Achieve Ultrafast Separations with Agilent InfinityLab Poroshell 120 EC-C18, 1.9 µm Columns

Application Note

Agriculture, Food Testing, Small Molecule Pharmaceuticals

Author

Anne Mack
Agilent Technologies, Inc.

Abstract

Ten compounds were baseline-separated in 0.3 minutes on a 2.1 × 50 mm, 1.9 µm Agilent InfinityLab Poroshell 120 column with a minimum resolution of 2.2. Compared to the same analysis performed on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm column, the InfinityLab Poroshell column was able to run 120x faster while saving 98 % of the mobile phase. Both columns have similar selectivity, enabling easy method transfer.

Agilent Technologies

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 very 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 alkyphenones compared to a traditional 5 µm analysis. Significant time, solvent, sample, and money savings are demonstrated with the use of InfinityLab 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 lists the two Agilent LC columns that were 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.

Ten compounds are analyzed in this work. Nine alkyphenones were purchased as a standard solution from Agilent. Thiourea, purchased from Sigma-Aldrich, was added to the standard to serve as a void volume marker. Acetonitrile was purchased from Honeywell (Burdick and Jackson). Water was 0.2 µm filtered 18 MW from a Milli-Q system (Millipore).

Table 1. UHPLC System Configuration

Agilent 1290 Infinity LC System Configuration	
Agilent 1290 Infinity Binary Pump (G4220A)	35 µL Solvent mixer: 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 x 220 mm, SV/SLV (5067-4784)
Agilent 1290 Infinity Diode Array Detector (G4212A)	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 × 250 mm, 5 µm (959990-902) Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm (699675-902)

Table 2. UHPLC Method Parameters

Column	Mobile phase	Flow rate (mL/min)	Gradient	Injection volume (µL)	Sample	Thermostated column compartment (°C)	Diode array detector
Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm	A) water B) acetonitrile	1.0	35–95 %B in 36 minutes	20	p/n 5188-6529 (~1 mL) + 0.1 mL of 1 mg/mL thiourea	25	254 nm, 80 Hz
Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm		0.5	35–95 %B in 3 minutes	1			254 nm, 80 Hz
Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm (fast)		2.3	50–100 %B in 0.3 minutes	1		60	254 nm, 160 Hz

Results and Discussion

The original separation of 10 compounds is demonstrated on a totally porous particle Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm column. This separation was accomplished in 36 minutes with a minimum resolution of 6.1, and is shown in Figure 1, which also shows the same LC method transferred to a superficially porous particle Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm column. ZORBAX Eclipse Plus C18 and InfinityLab Poroshell 120 EC-C18 have very similar bonded chemistries for highly correlated overall selectivity, so no changes to the method were needed. However, because the columns were different dimensions, the gradient, flow rate, and injection volume were geometrically scaled for the smaller column volume. The flow rate was increased for the InfinityLab Poroshell 1.9 µm column to run at the optimal flow rate for this smaller particle. The resulting chromatogram is 12x faster, uses 96 % less mobile phase, 95 % less sample, and still maintains a minimum resolution of 5.5.

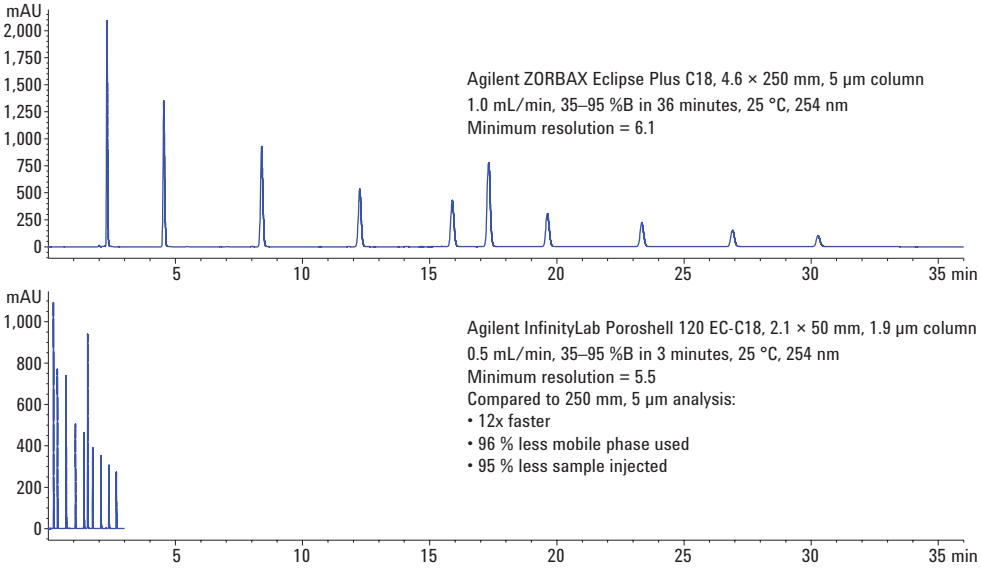

Figure 1. An Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm column analysis of alkylphenones is improved by transferring to a high performance Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm column, saving significant time, sample, solvent, and money.

The benefit of using small superficially porous particles is that they offer very high efficiency and resolution. This high performance can be used to run ultrafast analyses, if your LC system pressure limit allows. In Figure 2, the InfinityLab Poroshell 1.9 µm 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 2.3 mL/min, generating 1,150 bar. The gradient was slightly adjusted from the previous methods to ensure the fastest possible separation from this column with this 10-component sample. The result was that all 10 compounds separated in 0.3 minutes with a minimum resolution of 2.2. Compared to the Eclipse Plus 250 mm, 5 µm method, this analysis was 120x faster, and used 98 % less mobile phase while maintaining the minimum resolution.

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

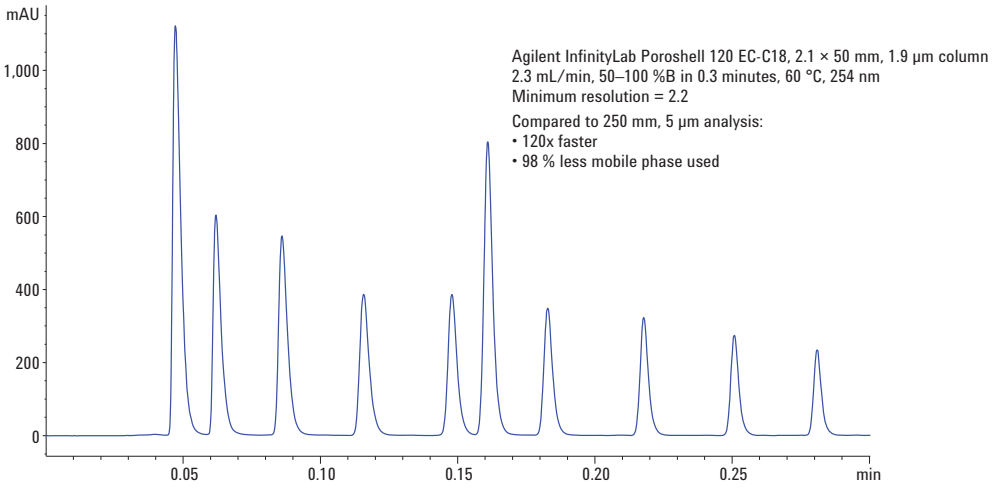

Figure 2. Additional time, solvent, and money can be saved by operating the high-performing Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm column near its pressure limit without compromising method performance.

Table 3. Comparison of Alkyphenone 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, 5.0 µm	181	6.1	134	36	Original 5 µm analysis	36	Original 5 µm analysis
Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm	420	5.5	91	3.0	12x Faster than original 5 µm analysis	1.5	Uses 96 % less mobile phase than original 5 µm analysis
Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm (ultrafast)	1150	2.2	43	0.3	120x Faster than original 5 µm analysis	0.69	Uses 98 % less mobile phase than original 5 µm analysis

Conclusions

The highly efficient Agilent InfinityLab Poroshell 120, 1.9 µm column can be used to improve existing methods using traditional columns, such as an Agilent ZORBAX Eclipse Plus C18, 5 µm column. The InfinityLab Poroshell and ZORBAX families offer similar bonded phase chemistries so that methods are easily transferred, often requiring no additional method development. When LC system pressure limits allow, the InfinityLab Poroshell 1.9 µm column can be pushed to accomplish an ultrafast separation, which will save significant time, solvent, sample, and money compared to traditional LC columns.

References

1. A. Gratzfield-Hugsen, E. Naegele. Maximizing efficiency using Agilent Poroshell 120 Columns. *Agilent Technologies Application Note*, publication number 5990-5602EN, **2016**.
2. V. R. Meyer. Practical High Performance Liquid Chromatography. Fourth Edition, p. 34. Wiley (2004).
3. Transfer of Methods between Poroshell 120 EC-C18 and ZORBAX Eclipse Plus C18 Columns. *Agilent Technologies Application Note*, publication number 5990-6588EN, **2011**.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
November 2, 2016
5991-7540EN

Agilent Technologies