Fast Analysis of Environmental Phenols with Agilent Poroshell 120 EC-C18 Columns

Application Note

Environmental

Authors

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Abstract

A method separating nine phenol compounds originally developed on a 4.6 × 100 mm, 5-µm column is transferred to an Agilent Poroshell 120 EC-C18 4.6 × 100 mm, 2.7 µm column. The gradient and flow rate are scaled, maintaining a constant retention index in order to determine the optimal flow rate for each column evaluated. Simple guidelines for transferring a method are provided. The new separation reduces the analysis time from 20 minutes to less than 3 minutes with a higher peak capacity, and should not require any changes to sample preparation since both columns use 2 µm frits. The method is further scaled to a 50 mm column that produces slightly less resolution than found with the 100 mm column, but with an analysis time of only 2 minutes with an equivalent peak capacity to the original method. While pressure is increased from the original method, it is still below 400 bar and can be easily transferred to any HPLC system.

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Introduction

The presence of phenols in the environment is caused by their generation from many industrial processes. These processes include the manufacture of phenolic resins, antioxidants, pesticides and the combustion of wood, coal and petroleum. Some phenols are germicidal and are used in formulating disinfectants, and other phenols are formed during natural processes. When phenolic compounds are discharged into the environment they can present a serious hazard by contaminating water because they cause devastation to the majority of the aquatic organisms and induce bioaccumulation in the food chain at trace levels. Others possess estrogenic or endocrine disrupting activity. Frequently, these compounds find their way into the environment as water pollution. The analytical determination of phenol and substituted phenols is necessary because of their toxicity, persistency, and widespread use in industry. Many phenols are on the priority pollutant list.[1]

Agilent Poroshell 120 EC-C18, 2.7 µm columns perform similarly to sub-2-µm totally porous materials, but since they use 2-µm column frits like those found on 5-µm columns, they require no additional sample preparation. This allows a more seamless method transfer to new Poroshell 120, from established methods using 5-µm columns.[2,3,4]

In this work, a gradient method is transferred and optimized from a 4.6 × 100 mm, 5-µm column to an Agilent Poroshell 120 EC-C18, 4.6 × 100 mm column. Gradient time was decreased from 20 minutes to 3 minutes, and a higher peak capacity resulted. Time can be further reduced using a 4.6 × 50 mm column, while maintaining the peak capacity of the original method.

Experimental

An Agilent 1200 Rapid Resolution liquid chromatography (RRLC) system was used for this work:

- G1312B Agilent Binary Pump SL with mobile phase A: 0.1 % Formic Acid in water and B: Acetonitrile. The gradient started at 5% B, held at that concentration; then ramped to 40 % B, held at that concentration, and then re-equilibrated to the initial condition. Gradient times vary depending on column dimensions and flow rate (Tables 1 and 2). The system is configured with the pulse damper and standard mixer installed.
- G1367C Agilent Automatic Liquid Sampler (ALS) SL. Injection volume was 20 µL and 10 µL for the 4.6 × 100 mm and 4.6 × 50 mm columns respectively.
- G1316B Agilent Thermostatted Column Compartment (TCC) SL with temperature set to 35 °C.
- G1315C Agilent Diode Array Detector (DAD) SL with the signal set to 270, 4 nm and reference set to 360, 100 nm, using a G1315-60024 micro flow cell (3-mm path, 2-µL volume).
- Agilent ChemStation version B.04.01 was used to control the HPLC and process the data.

Five Agilent columns were used in this work:

- Agilent Poroshell 120 EC-C18,4.6 × 100 mm, 2.7 µm – Agilent p/n: 695975-902
- Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm
  - Agilent p/n: 699975-902
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 1.8 µm
  - Agilent p/n: 959964-302
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 3.5 µm
  - Agilent p/n: 959961-902
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm
  - Agilent 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. Thiourea was used as a void marker in all samples to determine t_0.

The following compounds were purchased from Sigma Aldrich: hydroquinone, resorcinol, phenol, 4-nitrophenol, p-cresol, o-cresol, 2,3 dimethylphenol, 2,5 dimethylphenol, 1-napthol. Formic acid was purchased from Sigma Aldrich (Bellefonte, PA). Acetonitrile was purchased from Honeywell, Burdick and Jackson High Purity, (Muskegon, MI). Water used was 18 M-Ω Milli-Q water (Bedford, MA).

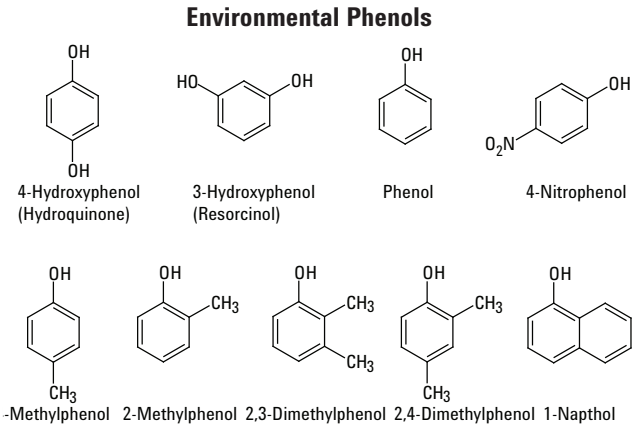

Figure 1. Compounds of interest.

Results and Discussion

The original work shows an excellent separation of phenols commonly identified in drinking and surface water. The objective in this work was to chromatographically improve the method; either by increasing the peak capacity of the analysis or substantially shortening the chromatography. A change in the acidic modifier from acetic acid to formic acid is made. The use of formic acid instead of acetic acid lowers the pH of the mobile phase slightly and can allow cleaner baselines. As discussed in reference 5, once a separation has been optimized (selectivity and retention index), it is possible to further improve the chromatography by varying column length, particle size and flow rate. However the k* value must be maintained, while varying these column conditions so as not to lose selectivity while gaining peak capacity.

Equation 1: $k^* = (t_g F)/(d/2)^2 L(\Delta\%B)$

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

As shown in a previous note, the initial gradient is scaled keeping column volumes constant, and preserving method selectivity. In this case, the flow rate is varied between 0.5 mL/min and 3.5 mL/min at 0.5 mL/min intervals. Using Equation 1 as a guideline, the conditions listed in Table 1 are developed. These conditions were calculated manually but could just as easily have been calculated using the Agilent 1200 Series Rapid Resolution LC Method Translator and Cost Savings Calculator [6]. These conditions are the basis of the chromatographic programs used for the 100 mm columns in this study. As can be seen, all steps in the program are proportionately shortened as the flow rate is increased. In the resulting chromatographs in Figures 2a and 2b, elution order remains similar for the scaled chromatograms of both the 5 µm Agilent ZORBAX Eclipse Plus C18 and the 2.7 µm Agilent Poroshell 120 EC-C18 columns. In addition to these columns, the same experiments are performed on 3.5 µm Agilent ZORBAX Eclipse Plus C18 and 1.8 µm Agilent ZORBAX Eclipse Plus C18 columns with the resulting chromatograms depicted in Figures 3a and 3b respectively. The pressure generated using the 4.6 × 100 mm, 1.8-µm column approaches 550 bar at 2 mL/min. Therefore, no further experiments are considered. In all cases, the separation elution order is maintained. The peak capacity of each chromatogram is calculated using Equation 2. Peak capacity in the simplest terms, is the number of peaks of a given width that can fit between the first and last peaks.

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**

Similar selectivity allows convenient method transfer between Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 column

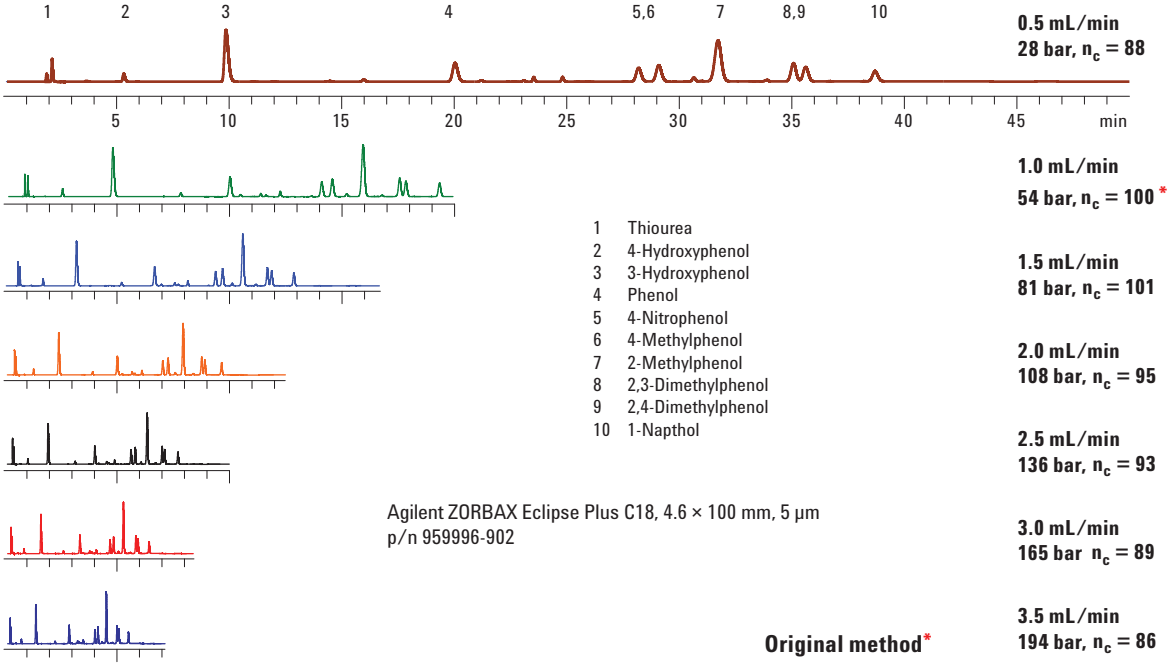

Figure 2a. Scaled chromatography using Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm column.

Similar selectivity allows convenient method transfer between Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 column

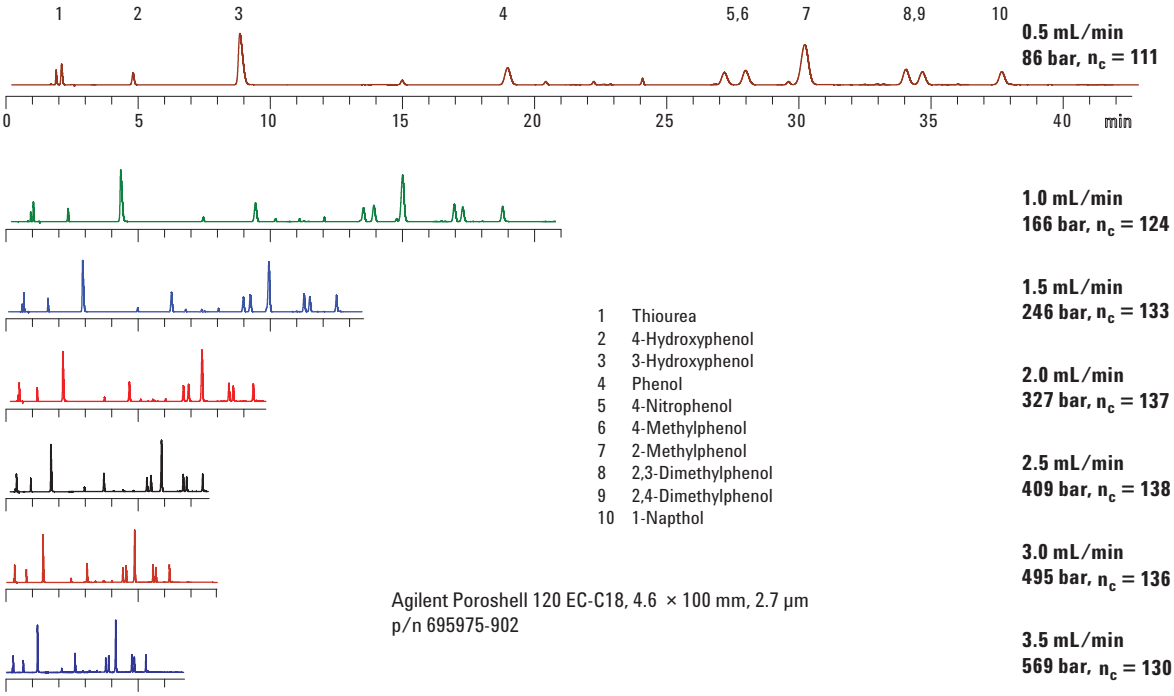

Figure 2b. Scaled chromatography using Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm column.

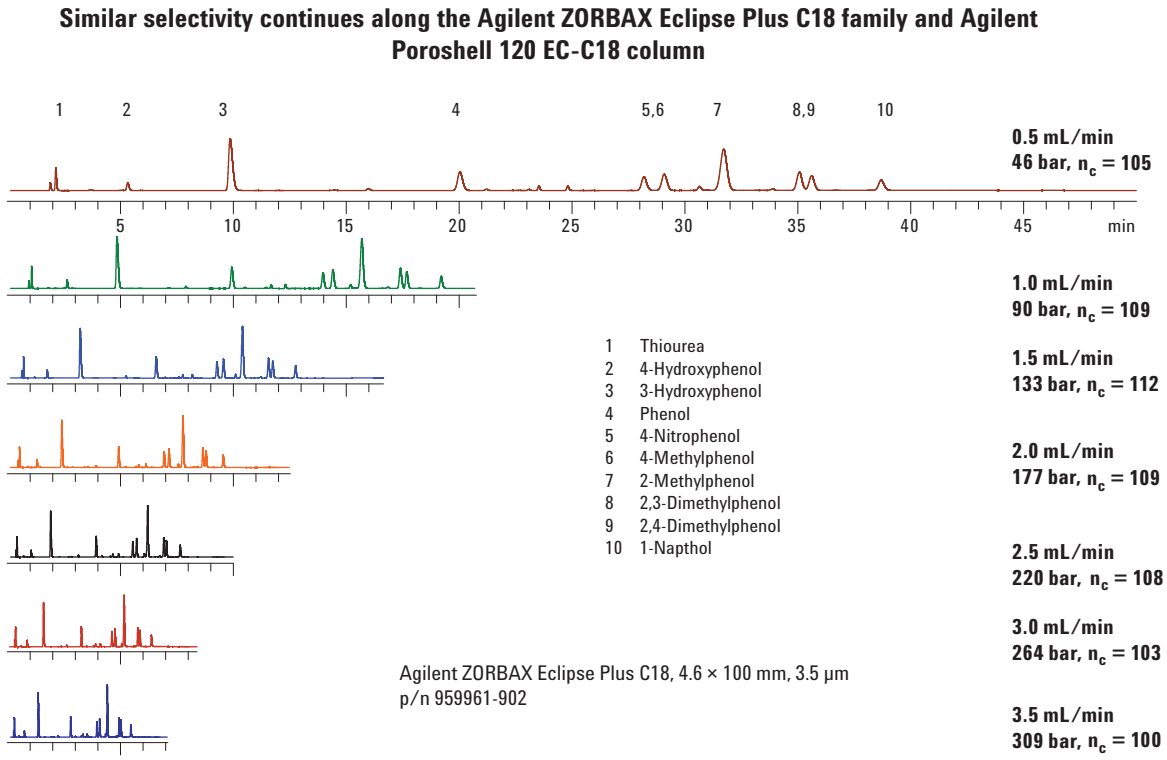

Figure 3a. Scaled chromatography using, Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 3.5 µm column.

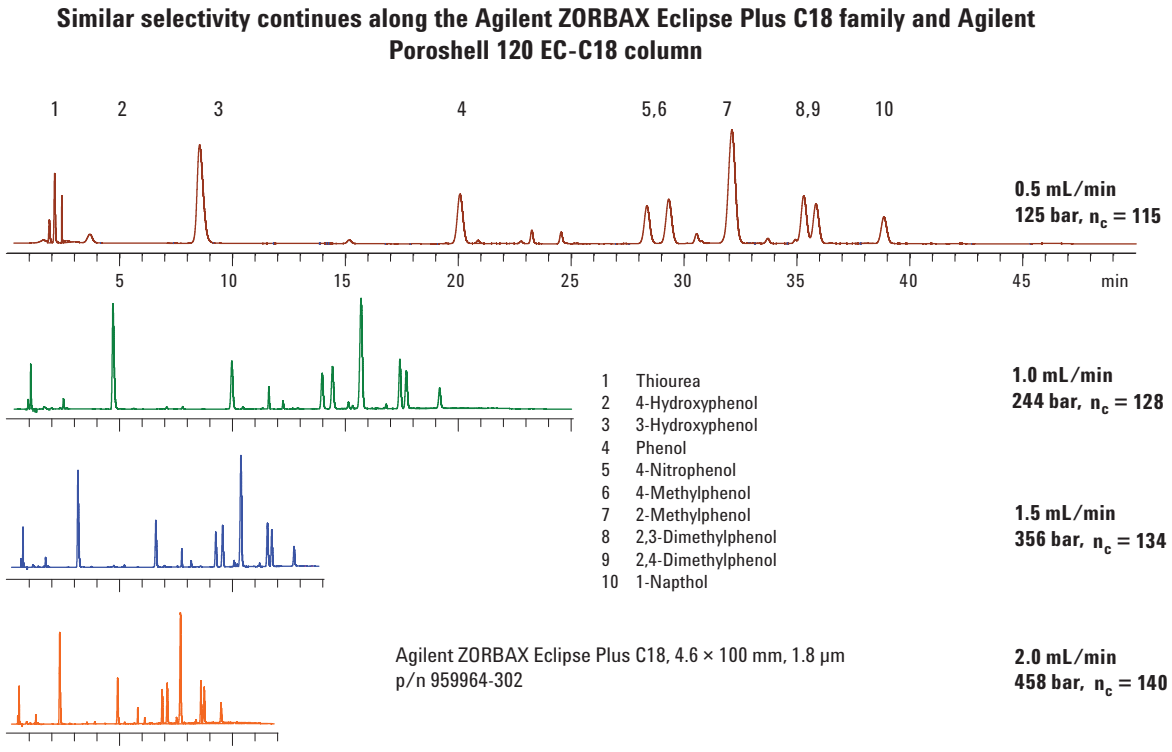

Figure 3b. Scaled chromatography using, Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 1.8 µm column.

Equation 2:

Conditional Peak Capacity $n_c = (t_{R,n} - t_{R,1})/w$

Where:

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

w is the 4σ peak width

$w = (W_{½} / 2.35) \times 4$

$W_{½}$ = peak width at half height

Peak capacity for each of the chromatograms shown in Figures 2 a and b and 3 a and b are plotted in Figure 4. The highest peak capacity is found for the 1.8 µm Agilent ZORBAX Eclipse Plus C18 column at 2 mL/min. It is likely that the peak capacity would have been higher at faster flow rates. The 100 mm Agilent Poroshell 120 EC-C18 column generates the next highest peak capacity between 2 and 3 mL/min. Figure 5 indicates that a faster separation is possible with only a slight loss of peak capacity. The 3.5 µm column has an optimal peak capacity at approximately 1.5 mL/min, while 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 dimension, larger particle columns yield lower peak capacities at lower optimal flow rates. The chromatographic scaling experiment is also carried out using a 50 mm Agilent Poroshell 120 EC-C18 column, with the gradient conditions summarized in Table 2, and the results depicted in Figure 5. A comparison between the peak capacities of 50 mm and 100 mm Agilent

Peak Capacity of Agilent Poroshell 120 Column is similar to 1.8 µm column of same size and higher than 3.5 or 5 µm columns of similar size, Peak capacity of totally porous particles is inversely proportional to their size.

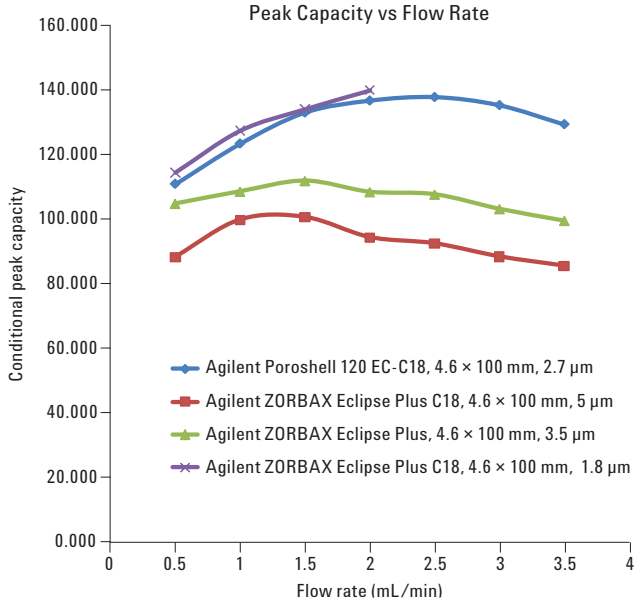

Figure 4. Peak capacity of Agilent Poroshell 120 EC-C18 compared to totally porous Agilent ZORBAX Eclipse Plus C18 columns of different particle sizes at varied flow rates.

Agilent Poroshell 120 EC-C18, 4.6 x 50 mm column can provide peak capacity of original method at 182 bar in 3 minutes instead of 20 minutes

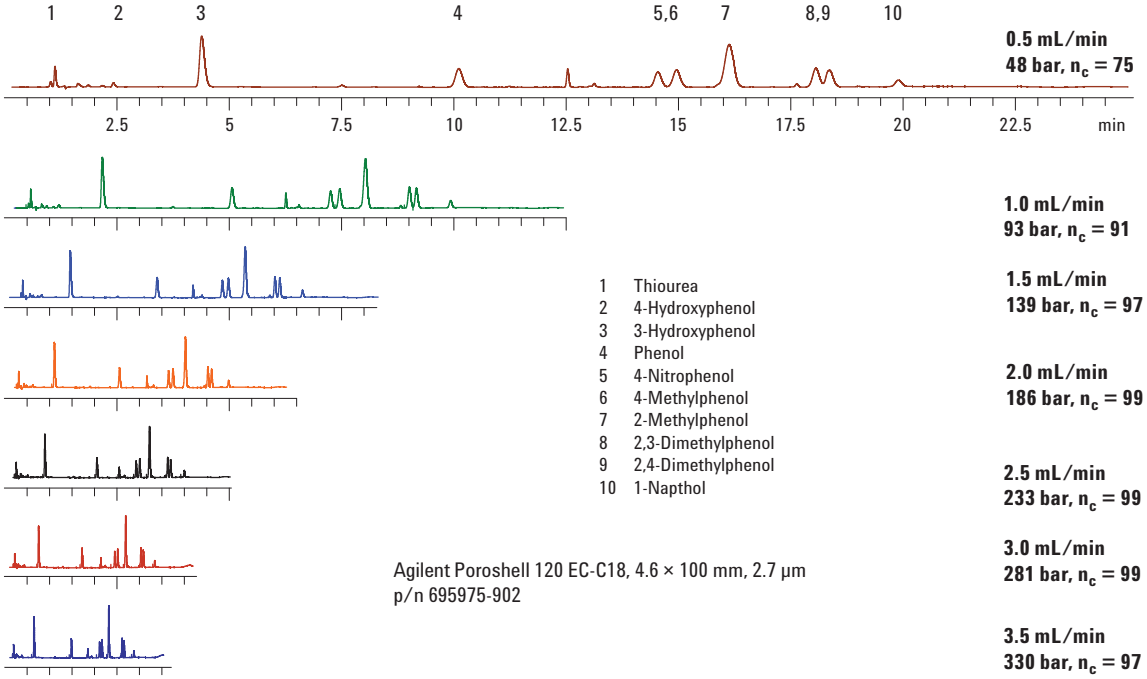

Figure 5. Scaled Chromatography using Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm column.

Table 2. Gradient Program Used With 4.6 × 50 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**

Poroshell 120 EC-C18 columns is shown in Figure 6. The optimal peak capacity of the 50 mm Agilent Poroshell 120 EC-C18 column is found to be between 2 and 3 mL/min, at 3 min. The last peak is eluted at 2 min with a peak capacity equivalent to the peak capacity of the original 20-min 4.6 × 100 mm, 5 µm method.

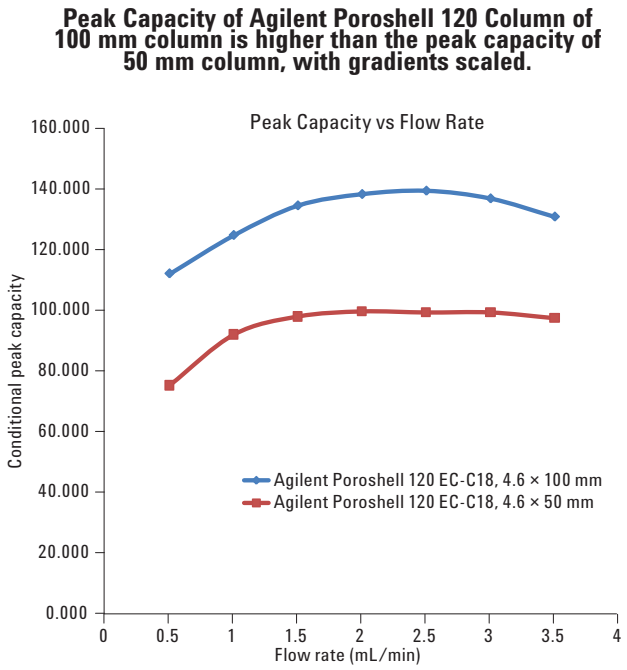

Figure 6. Peak Capacity of 50 and 100 mm Poroshell 120 EC-C18 at varied flow rates.

Conclusion

HPLC columns packed with superficially porous particles offer many advantages over columns packed with conventional, fully porous particles. The superficially porous 2.7-µm Agilent Poroshell 120 EC-C18 offers similar efficiency and selectivity to the 1.8-µm Agilent ZORBAX Eclipse Plus C18 column, without the high back pressure.

While columns packed with larger 5-µm particles can yield excellent separations, many separations can be improved by implementing a column change and appropriate gradient scaling. Due to the similar selectivity between Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 columns, methods can easily be transferred from older Agilent ZORBAX Eclipse Plus C18 columns to new Agilent Poroshell 120 EC-C18 to decrease run time, improve throughput and increase peak capacity.

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