Transfer of Methods between Poroshell 120 EC-C18 and ZORBAX Eclipse Plus C18 Columns

Technical Overview

Introduction

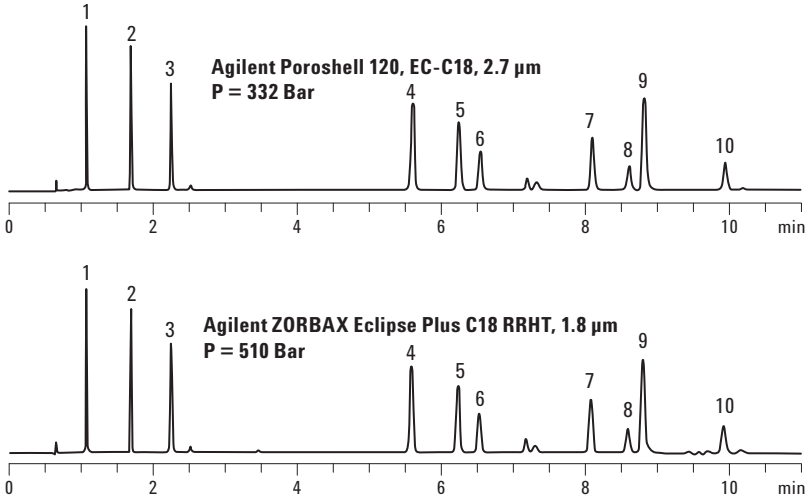
The developement of superficially porous particles has led to the possibility of method transfer from larger 5-µm totally porous particles, as well as from sub-2-µm totally porous particles. One of the benefits of transferring from larger particle columns is significant time savings, as the superficially porous particles are optimally run at a faster flow rate achieving similar resolution with a much shorter column length [1–4]. The high efficiency of superficially porous particles is similar to sub-2-µm totally porous particles because of the short mass transfer distance and substantially narrower particle size distribution. Transferring methods from totally porous sub-2-µm columns may also be desirable. Many development laboratories have chosen to use sub-2-µm columns. However, in some cases the higher operating pressure required of sub-2-µm methods may not be transferable to all HPLC systems. In many cases methods using sub-2-µm columns can be directly transferred to superficially porous particle columns, without adjustment. This is particularly true when columns like the Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 are manufactured to have similar bonding chemistries and use similar retention mechanisms. Additionally, superficially porous particle columns can perform the same analysis as sub-2-µm columns, while generating less backpressure. This allows analysts to increase flow rates for higher throughput, or to increase column length to enhance resolution without exceeding the system pressure limits.

One asset of the Agilent ZORBAX family of HPLC columns is the scalability of methods between particle sizes. This allows a quick and reliable transfer of methods from method development to preparative lab and high throughput analysis.

Agilent Technologies

Several recent comparisons of Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 have shown very similar chromatography. Poroshell 120 was designed to deliver 90 % of the efficiency of sub two micron columns such as Eclipse Plus C18 at approximately 60 % of the pressure. Superficially porous particles found in Poroshell 120 have the low pressure benefits of larger particles while achieving the performance of sub two micron particles.

Examples of this chromatographic similarity are shown using environmental phenols in Figure 1 with 0.1 % Formic acid and in Figure 2 in the analysis of soft drink additives using 10 mM ammonium acetate pH 4.8. In both cases, the retention order of the compounds are the same. The similarity of these two examples leads to the larger question, how similar are Poroshell 120 EC-C18 and Eclipse Plus C18, in terms of selectivity over a wider range of operating conditions and with a larger set of compounds including acids bases and neutral materials.

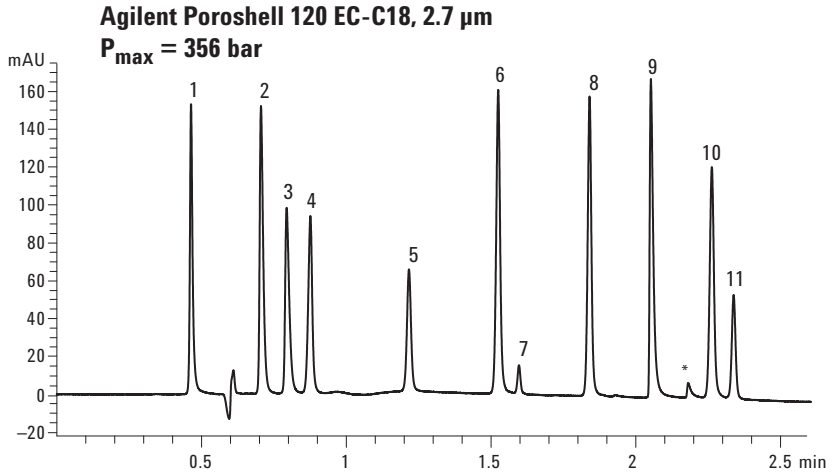


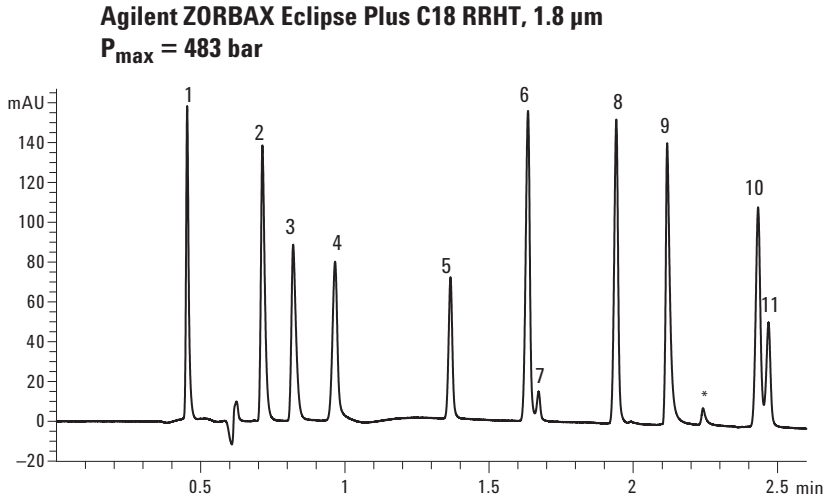
Conditions

Columns	Agilent Poroshell 120 EC-C18, 4.6 mm × 100 mm, 2.7 µm Agilent p/n 689975-902 Agilent ZORBAX Eclipse Plus RRHT C18, 4.6 mm × 100 mm, 1.8 µm Agilent p/n 959964-902
Mobile phase	A: 0.1% Formic acid B: MeCN + 0.1% Formic acid
Temperature	40 °C
Detection	275 nm
Injection volume	10 µL
Flow	2 mL/min
Initial	8% B, 10 min 30% B

1. Hydroquinone	6. o-cresol
2. Resorcinol	7. 2-Nitrophenol
3. Catechol	8. 2,3 Dimethyl phenol
4. 4-Nitrophenol	9. 2,5 Dimethyl phenol
5. p-cresol	10. 1-Naphtol

Figure 1. Comparison of Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 using acetonitrile and formic acid mobile phase for the analysis of environmental phenols.





Conditions

Columns	Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm Agilent p/n 695975-302 Agilent ZORBAX Eclipse Plus C18 RRHT, 3.0 mm × 100 mm, 1.8 µm Agilent p/n 959964-302
Mobile phase	A: 20 mM Ammonium acetate, pH 4.80 B: Acetonitrile
Gradient	14% B at t_0, ramp to 52% B in 2.1 min
Flow rate	0.851 mL/min
Temperature	30 °C

1. Ascorbic Acid
2. Acesulfame K
3. Saccharin
4. p-Hydroxybenzoic Acid
5. Caffeine
6. Benzoic Acid
7. Aspartame
8. Sorbic Acid
9. Quinine
10. Dehydroacetic Acid
11. Methylparaben

* Quinine Impurity

Figure 2. Comparison of Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 using acetonitrile and ammonium acetate mobile phase for the analysis of soft drink addities.

Experimental

Method development is often based upon the use of a generic gradient. Using a short Agilent Poroshell 120 EC-C18, 4.6 x 50 mm column, several different mobile phases can be quickly evaluated. The generic gradient used in this work is run at 2.0 mL/min, starts at 5% organic and increases to 95% organic over 2 min and holds at this concentration for 1 min. Mass spectrometer compatible mobile phases consisting of volatile buffers such as ammonium formate buffer and ammonium acetate buffer are used. These buffers were prepared by dissolving sufficient ammonium formate or ammonium acetate in water to produce 10 mM solutions and titrating the solutions to the desired pH with the appropriate concentrated acid. The pH of these buffers covers a range between 3 and 6.5.

An Agilent 1200 Method Development Solution LC system was used for this work:

- G1312B Binary Pump SL
- G1367D Automatic Liquid Sampler (ALS) SL
- Two G1316C Thermostatted Column Compartments (TCC) SL
- G1315C Diode Array Detector (DAD) SL, using a G1315-60024 micro flow cell (3-mm path, 2-µL volume)
- ChemStation version B.04.01 was used to control the HPLC and to process the data.

Correlation data was calculated and plotted using Microsoft Excel 7.0.

Four Agilent Poroshell 120 EC-C18 columns were used in this work:

- Agilent Poroshell 120 EC-C18, 4.6 mm × 50 mm, 2.7 µm p/n 699975-902
- Agilent Poroshell 120 EC-C18, 3 mm × 100 mm, 2.7 µm p/n 695975-302
- Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 µm p/n 959943-902
- Agilent ZORBAX Eclipse Plus C18, 3 mm × 100 mm, 1.8 µm p/n 959964-302

Table 1 summarizes the list of compounds studied for this work. These compounds were prepared in water or 50/50 water/acetonitrile and injected individually.

Table 1. Sixty-six Compounds Including Acids, Bases and Neutrals Prepared in 50/50 MeCN/Water and Injected onto 4.6 x 50 mm Columns Individually

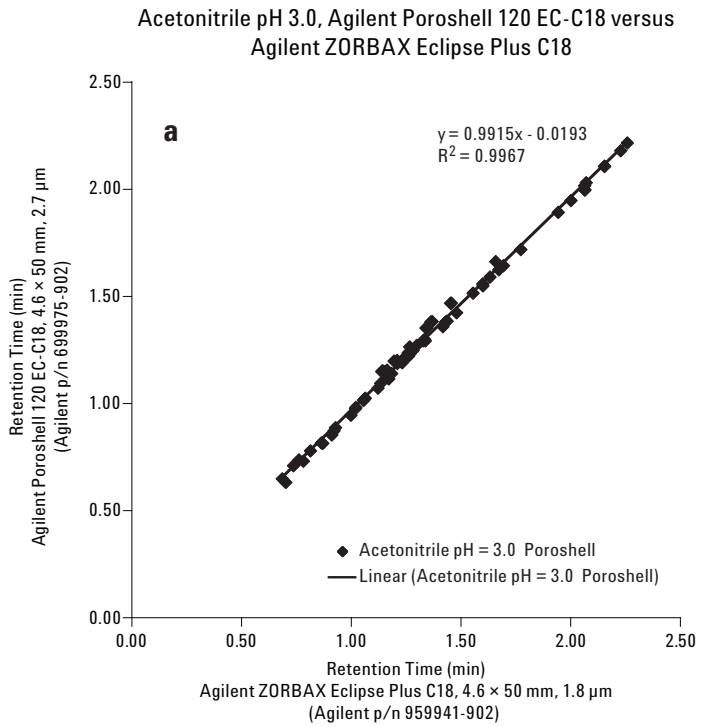
List of tested compounds	
furazolidone	phenacetin
chloramphenicol	acetanilide
impramithue	phenol
norethindrail	resorcial
cortisone acetate	hydroquinone
chloramphenicol	4 nitro phenol
busirone hydrochloride	o cresol
benzocaine	1 napthol
pyrimethamine	imipramine hydrochloride
sulfaquinoxaline	3 4 dihydroxy l phenyl alanine
sulfamonomethoxine	dl phenyalanine
nimopidin	ephedrine hydrochloride
sulfadimethoxine	loperamide
sulfamethoxazole	dibenzofuran
sulfachloropyridazine	procaine hydrochloride
sulfamethoxypyridazine	exonazole nitrate
sulfamethizole	gembigrozil
sulfamerazine	beta estradiol
sulfathiazole	metoprolol
sulfadiazine	protriptyline
benzaldehyde	hydroxy sophthalic
phenanthrene	flufenamic acid
biphenyl	pramoxine hydrochloride
acenaphthene	naproxen
methoxy naphthalene	diphenhydramine
dimethoxy benzene	diflunisal
alpha hydroxyprogesterone	nisoldipin
progesterone	diclofenac
prednisolone	hydrocortisone
deoxycorticosterone	procainamide hydrochloride
chlorphenamine	lidocaine
berberine	terfenaine
chlortetracycline hydrochloride	chlorpheniramine maleate

Discussion

Differences in column performance have been studied by many including Wilson, Nelson, Gilroy, Dolan, Snyder and Carr [5,6]. The United States Pharmacopeia lists many columns [7] and a tool to determine how interchangeable columns may be. Characteristics such as silica chemistry and bonding can change selectivity. Silanol activity affects peak shape dramatically through secondary interactions. It also can affect selectivity through H-bonding or ion-exchange. These effects become more pronounced at higher pH than at lower pH [8]. Both Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 Columns are made from silica produced by Agilent at the same facility that makes the final columns. Both are intended to be highly inert columns and have been designed to yield excellent peak shape with basic compounds. In addition to the effect of pH, silanol activity can also be affected by differences in solvent. Methanol is an H-bonding solvent that has weaker elution strength than aprotic acetonitrile [10]. By choosing a wide range of conditions, it is more likely that differences in selectivity will be revealed.

Figure 3 shows similar retention of 66 compounds on Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 columns using a generic gradient analysis with a variety of compounds from different chemical classifications. The high correlation coefficient (R^2) indicates a high degree of similarity between the interactions involved in the separation on the two Agilent C18 columns, while a slope of approximately 1 implies similar interaction strengths [9,10].

Generic Gradients using Acetonitrile, Buffered with 10 mM Ammonium Formate or Ammonium Acetate between pH 3 and 6.5



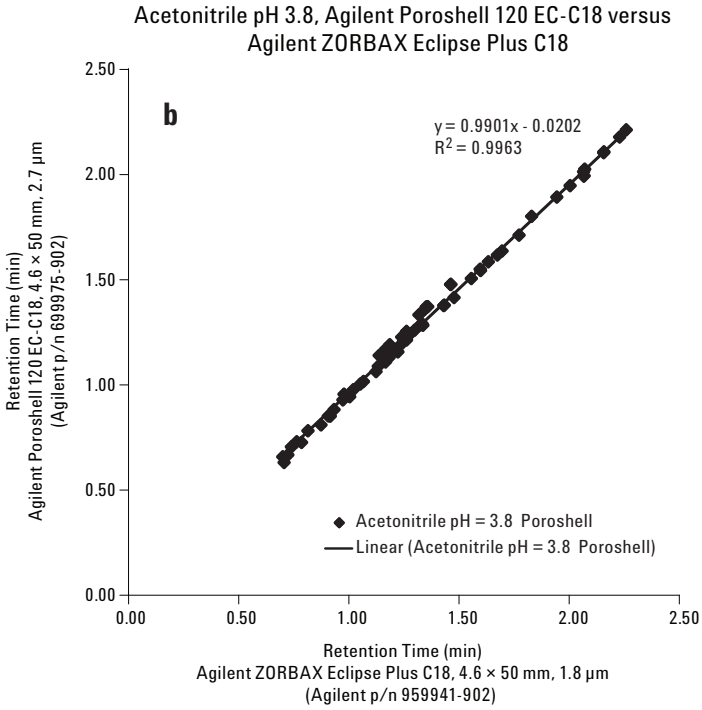
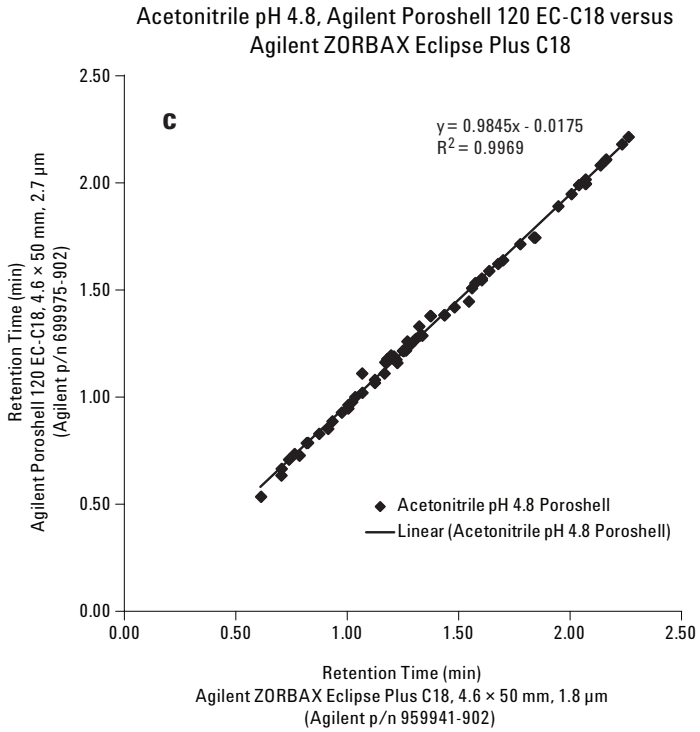
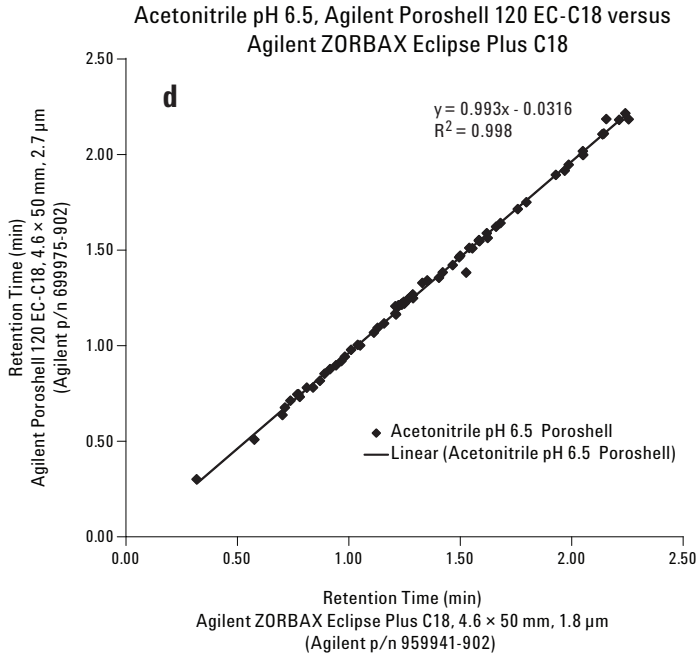


Figure 3. Scatter plot of retention time of 66 compounds on Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm versus Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 1.8 µm. (continued)





Conditions

Mobile phase	A: 10 mM Buffer B: Organic (ACN)
Gradient	5% B at t_0, ramp to 95% B in 2 min, hold 95% B for 1 min
Flow rate	2 mL/min
Sample	1 µL of 1 mg/mL standard in H_2O or H_2O/ACN

Figure 3. Scatter plot of retention time of 66 compounds on Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm versus Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 1.8 µm.

Figure 4 shows scatter plots of the retention times of 66 compounds on Agilent Poroshell 120 EC-C18 versus Agilent ZORBAX Eclipse Plus C18 columns at different pH values between 3 and 6.5 in acetonitrile. Figure 2 shows scatter plots at different pH values between 3 and 6.5 in methanol. The slope and R^2 values for these combinations are summarized in Table 2. As illustrated, the correlation between the two plots is quite good. While retention times sometimes change with the ionic compounds, the changes are proportional on both columns. A slight difference in the slopes of the correlation curves may indicate some difference in H bonding interaction between Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 when comparing the acetonitrile and methanol data (slope of 0.99 and slope of 1.01), but this is not likely to cause any problems in method transfer and is only measureable given the large number of experiments and compounds studied.

Generic Gradients using Methanol, Buffered with 10 mM Ammonium Formate or Ammonium Acetate between pH 3 and 6.5

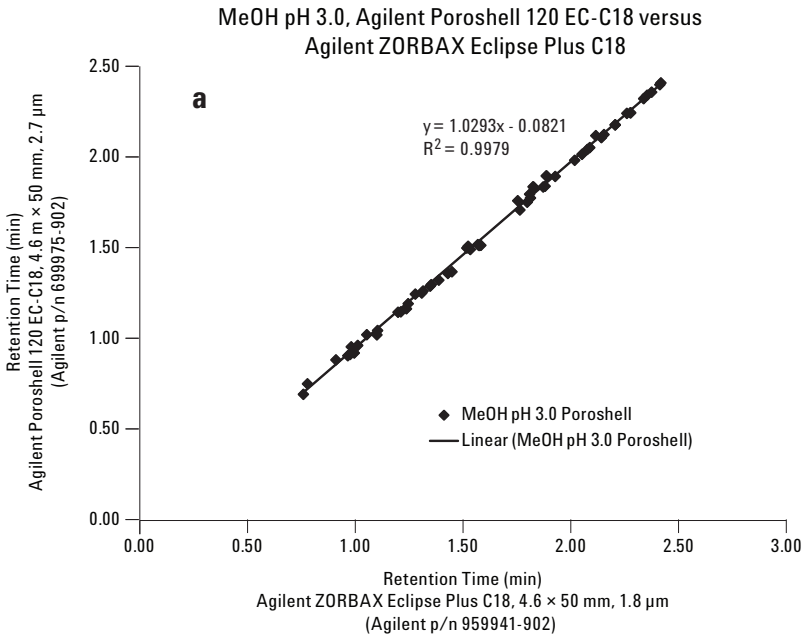
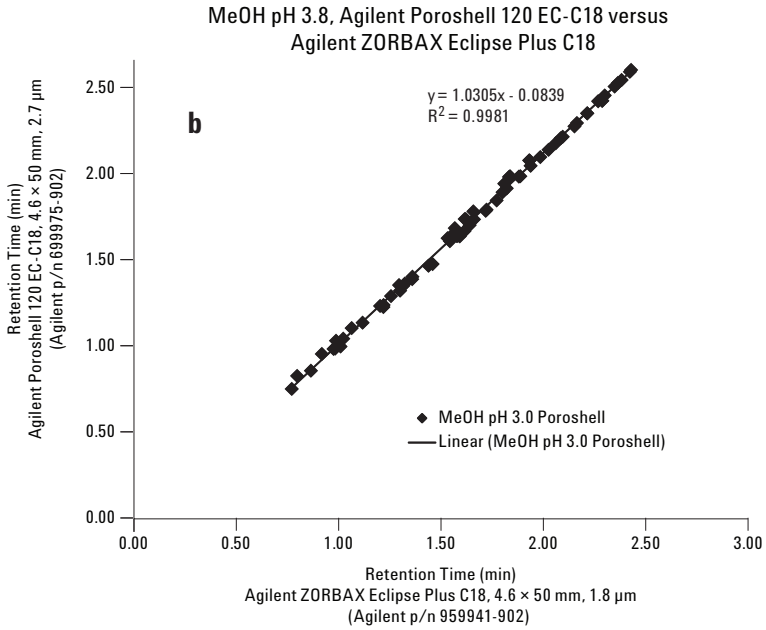


Figure 4. Scatter plot of retention time of 66 compounds on Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm versus Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 1.8 µm. (continued)



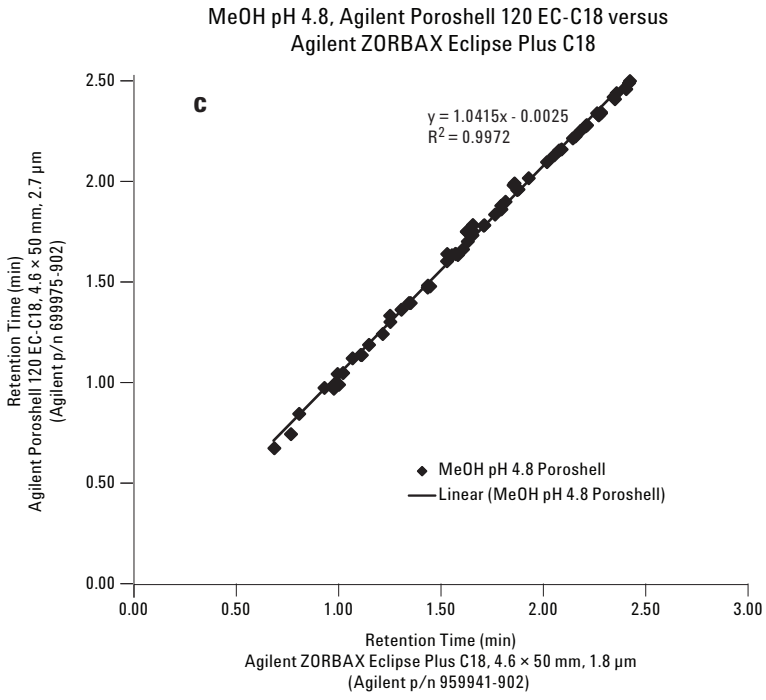
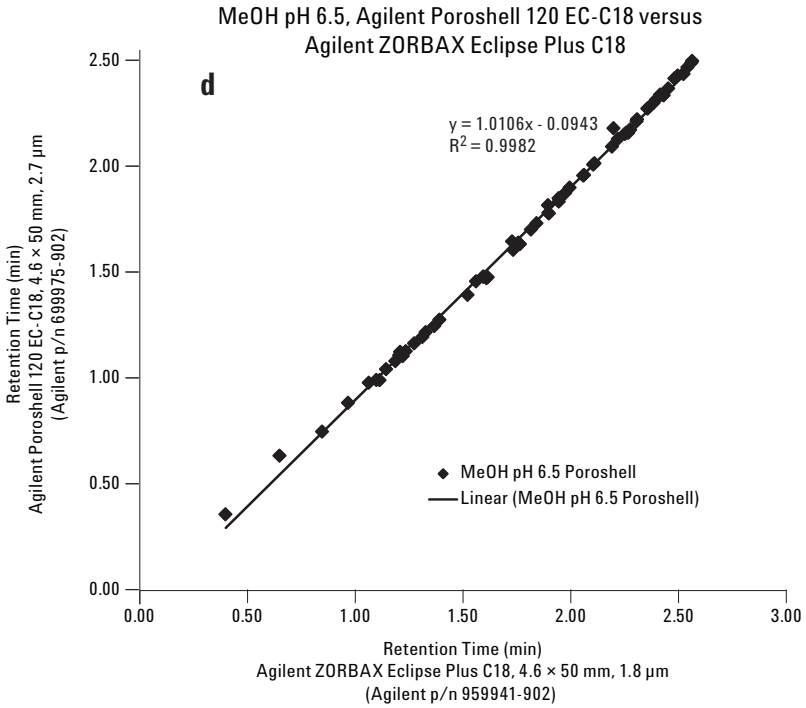


Figure 4. Scatter plot of retention time of 66 compounds on Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm versus Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 1.8 µm. (continued)



Conditions

Mobile phase:	A: 10 mM Buffer
	B: Organic (MeOH)
Gradient:	5% B at t_0, ramp to 95% B in 2 min, hold 95% B for 1 min
Flow rate:	2 mL/min
Sample:	1 µL of 1 mg/mL standard in H_2O or H_2O/ACN

Figure 4. Scatter plot of retention time of 66 compounds on Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm versus Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 1.8 µm.

Table 2. Summary of Correlation Data

Acetonitrile	Methanol
a. pH =3.0 y = 0.9915x -0.0193 R² = 0.9967	a. pH =3.0 y = 1.0293x -0.0821 R² = 0.9979
b. pH =3.8 y = 0.9901x -0.0202 R² = 0.9963	b. pH =3.8 y = 1.0305x -0.0839 R² = 0.9981
c. pH =4.8 y = 0.9845x -0.0175 R² = 0.9969	c. pH =4.8 y = 1.0415x -0.002 R² = 0.9972
d. pH =6.5 y = 0.993x -0.0316 R² = 0.998	d. pH =6.5 y = 1.0106x -0.0943 R² = 0.9982

Another benefit of the Agilent Poroshell 120 columns over sub-2-µm columns is lower operating pressure. The pressure is related to the particle size of the column; larger particles naturally yield lower pressure than smaller particles. In addition to the particle size, the pressure generated inside a column is dependent upon several other factors including solvent linear velocity, and solvent viscosity at a given composition and temperature. While this is a gradient study, the most viscous solvent composition in this study occurs between 40/60 and 50/50 methanol/water. At 25 °C the viscosity of this solvent is 1.62 cP. The most viscous acetonitrile composition is 10/90 acetonitrile/water. At 25 °C the viscosity of this solvent is 1.01 cP [11]. As indicated in the references the viscosity of the solutions is inversely dependent on the temperature. The pressure verses linear velocity graphs for Agilent Poroshell 120 EC-C18 columns and Agilent ZORBAX Eclipse Plus C18 1.8 µm columns are shown for both solvent pairs as Figures 5 and 6. In this case 100 mm columns are used. As stated earlier, this benefit can allow the use of longer columns achieving the same pressure (and larger injection volumes), or higher flow rates.

Differences in selectivity are more likely to occur in cases where the pore size difference becomes more important, typically for compounds between 1500 and 2500 mw. Compounds such as PAHs that involve shape selectivity may also be problematic.

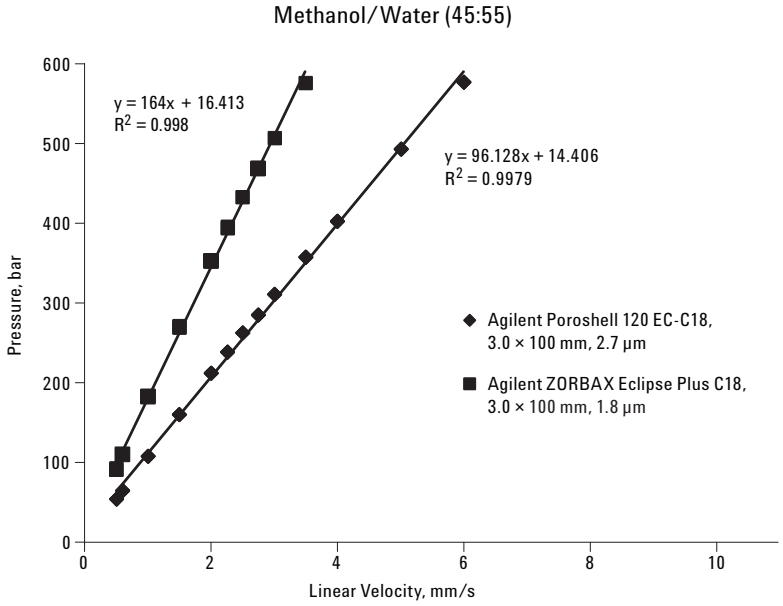


Figure 5. Pressure measured at varied linear velocities indicates lower operating pressure for Agilent Poroshell 120 than an a 1.8 µm column of similar length.

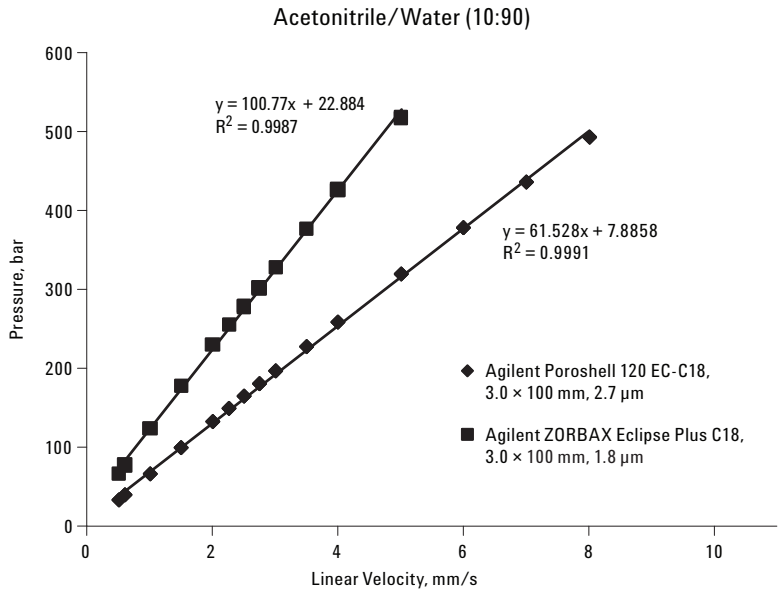


Figure 6. Pressure measured at varied linear velocities indicates lower operating pressure for Agilent Poroshell 120 than an a 1.8 µm column of similar length.

Conclusions

This work has demonstrated the equivalence of selectivity between Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 columns across a wide range of pH and mobile phase conditions. Both column chemistries are manufactured using similar materials with similar proprietary bonding chemistries. Both columns were designed to achieve excellent peak shapes for bases without sacrificing low pH peak shape and performance for other compounds. The benefit of using Agilent Poroshell 120 EC-C18 columns is high efficiency at a lower backpressure. Based on this work, it is expected that if the need arises methods developed on Agilent ZORBAX Eclipse Plus C18 columns can be reliably transferred to Agilent Poroshell 120 EC-C18 columns and conversely with low risk.

References

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