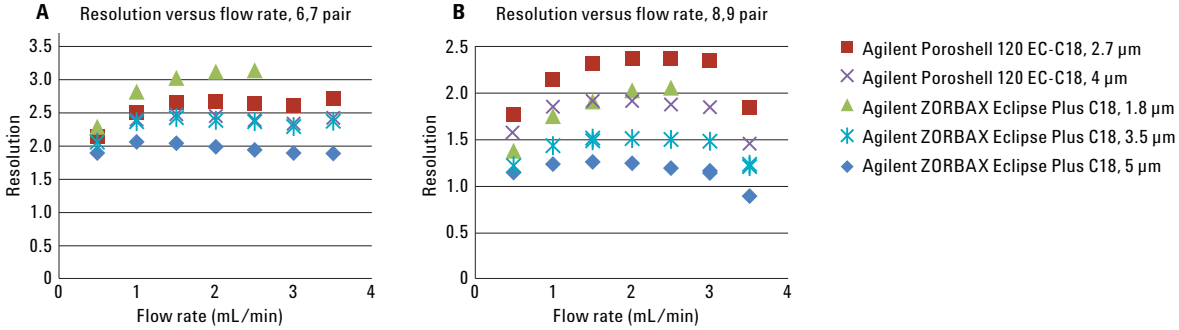

Figure 5. Resolution optimization between 0.5 and 3.5 mL/min.

Conclusions

HPLC columns packed with superficially porous particles offer many advantages over columns packed with conventional, fully porous particles. The Agilent Poroshell 120 EC-C18, 4 µm column offers a substantial increase of efficiency and peak capacity compared to 5 µm totally porous columns.

The superficially porous 2.7 µm Poroshell 120 EC-C18 offers similar efficiency and selectivity to the 1.8 µm Agilent ZORBAX Eclipse Plus C18, without the high backpressure. Due to the similar selectivity between Poroshell 120 EC-C18 and ZORBAX Eclipse Plus C18 columns, methods can be easily transferred to decrease run time, improve throughput, and increase peak capacity.

For More Information

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