



Optimizing Your LC Performance When Transferring A Method from a Conventional Column to a Rapid Resolution High Throughput (RRHT) 1.8 μ m Column

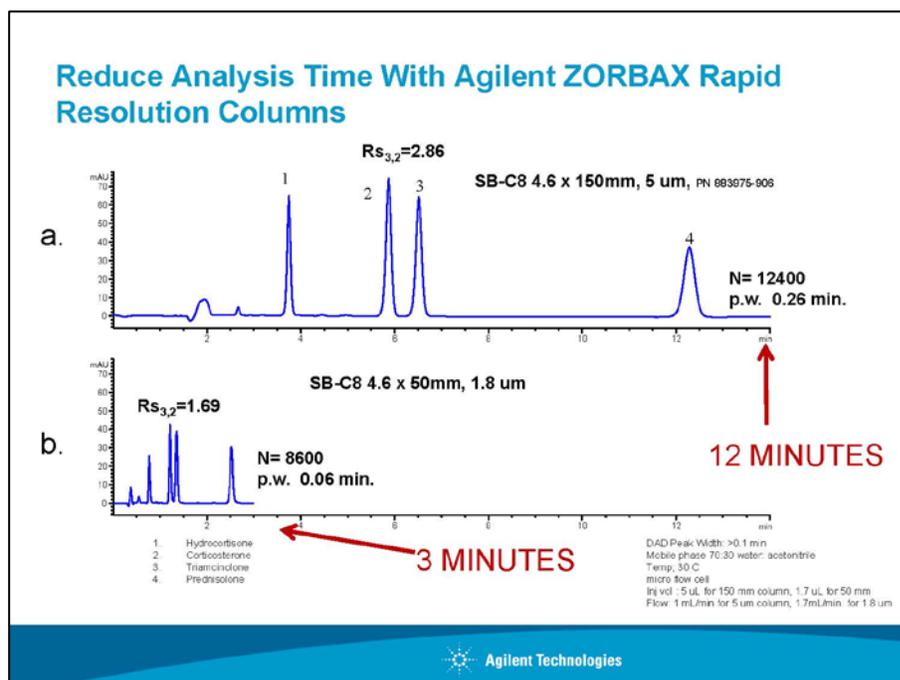
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Scaling your method from a conventional 5 μ m column to an **Agilent ZORBAX Rapid Resolution High Throughput (RRHT) 1.8 μ m** column using your current instrumentation, with a few minor modifications, can save your lab time and money.

For an isocratic method, time savings will be proportional to the change in column length. For example, scaling from a 150 mm column to a 50 mm column saves two-thirds of analysis time.

See Agilent Publication 5989-2908EN "**Plug & Play**" *Fast and Ultra-Fast Separations Using 3.5- μ m ZORBAX Rapid Resolution and 1.8 μ m Rapid Resolution High Throughput (RRHT) Columns*" for general information. To locate and print a publication, go to www.chem.agilent.com, select "literature library" and "online literature" and type in the publication number in the Keyword Search box.

Agilent ZORBAX RRHT columns, with 1.8 μ m particles in shorter column lengths, decrease the analysis time compared to conventional columns. The chromatograms below illustrate this time-savings for one example.



John demonstrates Agilent flexible capillaries in the video. The flexible capillaries are very flexible and easy to use, and have finger-tight fittings. Capillary kits are available, pre-packed with a variety of capillaries you'll need.

For RRHT optimization, look for these kits: For the 1100, use capillary kit #5065-9947; For the 1200, use capillary kit #G1316-68716. Finger-tight fittings: part number 0100-1516.

View kits at www.agilent.com/chem/capillarykits

For isocratic methods, when scaling to an **Agilent ZORBAX Rapid Resolution High Throughput (RRHT) Column**, four things can help optimize your method:

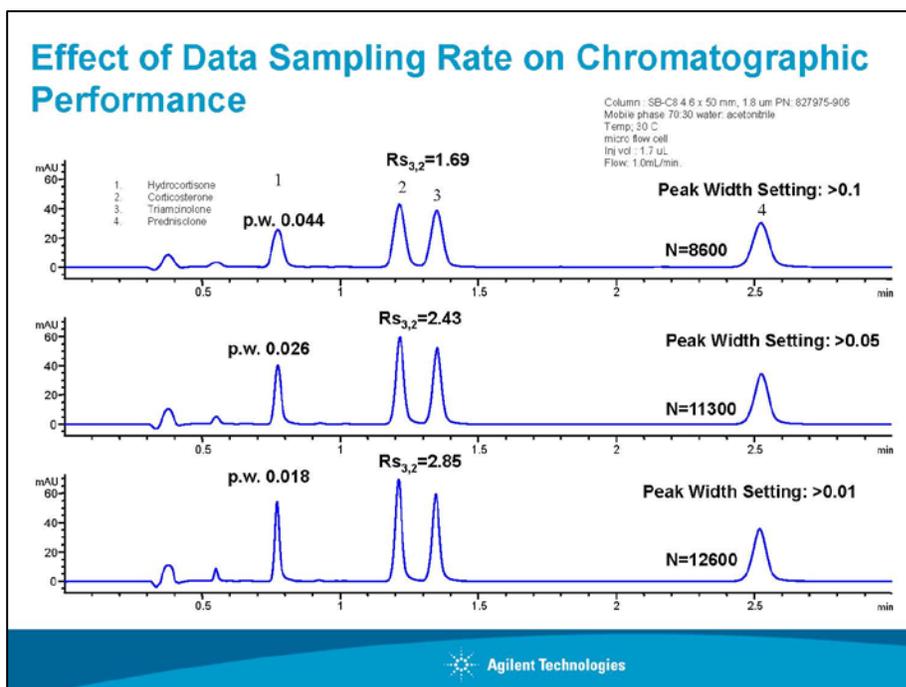
- 1) Adjust flow rate
- 2) Minimize tubing length
- 3) Adjust data collection rate
- 4) Reduce injection volume

For more information, see publication # 5988-9251EN "**The Influence of Sub-Two Micron Particles on HPLC Performance**"

Increase your flow rate, starting with the flow rate specified in your original method. Be sure to watch how it affects your pressure, which should not go above 350 bar for a standard LC.

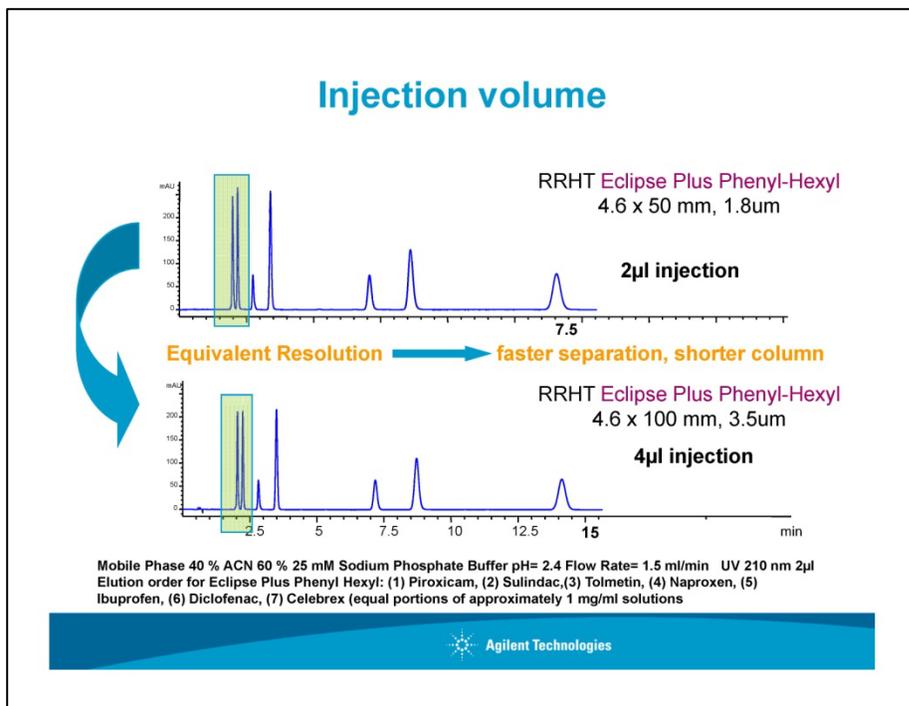
Tip: Tubing that is too long creates extra-column volume, and contributes to peak broadening. Ordering lengths that are just the right length is an easy way to improve your chromatograms. This is a good practice for all chromatography, not just for use with RRHT columns.

A chromatogram with wide-looking or low efficiency peaks may indicate your data collection rate is too slow. Adjusting it to meet the faster analysis of the RRHT column will improve your chromatogram.



See Agilent publication #5989-5810EN, "**Optimize Data Sampling Rate to Take Advantage of RRHT Columns**", for more information.

Tip: When scaling a method, adjust your injection volume to match the change in volume in your new column. On the same diameter columns, it will be proportional to the change in column length. Adjust the peak width setting and do a sample run until you optimize the peaks. Note the optimal peak width setting and make it part of your method.



For scaling from 5 µm or 3.5 µm to a RRHT 1.8 µm column in a smaller length and 2.1 mm ID, make the following additional modification:

- 1) Switch all tubing from the standard green (.17mm) to red (.12 mm), including at the autosampler needle seat.
- 2) Change the flow cell to a micro flow cell

You should use red tubing (0.12 mm ID) for 2.1 mm ID columns, because its smaller volume is better suited for the narrower column. This improves overall chromatographic performance.

Change green .17 mm tubing to red .12 mm tubing:

- 1) The needle seat
- 2) The autosampler to the TCC (thermal column compartment)
- 3) The TCC to the column and
- 4) The column to the flow cell

To find the right needle seat for your system, visit www.agilent.com/chem/needleseat or see the **Essential Chromatography and Supplies Catalog**. You can order the **Essential Chromatography and Supplies Catalog**, and other helpful guides, at www.agilent.com/chem/guides

Steps for changing the needle seat:

- 1) In ChemStation window, from the Method & Run Control view, go to the "Diagnosis" view (look at top tabs)
- 2) Under the Maintenance menu, scroll down to the Wellplate Sampler (WPS) then over to "WPS Maintenance Positions".
- 3) Select "start" under "Change needle seat". You'll see the injector needle move away.
- 4) Gently pry up the injector seat with a small screwdriver. Do not pry the ring at the top of the injector seat.
- 5) Change capillary by pulling it through, and threading the new needle seat in. Lightly tighten the capillary fitting with a small wrench.
- 6) Close the door and select "end". The needle will reposition.

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

There are kits with all the parts you need to adjust the LC for RRHT columns, including columns, tubing and flow cells. The one for the 2.1 mm ID columns is part number 5188-5328. This particular kit is for Diode Array Detectors and Mass Spec.

It's especially important to filter your samples when using RRHT columns! Uniprep filters are a great way to combine several steps into one and make the process easy. For more information about the uniprep filters, go to www.agilent.com/chem/uniprep.

SUMMARY

For isocratic methods, when scaling to a RRHT column with a 4.6 mm ID, four things can help optimize your method:

- 1) Adjust flow rate
- 2) Minimize tubing length
- 3) Adjust data collection rate
- 4) Reduce injection volume

For scaling from 5 μ m or 3.5 μ m to a RRHT 1.8 μ m column in a smaller length and 2.1 mm ID, make the following additional modifications:

- 1) Switch all tubing from the standard green (.17mm) to red (.12 mm), including at the autosampler needle seat.
- 2) Change the flow cell to a micro flow cell.

See "***Step-by-step upgrade of Agilent 1100 Series LC systems to Agilent 1200 Series Rapid Resolution LC systems for higher performance***", parts 1 and 2, publication numbers 5989-6336EN and 5989-6337EN, for more information about available upgrades to your current instrument.

Please contact Agilent technical support (lc-column-support@agilent.com) for additional assistance.