# Maximizing efficiency using Agilent Poroshell 120 columns

## 100000 plates in less than 5 min 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 Poroshell 120 columns offer:

- Lower back pressure
- Highest efficiency
- Comparable volume capacity


## 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 back pressures are obtained, due to the small particle sizes. In many cases, especially for long sub-2-µm columns, the LC instrumentation must allow back pressures of >400 bar.

The superficially porous particle technology offers an alternative for very high resolution analyses[1], because these columns show significantly less back pressure. 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 back pressure.

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This Application Note demonstrates that the coupling of three long Agilent Poroshell 120 columns results in extremely high efficiencies. It is also demonstrated that the back pressure 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.

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

The ChemStation software revision B.04.02 was used.

# 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 about 2.7 µm. The 2.7 µm superficially porous particles provide 40–50% lower back pressure 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

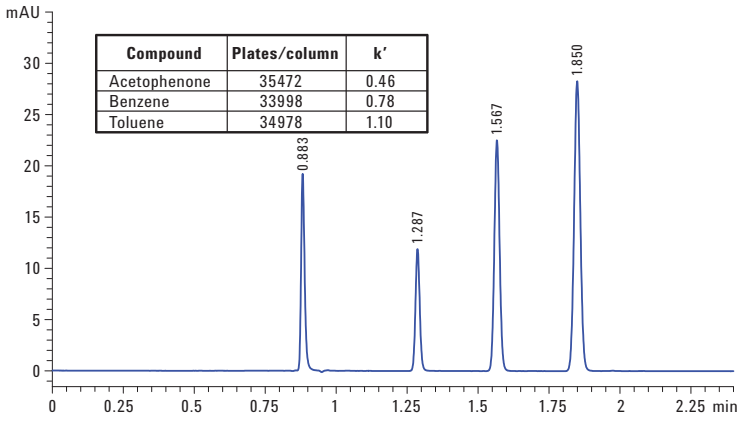
The following experiments evaluated the performance of the Agilent Poroshell 120 columns. The internal diameter was 4.6 mm and the column length 150 mm for all columns used.

- 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):

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

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




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

Chromatographic conditions:

| | |
|---|---|
| Column: | Agilent 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 Poroshell 120 150 mm × 4.6 mm column.**

## 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 35000 plates/column for toluene under the chromatographic conditions specified.

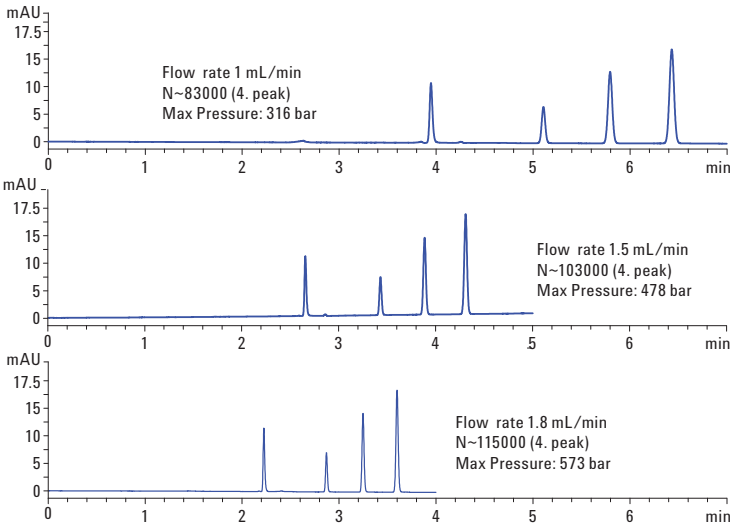
## Evaluation of plate numbers for three coupled columns

The plate number for one column is approximately 35000 plates. The expectation is that three columns deliver a plate number of 105000 plates. Column coupling was done using stainless steel capillaries, 90 mm × 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 80000 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 1200 bars.

At 1.5 mL/min flow rate the obtained plate number of approximately 103000 plates is close to the expected value.

The best result for toluene with approximately 115000 plates was obtained at 1.8 mL/min with a retention time < 5 min (Table 1).




Chromatographic conditions:

| | |
|---|---|
| Sample: | Thiourea, Acetophenone, Benzene, Toluene |
| Column: | Three coupled Agilent 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 Poroshell 120 150 mm × 4.6 mm columns at different flow rates.**

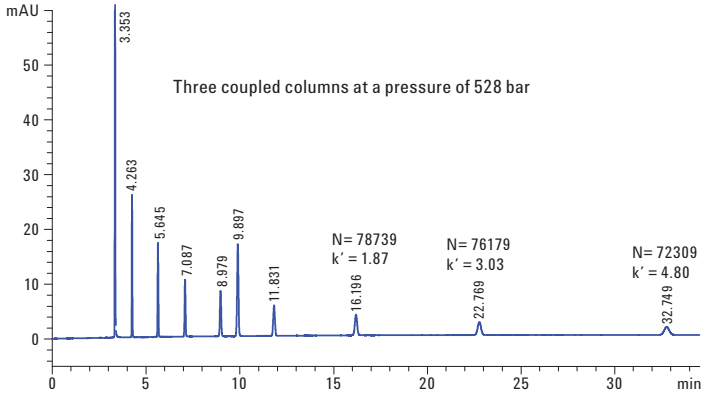
| Compound | Plates | k' |
|---|---|---|
| Acetophenone | 114120 | 0.29 |
| Benzene | 109931 | 0.46 |
| Toluene | 114800 | 0.62 |

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

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

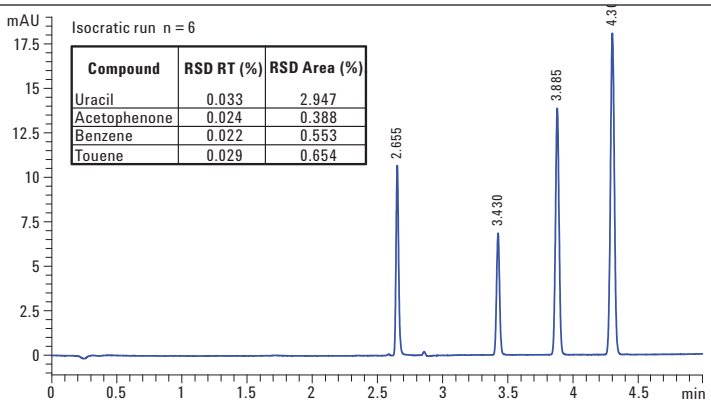
Precision for isocratic conditions at 1.5 mL/min was evaluated and results are shown in Figure 4 together with an overlay of six consecutive runs. The precision of retention times is < 0.034% RSD, and the precision for areas is < 0.66% RSD, except for uracil.




Chromatographic conditions:

| | |
|---|---|
| Sample: | Thiourea+Test sample: Set of nine compounds, 100 ng/uL 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 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:

| | |
|---|---|
| Sample: | Uracil, Acetophenone, Benzene, Toluene |
| Column: | Three coupled Agilent 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 |

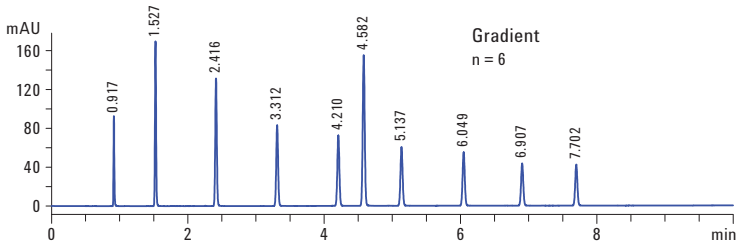
**Figure 4**
**Overlay of six consecutive runs using isocratic conditions and precision data for retention times and areas.**

## 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 min. 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 Retention time (%) | 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:

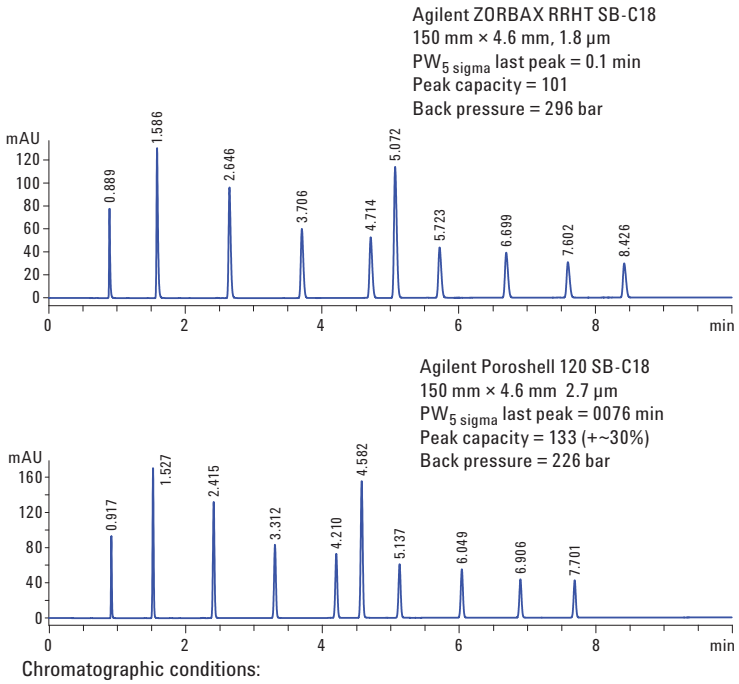
| | |
|---|---|
| Sample: | Thiourea + Test sample: Set of nine compounds, 100 ng/µL each, dissolved in water/ACN (65/35)<br>1. Acetanilide, 2. Acetophenone, 3. Propiophenone,<br>4. Butyrophenone (200 ng/µL), Benzophenone,<br>6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone,<br>9. Octanophenone |
| Column: | Agilent Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm |
| Mobile phase: | Water and ACN |
| Gradient: | At 0 min 35% ACN, at 10 min 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 mm × 4.6 mm id columns were compared analyzing a set of 10 compounds (Figure 6).

The Agilent Poroshell 120 column shows shorter elution times, and smaller peak width, which results in a higher peak capacity for the porous shell column. The Agilent 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 Agilent Poroshell 120 column compared to the sub-2-µm column for the conditions used.




Chromatographic conditions:

| | |
|---|---|
| Sample: | Thiourea + Test sample: Set of nine compounds, 100 ng/µL each, dissolved in water/ACN (65/35)<br>1. Acetanilide, 2. Acetophenone, 3. Propiophenone,<br>4. Butyrophenone (200ng/µL), Benzophenone,<br>6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone,<br>9. Octanophenone |
| Column: | Agilent ZORBAX RRHT SB-C18, 150 mm × 4.6 mm, 1.8 µm,<br>Agilent Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm |
| Mobile phase: | Water and ACN |
| Gradient: | 0 min 35% ACN, 10 min 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.**

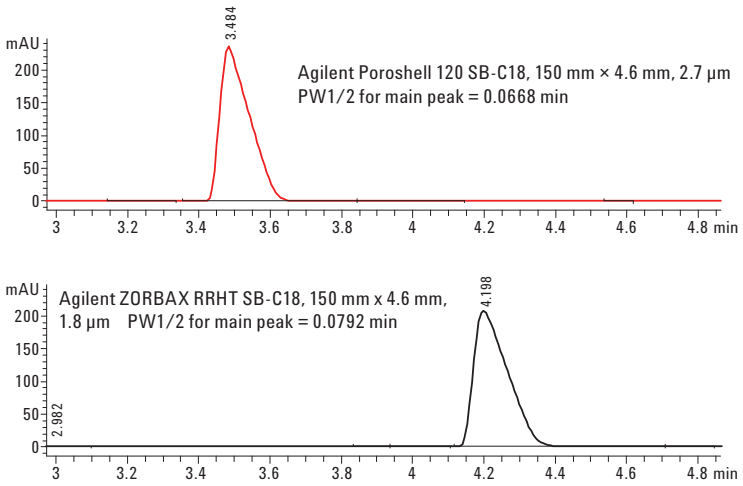
## Comparison of volume capacity

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 Poroshell 120 column was somewhat lower because in this case the peak eluted earlier. The peak width is typically smaller.

## Comparison of signal-to-noise

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




Chromatographic conditions:

| | |
|---|---|
| Test Sample: | Tramadol 2,022 mL/mL containing impurities, |
| Column: | Agilent Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm<br>Agilent ZORBAX RRHT SB-C18, 150 mm × 4.6 mm, 1.8 µm |
| Pump: | |
| Solvent A: | Water + 0.2 % TFA and Solvent B: ACN + 0.16% TFA |
| Gradient: | 17 to 45% B in 5 min, Stop Time 7 min, Post time 3 min |
| 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 shows an overlay of a section of the complete chromatograms. The red trace represents the Poroshell 120 chromatogram and the black trace represents the sub-2-µm chromatogram.

In Table 2, the signal-to-noise calculations for both columns are combined. Impurity 1 and 2 were analyzed on the Poroshell 120 column and on the sub-2-µm column.

## Conclusion

Porous shell columns represent a real alternative to sub-2-µm columns. The lower back pressure allows flow rates of 1 mL/min for a 4.6 mm x 150 mm, 2.7 µm column without exceeding the 400 bar limit. In this case, 35000 plates are achievable or more than 235000 plates/meter.

Column coupling of three 4.6 mm × 150 mm columns result in a plate number of 100000 plates in under 5 min without exceeding the 600 bar limit.

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

Typically for Agilent Poroshell 120 columns shorter elution times than that of the similar sub 2-µm banded 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. J. M. Cunliffe, T. D. Maloney, "Fused-core particle technology as an alternative to sub-2-µm particles to achieve high separation efficiency with low back pressure", *J. Sep. Sci.* 2007, 30, 3104-3109

2. F. Griiti, A. Cavazzini, N. Marchetti, G. Guiochon," Comparison between the efficiencies of columns packed with fully and partially porous C18-bonded silica materials", *Journal of Chromatography A*, 1157, 289-303, 2007

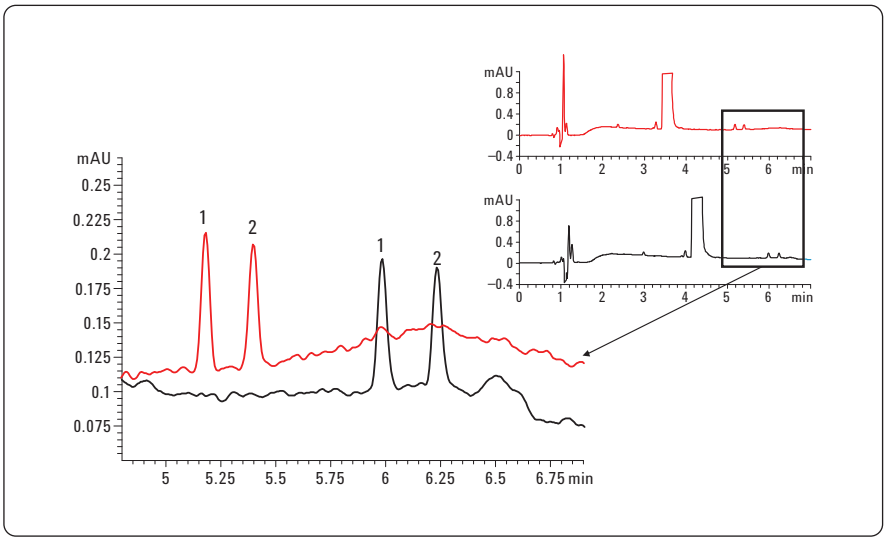




**Figure 8**
**Comparison signal-to-noise ratio, red represents the porous shell column and black trace represents the 1.8 µm particle column. Modifier TFA was used.**

| Peak | Poroshell 120 S/N | 1.8 µm S/N |
|---|---|---|
| 1 | 14 | 13.6 |
| 2 | 12.8 | 12 |

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

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