Transferring a Method from a Conventional 3.5 µm or 5 µm to an Agilent Poroshell 120 Column to Improve Analytical Speed and Resolution

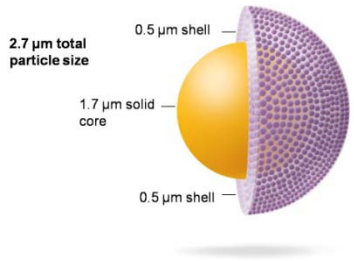
Ron Majors is Senior Scientist for Agilent's Columns and Supplies Division; John Henderson is an Agilent Applications Scientist.

Instrument pressure limits are getting higher, to take advantage of trends to new smaller particles, and all the speed and resolution advantages they provide. New instrumentation can provide significant new features and sensitivity to improve analyses. Find out more about the latest advances at Agilent at www.agilent.com/chem/infinity.

However, if you cannot upgrade your instrument in the nearterm, transferring your methods from conventional 3.5 or 5 µm columns to new **Agilent Poroshell 120** columns can save your lab time and money *today.*

What is Poroshell 120?

Poroshell 120 particles are manufactured by Agilent using a proprietary process that creates a superficially porous shell around a solid core. These are different than sub-2 µm particles.



Poroshell 120 particles are 2.7 µm in total size, with a 1.7 µm solid core and a 0.5 µm porous outer shell. So you get analytical performance of a sub-2 µm particle, but at the pressure of a 2.7 µm particle, usually less than 400 bar.

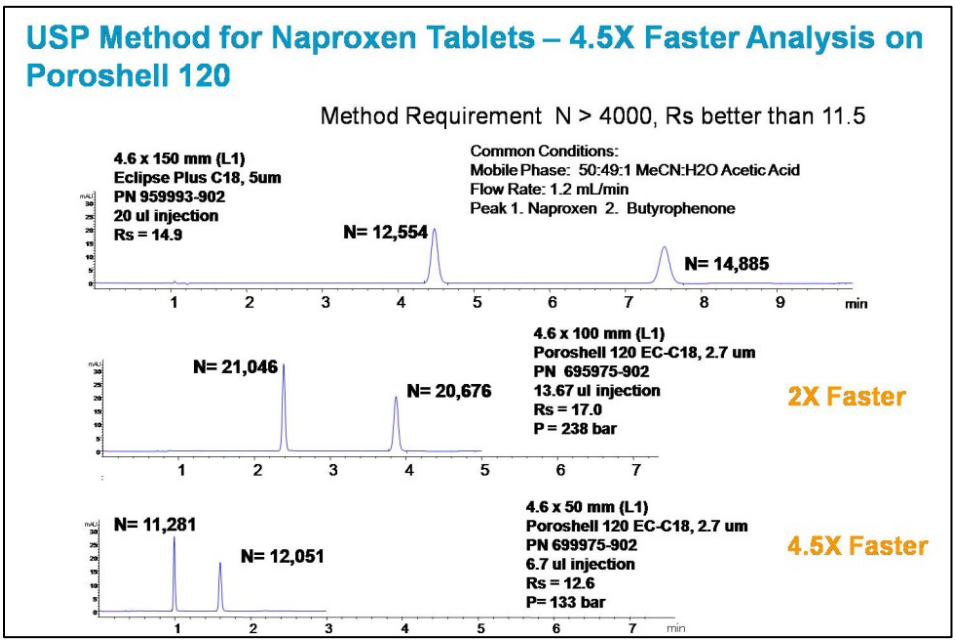
Poroshell 120 columns will work on virtually any HPLC, and enable you to scale methods from conventional 3 or 5 µm columns with a few minor modifications to the instrument.

Today, you can purchase Poroshell 120 columns in an endcapped EC-C18 phase and a non-endcapped StableBond SB-C18 phase. The selectivity will be the same, or very similar to, your current C18 column. More phases are coming soon.

Realizing Time Savings with Poroshell 120

See the following comparison examples of an analysis of Naproxen using a conventional 5 µm column, then two different Poroshell 120 columns. Ultimately, the time savings was 450% for this analysis! Additional savings include a 77% reduction in solvent usage.

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Transferring Your Method to Poroshell 120

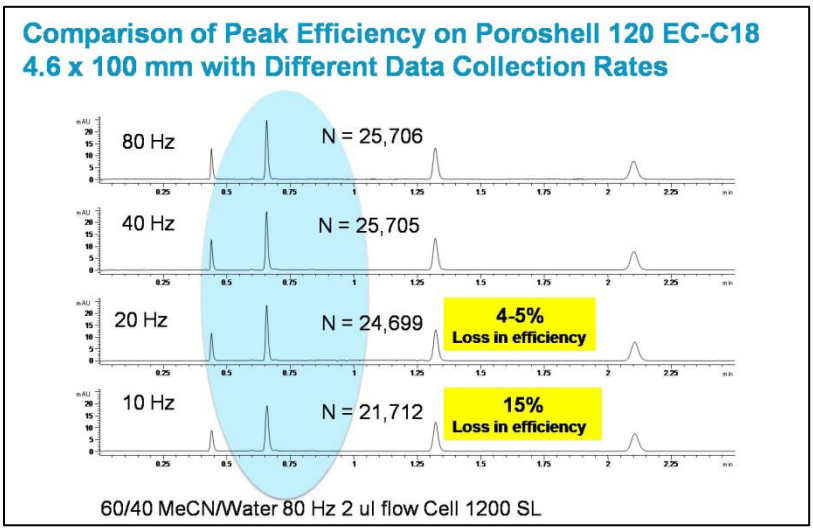
It's important to pay attention to the instrument configuration and method parameters to ensure you get the best results.

The key instrument parameters to address for optimized performance with Poroshell 120 are:
1) Detector response time/Data Collection Rate
2) Flowcell volume
3) Sample flow path tubing volume
4) Flow path fitting
5) Flow rate.

Set the Proper Data Collection Rate

Set the data collection rate to the fastest setting such that signal-to-noise (S/N) is not affected. This may not be the fastest setting on the detector but should be close. For best results when using small columns, collect data at 40 Hz or more. Newer Agilent detectors have data rates up to 80 Hz or 160 Hz and are great for Poroshell 120 columns, too. Slower data collection rate is practical when analytes retain longer (e.g., k'>4).

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Here is a comparison of chromatograms that illustrates how the data collection rate affects results. If the data collection rate is too slow, there is a loss of efficiency and differences in peak shape may result.

Looking at it another way, the table below illustrates the same point.

Response Time	Hz	Uracil	Phenol	4-Chloro-Nitrobenzene	Naphthalene
		Peak Width 0.008	Peak Width 0.011	Peak Width 0.021	Peak Width 0.031
0.02	80	16,240	21,641	25,706	25,061
0.1	40	15,414	22,028	25,705	25,080
0.2	20	12,755	18,647	24,699	24,506
0.5	10	6,965	12,658	21,712	23,872

Column: Poroshell 120 EC-C18, 4.6 x 100 mm; Instrument: 1200 SL 2µm flow cell; Flow Rate: 2.00 mL/min; Sample: 2 µL injection; Mobile Phase: 60:40 MeCN: Water

For narrower 2.1 mm ID columns, the same principles apply. Again, you'll see that you should collect data at at least 40 Hz. Slower data collection is practical when analytes retain longer (k'>4).

Flow Cell Selection:

Extra column volumes (ECVs) in your sample flow path can reduce the efficiency observed for Poroshell 120 columns. The flow cell volume is one of these ECVs. Smaller volume flow cells such as the semi micro (6 mm/5 µl) or micro (3 mm/2 µl) are recommended for best performance.

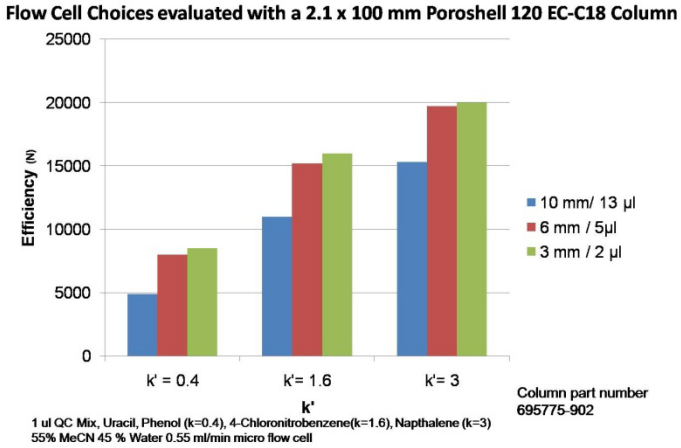
With 2.1 mm columns, it is best to use the 3 mm micro flow cell.

Standard flow cell (with RFID tag): 10 mm, p/n G1315-60022
Semi-micro flow cell (with RFID tag): 6 mm, p/n G1315-60025
Micro flow call (with RFID tag): 3 mm, p/n G1315-60024

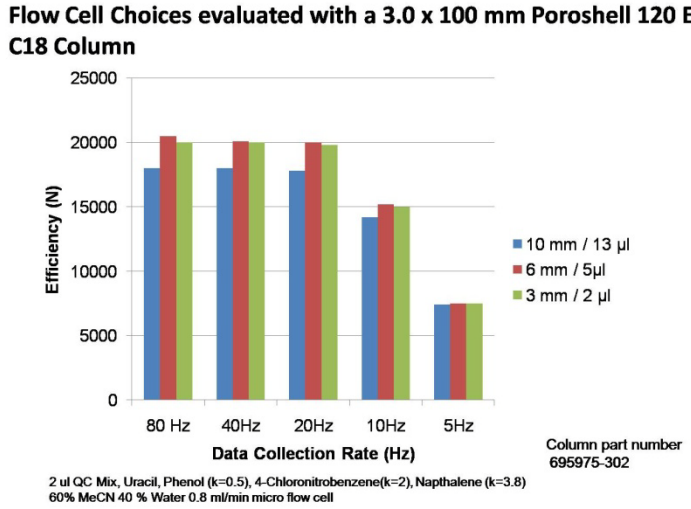
There are a number of different flow cells available, and your needs may vary depending on whether you are using an Agilent 1100 or 1200. Consult the ***Essential Chromatography and Supplies Catalog***, under

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LC and LC/MS, Detectors for more information. Contact Agilent technical support (lc-column-support@agilent.com) for assistance. You can request a catalog at www.agilent.com/chem/getguides.



There is a 30% loss of efficiency with a 10 mm (13 µL) standard flow cell. With 2.1 mm ID columns, it is best to use a 3 mm (2 µL) micro flow cell.



Larger flow cells, used at fast data collection speeds, can be used with large columns, as shown with this 3 x 100 mm Poroshell 120 EC-C18 column

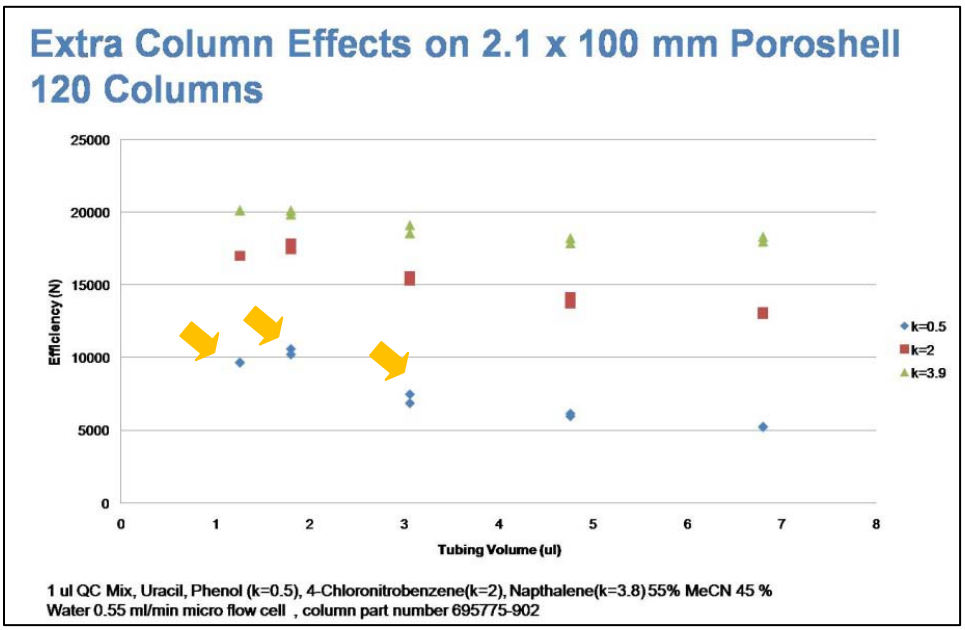
Minimize Extra Tubing Volume

Another ECV that can reduce column efficiency are the connecting capillary tubes. Use the shortest length of tubing possible for all connections. Use Red (0.12 mm id) tubing instead of Green (0.17 mm id) as it is only half the volume of the larger internal diameter (ID) green tubing.

Once you have optimized LC flow with the lower volume tubing you don't need to change it back for higher volume columns.

See the ***Agilent Essential Chromatography and Supplies Catalog*** for tables that indicate which tubing is appropriate for the LC connections.

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See here on this example using a 2.1 x 100 mm Poroshell 120 column, early eluters (k'< 3) are affected more severely by extra column broadening.

The autosampler needle assembly is an important part of the flow path that can be reduced in volume. It's easy to do this yourself:

1) If you are using Lab Advisor, select your instrument from the opening menu and select "tools".
2) Select the autosampler module, and a window will open that includes the needle seat option. Skip to Step 5.
3) For older instruments using ChemStation, go to the Method & Run Control view, then to the "Diagnosis" view (use top tabs)
4) Under the Maintenance menu, scroll down to the Wellplate Sampler (WPS) then over to "WPS Maintenance Positions".
5) Select "start" under "Change needle seat". You'll see the injector needle move away.
6) Gently pry up the injector seat with a small screwdriver. Do not pry the ring at the top of the injector seat.
7) Change capillary by pulling it through, and threading the new needle seat in. Properly tighten the capillary fitting with a small wrench.
8) Close the door and select "end" in ChemStation window. The needle will reposition.

Capillary kits are available, packaged with an assortment of usefully sized. For RRHT optimization, look for these kits:

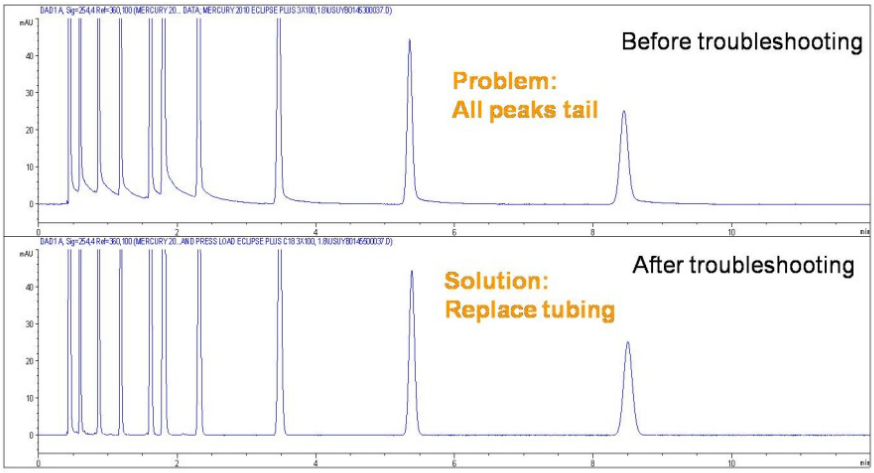
For the 1100, use capillary kit #5065-9947; For the 1200, use capillary kit #G1316-68716
To view kits, go to www.agilent.com/chem/capillarykits

Ensure Proper Connections:

Poorly installed fittings can lead to poor chromatographic results, due to ECVs and unswept dead volumes, as this example shows:

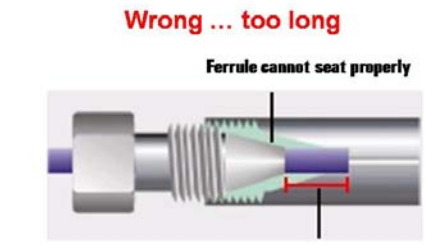
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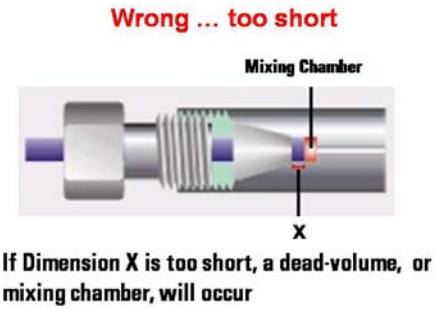
LC troubleshooting: poor peak shape, improperly swaged tubing



Cause: Capillary tubing connecting ALS and column was swaged improperly on the ALS end (ferrule was flush with end of tubing, causing a void).

Be sure the tubing is not too long or too short into the ferrule.





Alternately, finger-tight fittings are more convenient, allowing for adjustment of the end-fitting properly without wrenches.



Use polyketone fittings on column connections with Poroshell 120 (PN 5042-8957). Polyketone fittings, and Poroshell 120 columns, can withstand 600 bar pressures.

Note that PEEK fittings can only withstand pressures up to 400 bar.

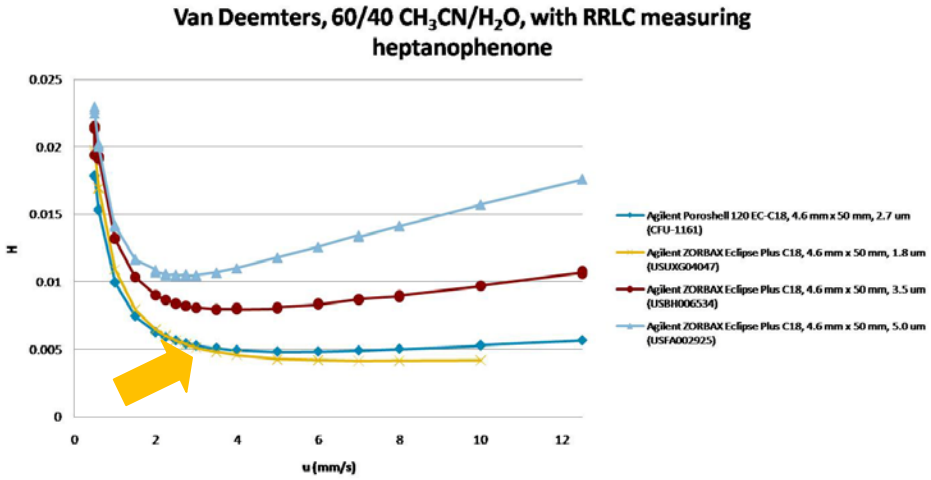
Optimize the Flow Rate:

Optimize the flow rate to achieve the desired efficiency (N) or peak capacity (Pc)

Suggested starting flow rates for Poroshell 120:
2.1 mm ID: start with 0.42 mL/min.
3.0 mm ID: start with 0.85 mL/min.
4.6 mm ID: start with 2 mL/min.

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You'll want to use a higher flow rate with Poroshell 120. Increase flow rate (F) 10% and compare efficiency (N) to the starting flow rate. If efficiency has increased, continue to increase F and compare efficiency N to determine the best flow rate.



As this van Deemter plot illustrates, the optimal flow rate for Poroshell 120 is faster than for 5 µm or 3.5 µm columns that they replace.

Other chromatographic considerations:

Properly scale the gradient conditions and the injection volume to the new smaller column volume to quickly transfer the method and avoid overloading.

Be sure to minimize sample dispersion in the column. To do this:
a. Use an injection solvent that is weaker than the mobile phase, especially when using an isocratic method.
b. Gradients can minimize dispersion but be aware of possible effects.
Poroshell 120 columns can achieve similar efficiencies as sub-2 µm columns with substantially less pressure.

Excellent results can be achieved on Agilent 1200 RRLC or 1290 Infinity

Summary:

In order to achieve best performance:

1. Optimize data collection rate (40 Hz detector with fast response time)
2. Use the smallest flow cell available (3mm micro flow cell works best when using 2.1 I.D. columns).
3. Minimize extra column volume: flow cell and connecting tubing are the biggest contributors, but needle seats should be replaced with smaller volume seats. Red tubing has half the volume of green tubing.
4. Take care to make proper tubing flowpath connections.
5. Optimize flow rates for best performance. Start with flow rate 2 ml/min on a 4.6 mm column, 0.85 ml/min on a 3.0 or 0.42 ml/min on 2.1 mm column.

If you need more help, contact Agilent technical support (lc-column-support@agilent.com) for assistance.

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