Improved Aromatic Acids Analysis: Saving Significant Time, Solvent, Sample and Money with 1.9 µm Agilent InfinityLab Poroshell 120 Columns

Application Note

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Abstract

Six aromatic acids were analyzed using a formic acid and acetonitrile gradient. The performance of four columns was evaluated for this separation. The original analysis was performed on a 4.6 × 250 mm, 5 µm totally porous particle Agilent ZORBAX Eclipse Plus C18 column, and compared to 1.9, 2.7, and 4 µm superficially porous particle Agilent InfinityLab Poroshell 120 EC-C18 columns in 2.1 × 50 mm format. The selectivity of all columns was similar, requiring no method development between column comparisons. All columns achieved successful separation of the aromatic acids, with a minimum resolution of 1.8. Compared to the long 5 µm column, the short 1.9 µm InfinityLab Poroshell 120 EC-C18 column was able to achieve better minimum resolution (2.9 versus 2.4), 12x faster, while using 96 % less mobile phase and 75 % less sample. Further method modifications showed that the 1.9 µm InfinityLab Poroshell 120 EC-C18 column can be pushed even faster to fully resolve these six aromatic acids in 0.35 minutes, with a minimum resolution of 2.3 at a speed 103x faster than the original analysis.

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Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. Superficially porous particle columns generate high efficiency at lower pressure, relative to their totally porous particle column counterparts[1]. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column[2]. The current trend with superficially porous particles is reducing particle size for further efficiency improvements. The higher efficiency can be used to speed up analyses or improve results by increasing resolution and sensitivity.

Within the Agilent family of LC columns, significant work has been done to ensure easy method transferability and scalability. Stationary phase chemistries are similar among totally porous particle Agilent ZORBAX and superficially porous particle Agilent InfinityLab Poroshell columns so that methods can easily be updated without needing further method development[3].

This work shows how small Poroshell particles can be used to improve a separation of aromatic acids compared to a traditional 5 µm analyses. Significant time, solvent, sample, and money savings are demonstrated with the use of Poroshell columns.

Experimental

An Agilent 1290 Infinity LC system was used in this experiment. The system was modified from its standard configuration to have low system volume and dispersion. Table 1 shows the configuration details and the four Agilent LC columns used in this experiment.

Table 2 shows the LC method parameters for all analyses. With the exception of the fast 1.9 µm analysis, all methods were geometrically scaled according to column volume to preserve the chromatographic separation from column to column.

Table 1. UHPLC system configuration.

Agilent 1290 Infinity LC System Configuration	
Agilent 1290 Infinity Binary Pump (G4220A)	35 µL Solvent mixer: Agilent Jet Weaver, 35 µL/100 µL (G4220-60006)
Agilent 1290 Infinity High Performance Autosampler (G4226A)	Seat assembly, ultralow dispersion, for Agilent 1290 Infinity Autosampler (G4226-87030) Autosampler to heater: capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784) Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (5182-0716) Cap, screw, blue, PTFE/red silicone septa, 100/pk (5182-0717) Vial insert, 250 µL, glass with polymer feet, 100/pk (5181-1270)
Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)	Heat exchanger, low dispersion, 1.6 µL, double (G1316-60005) Heater to column: InfinityLab Quick Connect assembly, 105 mm, 0.075 mm (5067-5961) Column to flow cell: capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784)
Agilent 1290 Infinity Diode Array Detector (G4212A)	Agilent Ultralow dispersion Max-Light cartridge flow cell, 10 mm (G4212-60038)
Agilent OpenLAB CDS ChemStation Edition, revision C.01.05 [35]	G4220A: B.06.53 [0013] G4226A: A.06.50 [003] G1316C: A.06.53 [002] G4212A: B.06.53 [0013]
Agilent LC Columns	Agilent ZORBAX Eclipse Plus C18, 4.6 x 250 mm, 5 µm (959990-902) Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm (699675-902) Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm (699775-902) Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 4 µm (699770-902)

Table 2. UHPLC method parameters.

Column	Mobile phase	Flow rate (mL/min)	Gradient	Injection volume (µL)	Sample	Thermostatted Column Compartment (°C)	Diode Array Detector
Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm	A) 0.2 % formic acid in water B) acetonitrile	1.0	8–35 %B in 36 minutes	20	0.01 mg/mL each of protocatechuic acid, 3,4-dihydroxyphenylacetic acid (DOPAC), 4-aminobenzoic acid (PABA), vanillic acid, syringic acid, salicylic acid in water Compounds are listed in elution order, see Figure 1 for structures.	25	280 nm, 80 Hz
Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm		0.5	8–35 %B in 3 minutes	5			
Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm							
Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 4 µm							
Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm (fast)		2.2	8–26 %B in 0.3 minutes			60	280 nm, 160 Hz

Six aromatic acids were analyzed in this work; their structures are shown in Figure 1. Each of these compounds, as well as the formic acid mobile phase modifier, was purchased from Sigma-Aldrich. Acetonitrile was purchased from Honeywell (Burdick and Jackson). Water was 0.2 µm filtered 18 MW from a Milli-Q system (Millipore).

Results and Discussion

The original separation of six aromatic acids was demonstrated on a 4.6 × 250 mm, 5 µm totally porous particle ZORBAX Eclipse Plus C18 column. This separation was accomplished in 36 minutes with a minimum resolution of 2.4, and is shown in Figure 2. Figure 2 also shows the same LC method transferred to a 2.1 × 50 mm, 1.9 µm superficially porous particle InfinityLab Poroshell 120 EC-C18 column. ZORBAX Eclipse Plus C18 and Poroshell 120 EC-C18 have similar bonded chemistries for highly correlated overall selectivity, so no changes to the method are needed. However, because the columns are different dimensions, the gradient, flow rate, and injection volume are geometrically scaled for the smaller column volume. Flow rate is also increased for the 1.9 µm Poroshell column to run at the optimal flow rate for this smaller particle. The resulting chromatogram is 12x faster, uses 96 % less mobile phase, 75 % less sample, and still maintains a minimum resolution of 2.9.

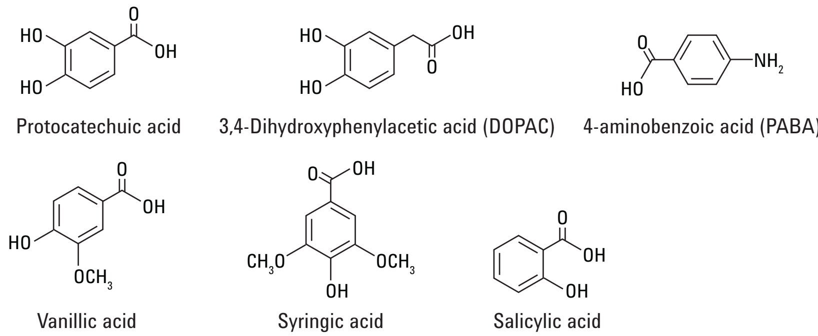

Figure 1. Compounds of interest

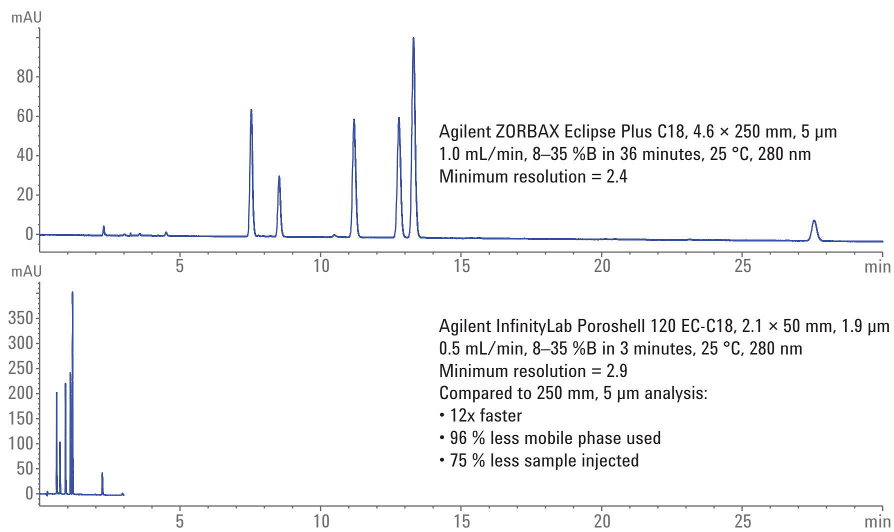

Figure 2. A 250 mm, 5 µm Agilent ZORBAX analysis of aromatic acids is improved by transferring to a high-performance 50 mm, 1.9 µm Agilent InfinityLab Poroshell column; minimum resolution is improved, while saving significant time, sample, solvent, and money.

The 1.9 µm Poroshell analysis generates 472 bar in pressure. This may be inhibitory for some analysts depending on their instrument pressure specifications. Within the Agilent Poroshell 120 family there are three particle size options: 1.9, 2.7, and 4 µm. These different particle sizes use the same bonded phases for easy method transferability. Figure 3 shows the same selectivity for all three particle sizes using this separation of aromatic acids; all method parameters are identical. As particle size increases, pressure decreases. However, this comes at the cost of reduced resolution and peak capacity with larger particle sizes. In this situation, an analyst should choose the column that best suits their needs, whether they are limited by the operating pressure of their existing instrumentation, or if they are looking for the best possible resolution.

The benefit of using small superficially porous particles is that they offer high efficiency and resolution. This high performance can be used to run ultrafast analyses, if your LC system pressure limit allows. In Figure 4, the 1.9 µm Poroshell column was pushed to its limit. High temperature was used to decrease the mobile phase viscosity so that the flow rate could be pushed to 2.2 mL/min, generating 1,150 bar. The gradient also was slightly adjusted from the previous methods to ensure the fastest possible separation from this column with this sample of aromatic acids. The result is that all six compounds separated in 0.35 minutes with a minimum resolution of 2.3. Compared to the 250 mm, 5 µm Eclipse Plus method, this analysis is 103x faster, and uses 98 % less mobile phase while maintaining the minimum resolution.

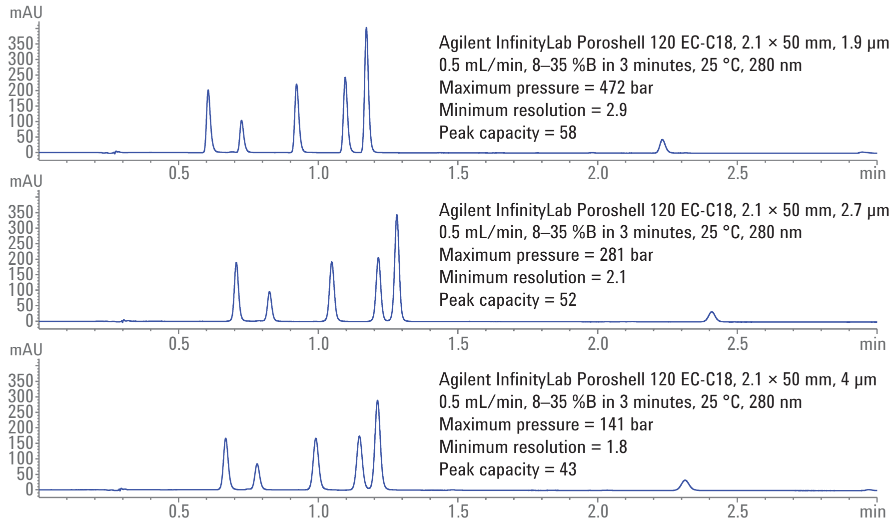

Figure 3. Similar selectivity among Agilent InfinityLab Poroshell particle sizes allows analysts to choose their column configuration based on instrument pressure limits or method performance requirements without needing to do additional method development.

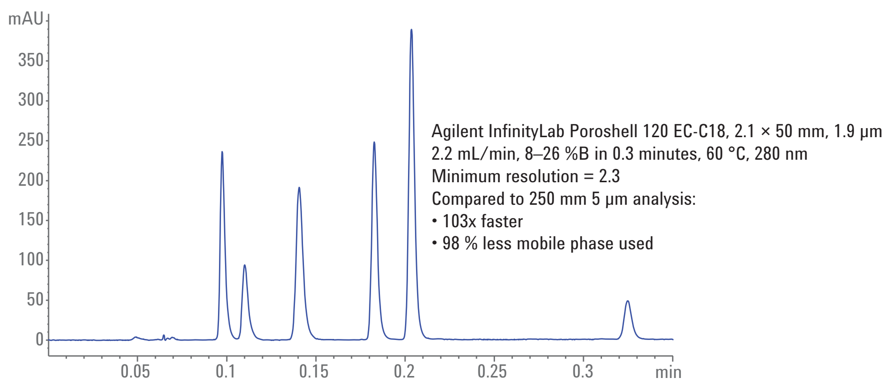

Figure 4. Additional time, solvent, and money can be saved by operating the highly performing 50 mm, 1.9 µm Agilent InfinityLab Poroshell column near its pressure limit without compromising method performance.

Table 3 shows a summary and comparison of all chromatographic results.

Table 3. Comparison of aromatic acid analyses with different LC columns.

Column	Pressure (bar)	Minimum resolution	Conditional peak capacity (nC)	Run time (min)		Mobile phase consumption (mL)	
Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm	193	2.4	88	36	Original 5 µm analysis	36	Original 5 µm analysis
Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm	472	2.9	58	3.0	12x Faster than original 5 µm analysis	1.5	Uses 96 % less mobile phase than original 5 µm analysis
Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm	281	2.1	52				
Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 4 µm	141	1.8	43				
Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm (ultrafast)	1150	2.3	37	0.35	103x Faster than original 5 µm analysis	0.77	Uses 98 % less mobile phase than original 5 µm analysis

Conclusions

The highly efficient 1.9 µm Agilent InfinityLab Poroshell 120 column can be used to improve existing methods using traditional columns, such as a 5 µm Agilent ZORBAX Eclipse Plus C18. The Poroshell and ZORBAX families offer similar bonded phase chemistries so that methods are easily transferred, often requiring no additional method development. If the pressure generated by the 1.9 µm particle is too high for LC system limits, there are also Agilent Poroshell 120, 2.7 and 4 µm particles available that offer the same selectivities. However, when LC system pressure limits allow, the 1.9 µm Poroshell column can be pushed to accomplish an ultra-fast separation, which will save significant time, solvent, sample, and money compared to traditional LC columns.

References

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