Maximizing Efficiency Using Agilent InfinityLab Poroshell 120 Columns

100,000 Plates in Less Than 5 Minutes Using Coupled Column Technology

Application Note

Food, Environmental, Chemical, Pharmaceutical

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Abstract

Columns based on superficially porous technologies are an alternative to sub-2 µm particle based columns. The combination of these columns with the Agilent 1290 Infinity LC system produces high efficiency separations. Agilent InfinityLab Poroshell 120 columns offer:

- Lower backpressure
- Highest efficiency
- Comparable volume capacity

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Introduction

Recently, sub-2-µm particle columns have gained a lot of interest, due to their high efficiency. They can be used at higher flow rates than those evaluated by the van Deemter equation. The loss in efficiency at higher flow rates is minor in comparison to the efficiency at the optimum flow rate. Run times and cycle times can be shortened and results obtained faster.

The drawback of these columns is that significantly higher backpressures are obtained, due to the small particle sizes. In many cases, especially for long sub-2 µm columns, the LC instrumentation must allow backpressures of > 400 bar.

The superficially porous particle technology offers an alternative for very high resolution analyses[1], because these columns show significantly less backpressure. The efficiency of these columns, compared to that of sub-2 µm particle columns is slightly lower. It is possible to obtain very high plate counts by coupling columns, due to less backpressure.

This Application Note demonstrates that the coupling of three long Agilent InfinityLab Poroshell 120 columns results in extremely high efficiencies. It is also demonstrated that the backpressure can be kept below 400 bar, unless special LC equipment is available. In that case higher flow rates are possible to save analysis and equilibration time. Finally, a comparison was made between one 2.7 µm porous shell column and one sub-2 µm particle size column.

Experimental

Equipment

An Agilent 1290 Infinity LC system equipped with a binary pump, autosampler, thermostatted column compartment and diode-array detector with a 10-mm path length cell was used for the experiments.

Columns

An Agilent ZORBAX Rapid Resolution HT 4.6 mm × 150 mm, 1.8 µm column and an Agilent InfinityLab Poroshell 120, 4.6 mm × 150 mm, 2.7 µm column were used. These columns can be used up to 600 bar.

Software

Agilent ChemStation software revision B.04.02

Results and Discussion

Potential benefits of superficially porous columns

Superficially porous column technology is based on particles with a solid core and a superficially porous shell. These particles consist of a 1.7-µm solid core with a 0.5-µm porous silica shell. In total, the particle size is approximately 2.7 µm. The 2.7 µm superficially porous particles provide 40–50 % lower backpressure and 80–90 % of the efficiency of a sub-2-µm totally porous particle. The superficially porous particles have a narrower particle size distribution than a totally porous particle. This results in a more homogeneous column and reduces diffusion in the column. At the same time the small particle and the porous shell allow for lower resistance to mass transfer. The result is higher flow rates without efficiency loss[1,2].

Configuring the system

The following experiments evaluated the performance of the Agilent InfinityLab Poroshell 120 columns. The internal diameter was 4.6 mm and the column length 150 mm for all columns used.

- Evaluation of the plate number of a single column at 1.5 mL/min
- Evaluation of the plate number for three coupled columns at 1.5 mL/min
- Evaluation of the plate number for three coupled columns at higher flow rates
- Precision of retention times using isocratic and gradient conditions
- Comparison of a porous shell versus a sub-2 µm particle column

Column efficiency (plate number) is typically measured using isocratic conditions. For a symmetrical peak use the following equation to calculate the plate number (N):

$N = 5.54 (RT/W)^2$

RT is the retention time, and W the peak width at half height.

Evaluation of plate numbers for single column

The following compounds were used to evaluate the plate number for a single column: uracil, acetophenone, benzene and toluene.

The resulting chromatogram and evaluated plate numbers are shown in Figure 1.

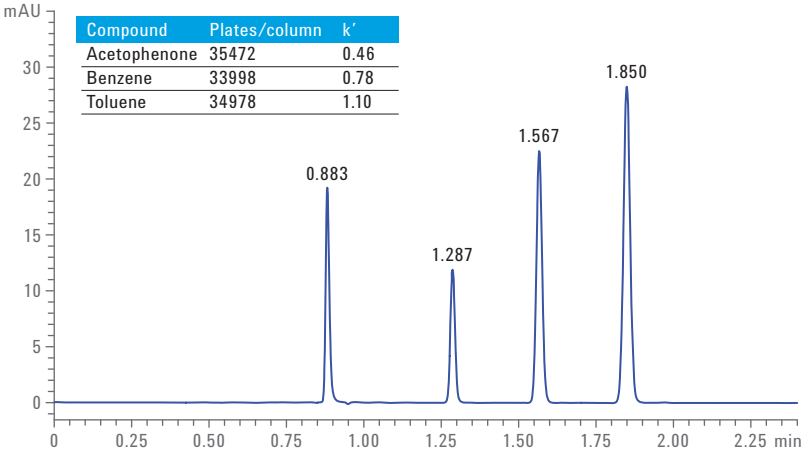
The result was approximately 35,000 plates/column for toluene under the chromatographic conditions specified.

Evaluation of plate numbers for three coupled columns

The plate number for one column is approximately 35,000 plates. The expectation is that three columns deliver a plate number of 105,000 plates. Column coupling was done using stainless steel capillaries, 90 × 0.12 mm. Plate numbers were evaluated for different flow rates.

The resulting chromatograms are shown in Figure 2. If a 400-bar LC system is used, about 80,000 plates can be obtained at 1 mL/min flow rate. However, higher flow rates and efficiencies can be obtained with this LC system, which allows pressures up to 1,200 bar.

At 1.5 mL/min flow rate the obtained plate number of approximately 103,000 plates is close to the expected value.

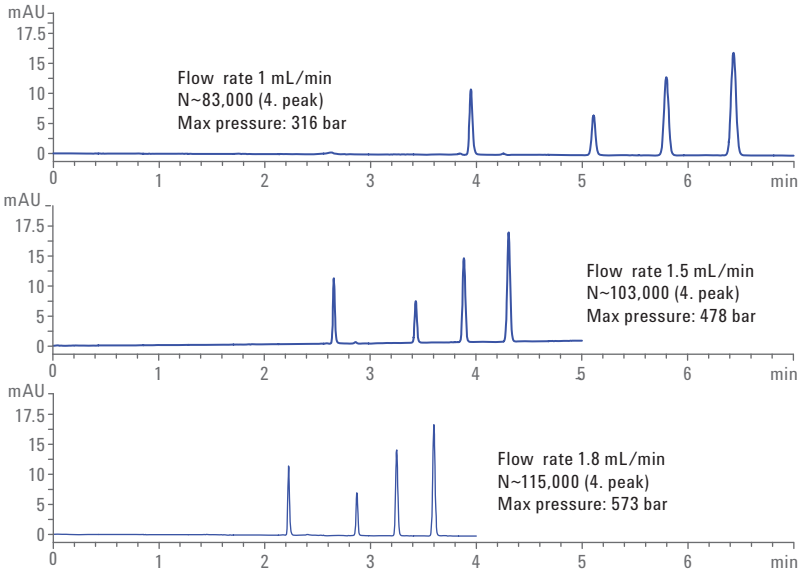


Compound	Plates/column	k'
Acetophenone	35472	0.46
Benzene	33998	0.78
Toluene	34978	1.10

Chromatographic conditions

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm
Sample	Thiourea, acetophenone, benzene, toluene
Mobile phase	Water:ACN = 30:70
Flow rate	1.5 mL/min
Injection volume	1 µL
Column temperature	50 °C
Detector	DAD 254 nm/10, Ref 360/100 nm, 20 Hz, standard cell

Figure 1. Chromatogram to evaluate N for the Agilent InfinityLab Poroshell 120, 4.6 × 150 mm column.



Chromatographic conditions

Parameter	Value
Sample	Thiourea, acetophenone, benzene, toluene
Column	Three coupled Agilent InfinityLab Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm columns
Mobile phase	Water:ACN = 20:80
Flow rate	1, 1.5, 1.8 mL/min
Injection volume	1 µL
Column temperature	60 °C
Detector	DAD 254 nm/10, Ref 360/100 nm, 20 Hz, standard cell

Figure 2. Two chromatograms to evaluate N for three coupled Agilent InfinityLab Poroshell 120, 150 mm × 4.6 mm columns at different flow rates.

The best result for toluene with approximately 115,000 plates was obtained at 1.8 mL/min with a retention time <5 minutes (Table 1).

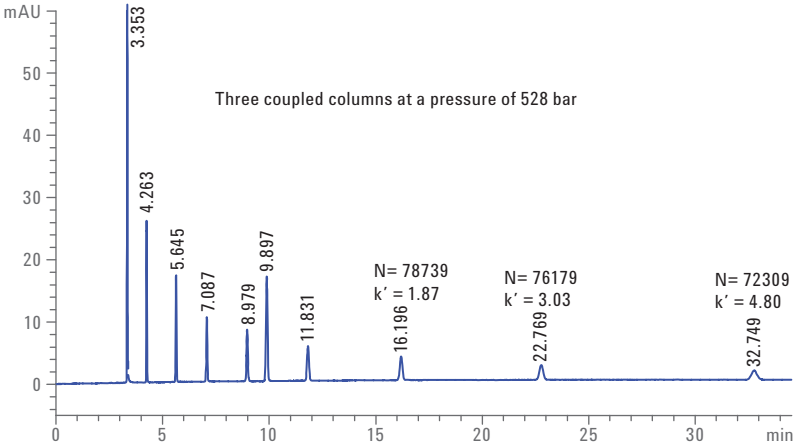
Table 1. Plate numbers at 1.8 mL/min flow rate.

Compound	Plates	k′
Acetophenone	114,120	0.29
Benzene	109,931	0.46
Toluene	114,800	0.62

For higher k′ values good results are obtained using three coupled columns. A flow rate of 1.2 mL/min was used. (Figure 3)

Precision of retention times using isocratic conditions

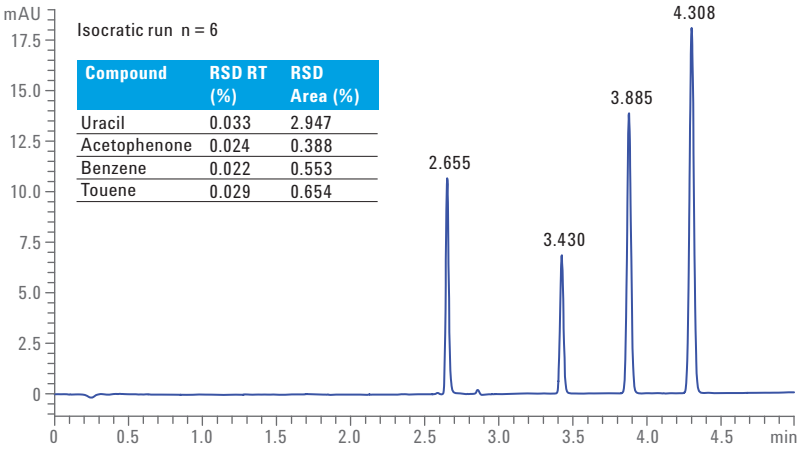
Precision for isocratic conditions at 1.5 mL/min was evaluated and results are shown in Figure 4 together with an overlay of six consecutive runs. The precision of retention times is <0.034 % RSD, and the precision for areas is <0.66 % RSD, except for uracil.



Chromatographic conditions

Parameter	Value
Sample	Thiourea + test sample: Set of nine compounds, 100 ng/µL each, dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone (200 ng/µL), 5. Benzophenone, 6. Valerophenone, 7.Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column	Three coupled Agilent InfinityLab Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm columns
Mobile phase	ACN/Water 60/40
Column temperature	60 °C
Flow rate	1.2 mL/min
Detector	DAD 254 nm/10 nm, ref 360/100 nm, 20 Hz, Standard cell

Figure 3. Plate numbers at higher k′ values for three coupled columns at 528 bar and 1.2 mL/min flow rate.



Chromatographic conditions

Parameter	Value
Sample	Uracil, Acetophenone, Benzene, Toluene
Column	Three coupled Agilent InfinityLab Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm columns
Mobile phase	Water:ACN = 20:80
Flow rate	1.5, mL/min
Injection volume	1 µL
Column temperature	60 °C
Detector	DAD 254 nm/10, Ref 360/100 nm, 20 Hz, standard cell

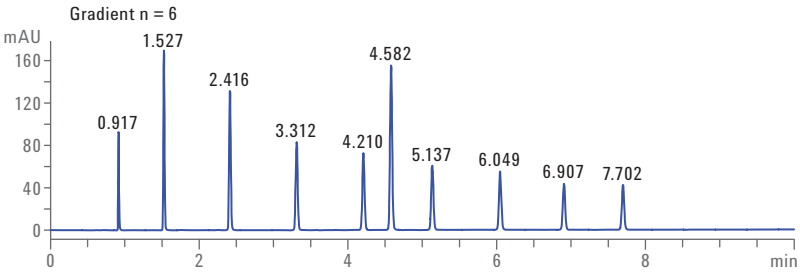
Figure 4. Overlay of six consecutive runs using isocratic conditions and precision data for retention times and areas.

Precision for retention times and areas using gradient conditions

The precision for gradient analysis was evaluated using a gradient from 35 to 95 % in 10 minutes. The results and the overlay of six consecutive runs are shown in Figure 5.

Excellent precision was achieved for retention times of all compounds (RSD < 0.04 %), except for Thiourea (Figure 5).

The RSDs for the areas of all compound peaks were less than 0.38 % for a 1-µL injection.



Peak	RSD RT (%)	RSD Area (%)
Thiourea	0.092	0.372
1	0.020	0.238
2	0.038	0.255
3	0.033	0.211
4	0.029	0.186
5	0.027	0.227
6	0.023	0.194
7	0.018	0.183
8	0.017	0.251
9	0.017	0.167

Chromatographic conditions

Parameter	Value
Sample	Thiourea + Test sample: Set of nine compounds, 100 ng/µL each, dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone (200 ng/µL), 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column	Agilent InfinityLab Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm
Mobile phase	Water and ACN
Gradient	At 0 minutes 35 % ACN, at 10 minutes 95 % ACN
Flow rate	1.5 mL/min
Injection volume	1 µL
Column temperature	60 °C
Detector	DAD 245/10 nm, Ref 400/100 nm, 20 Hz, standard cell

Figure 5. Overlay of 10 consecutive gradient runs and precision data for retention times and areas.

Comparison of the peak capacity of a porous shell column versus a sub-2 µm particle column

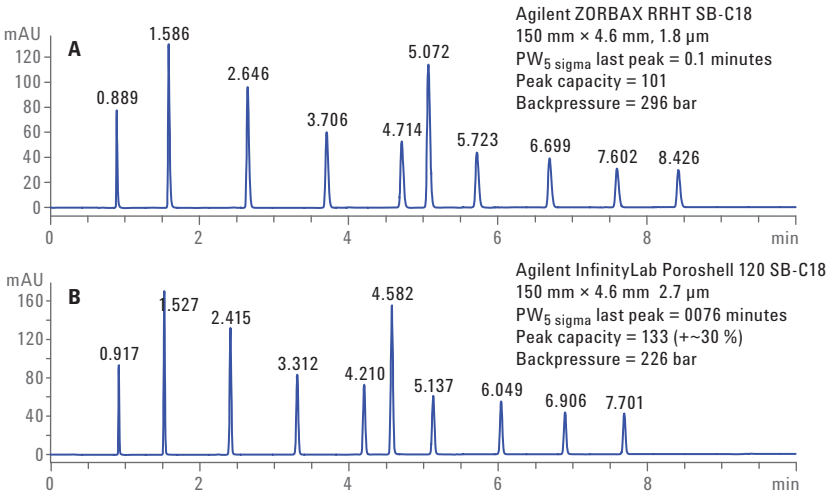
To illustrate the difference between porous shell and sub-2 µm columns, two 150 × 4.6 mm id columns were compared analyzing a set of 10 compounds (Figure 6).

The Agilent InfinityLab Poroshell 120 column shows shorter elution times, and smaller peak width, which results in a higher peak capacity for the porous shell column. The InfinityLab Poroshell 120 column shows 133 peaks with a higher peak capacity than the sub-2 µm column with a peak capacity of 101 peaks. This shows 30 % higher efficiency for the InfinityLab Poroshell 120 column compared to the sub-2 µm column for the conditions used.

Comparison of volume capacity

To test whether porous shell columns have the same or lower volume capacity than column packed with 1.8 µm particles, a highly concentrated sample was injected. The injection volume was 10 µL and the concentration was approximately 20 µg in 10 µL (Figure 7).

No significant differences were observed for the main peak using the selected conditions. The peak width for the InfinityLab Poroshell 120 column was somewhat lower because in this case the peak eluted earlier. The peak width is typically smaller.



Chromatographic conditions

Parameter	Value
Sample	Thiourea + Test sample: Set of nine compounds, 100 ng/µL each, dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone (200ng/µL), Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column	Agilent ZORBAX RRHT SB-C18, 150 mm × 4.6 mm, 1.8 µm Agilent InfinityLab Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm
Mobile phase	Water and ACN
Gradient	0 minutes 35 % ACN, 10 minutes 95 % ACN
Flow rate	1.5 mL/min
Injection volume	1 µL
Column temperature	60 °C
Detector	DAD 245/10 nm, Ref 400/100 nm, 20 Hz, standard cell

Figure 6. Chromatograms of a phenone mix analyzed on porous shell and sub-2 µm particle columns.

Comparison of signal-to-noise

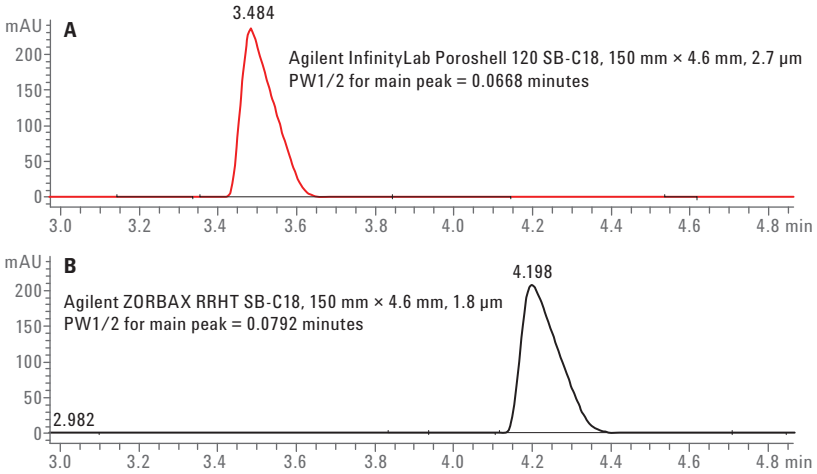
Impurities in a pharmaceutical drug were analyzed to evaluate the signal-to-noise ratio (S/N). The impurities were present in a 0.02–0.03 percentage range. The chromatographic conditions are listed in Figure 7.

Figure 8 shows an overlay of a section of the complete chromatograms. The red trace represents the InfinityLab Poroshell 120 chromatogram and the black trace represents the sub-2 µm chromatogram.

In Table 2, the S/N calculations for both columns are combined. Impurities 1 and 2 were analyzed on the InfinityLab Poroshell 120 column and on the sub-2 µm column.

Table 2. Comparison of signal-to-noise ratios for porous shell and 1.8 µm particle columns.

Peak	Agilent InfinityLab Poroshell 120 S/N	1.8 µm S/N
1	14	13.6
2	12.8	12



Chromatographic conditions

Parameter	Value
Test Sample	Tramadol 2,022 mL/mL containing impurities
Column	Agilent InfinityLab Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm
Pump	
Solvent A	Water + 0.2 % TFA
Solvent B	ACN + 0.16 % TFA
Gradient	17 to 45 % B in 5 minutes, Stop time: 7 minutes, Post time: 3 minutes
Flow rate	1.5 mL/min
Autosampler	
Injection volume	10 µL
Wash time	10 s
Thermostatted Column Compartment	
Temperature	30 °C
DAD	1290 270/10 nm, Ref. 360/100 nm, 20 Hz, Standard flow cell of 10-mm path length

Figure 7. Capacity comparison of porous shell and sub-2 µm columns; injection volume 10 µL = 20 µg.

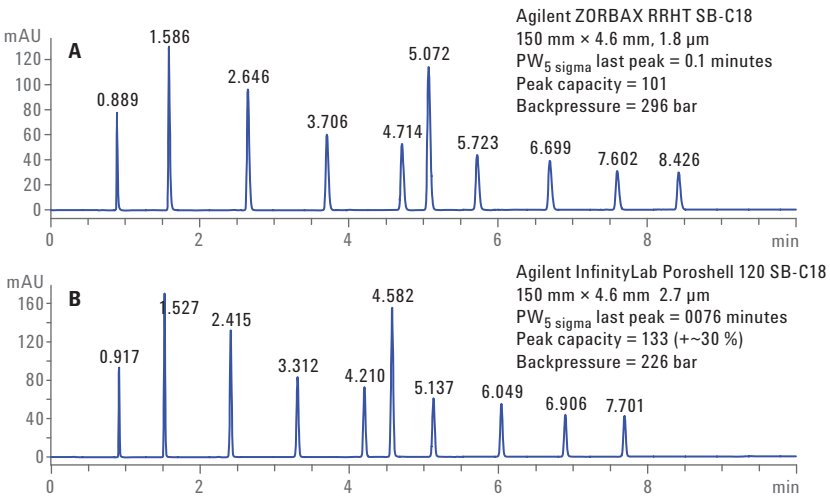


Figure 8. Comparison signal-to-noise ratio; red represents the porous shell column, and the black trace represents the 1.8-µm particle column. Modifier TFA was used.

Conclusion

Porous shell columns represent a real alternative to sub-2 µm columns. The lower backpressure allows flow rates of 1 mL/min for a 4.6 × 150 mm, 2.7 µm column without exceeding the 400 bar limit. In this case, 35,000 plates are achievable or more than 235,000 plates/meter.

Column coupling of three 4.6 × 150 mm columns result in a plate number of 100,000 plates in under 5 minutes without exceeding the 600 bar limit.

Agilent InfinityLab Poroshell 120 columns show excellent precision data for isocratic and gradient analysis.

Typically for InfinityLab Poroshell 120 columns shorter elution times than that of the similar sub 2-µm bonded phase columns can be expected if the same chromatographic conditions are applied. The shorter elution times result in smaller peak widths and consequently higher peak capacities.

References

1. Cunliffe, J. M.; Maloney, T. D. Fused-core particle technology as an alternative to sub-2-µm particles to achieve high separation efficiency with low backpressure. *J. Sep. Sci.* **2007**, *30*, 3104-3109.

2. Griiti, F.; *et al.* Comparison between the efficiencies of columns packed with fully and partially porous C18-bonded silica materials. *J. of Chromatog. A* **2007**, *1157*, 289-303.

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Published in the USA, June 1, 2016
5990-5602EN

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