Optimize Data Sampling Rate to Take Advantage of RRHT Columns

Application Brief

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Method-Development Troubleshooting

ZORBAX rapid resolution high throughput (RRHT) 1.8-µm columns deliver dramatic time savings and easy, reliable transfer of methods designed on 5-µm analytical columns. The smaller particle size significantly increases column efficiency and, coupled with shorter column lengths, maintains the resolution or separation of the analytes in shorter analysis time.

Transferring most method parameters, such as signal wavelength and bandwidth to RRHT methods, is straightforward. One detector parameter, however, that must usually be re-optimized to take advantage of the narrower peaks eluted by RRHT columns is the "peak width" parameter. This is sometimes called data sampling rate or response time and determines how often a detector-signal data point is recorded to construct the chromatogram. This does not affect the liquid chromatography, only the way it is recorded.

Figure 1A shows a method developed on a 5-µm sized column for corticosteroids transferred to a RRHT column. Injection volume is sized proportionally to column volume. Flow rate is easily optimized for smaller particles on isocratic methods as well. The peak width parameter, however, was not adjusted. The chromatographic performance of the RRHT column is far less than what is expected.

Generally, efficiency, or theoretical plates (N), is typically 70,000 plates/meter or 10,500 plates for a 4.6 mm × 150-mm long column packed with 5-µm particles. For a 4.6 mm × 50 mm, 1.8-µm column, one would expect similar plates per column. In practice, many factors influence efficiency, such as extra-column volume in the LC system, flow rate, temperature, and how well the column is manufactured.

For 4.6 mm × 150 mm, 5-µm columns, typical peak widths are 0.1 to 1 min wide, affording ample time for the DAD at its default ChemStation (Def_LC.M) setting of > 0.1 min to record enough data points across the peak for a good representation. Note the measured peak width (pw) of prednisone in Figure 1A is 0.26 min, so the DAD peak width setting is adequate. In Figure 1B, however, the measured peak width of prednisone is only 0.064 min, and is actually artificially broad from

Highlights

Optimizing the peak width DAD setting can dramatically improve efficiency, sensitivity, and resolution.

RRHT columns elute narrow peaks, compared to "analytical sized columns."

The Agilent 1200 DAD and MWD SL Series detectors operate at an 80-Hz sampling rate, offering the resolution to make the most of narrow peaks and the gain in speed of RRHT HPLC.

Suboptimal resolution and sensitivity can be caused by an incorrectly set detector method parameter, independent of the LC column's condition.

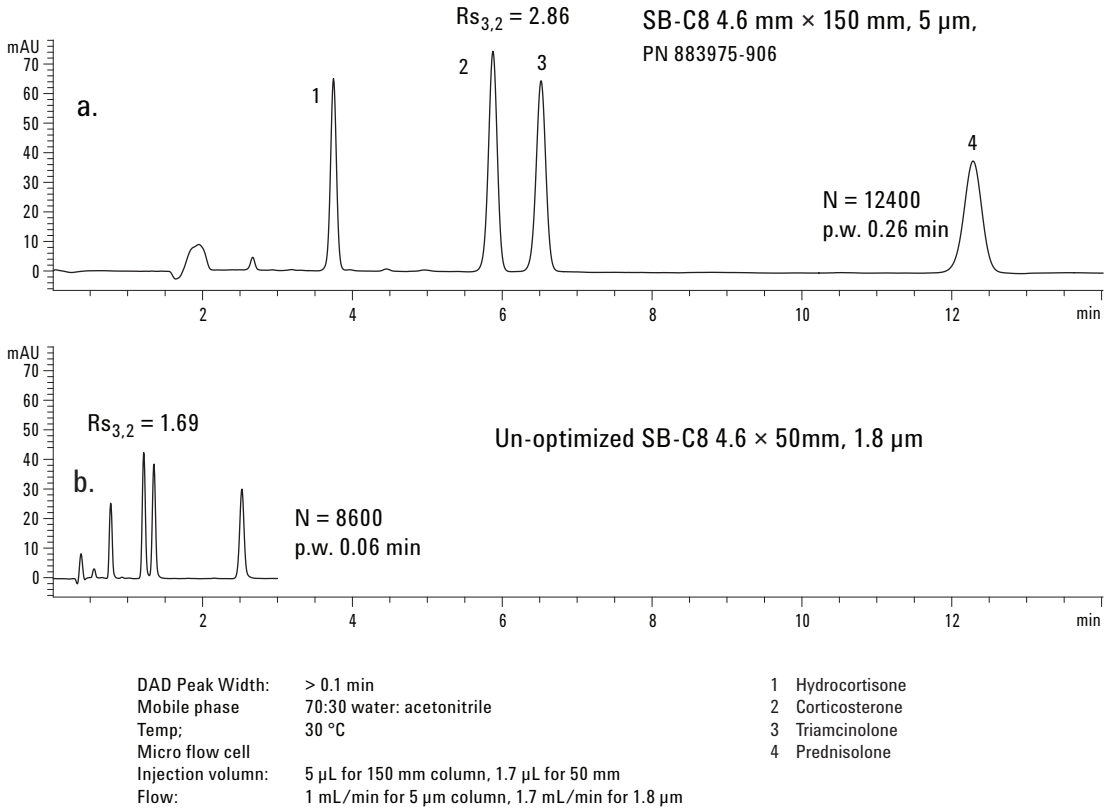
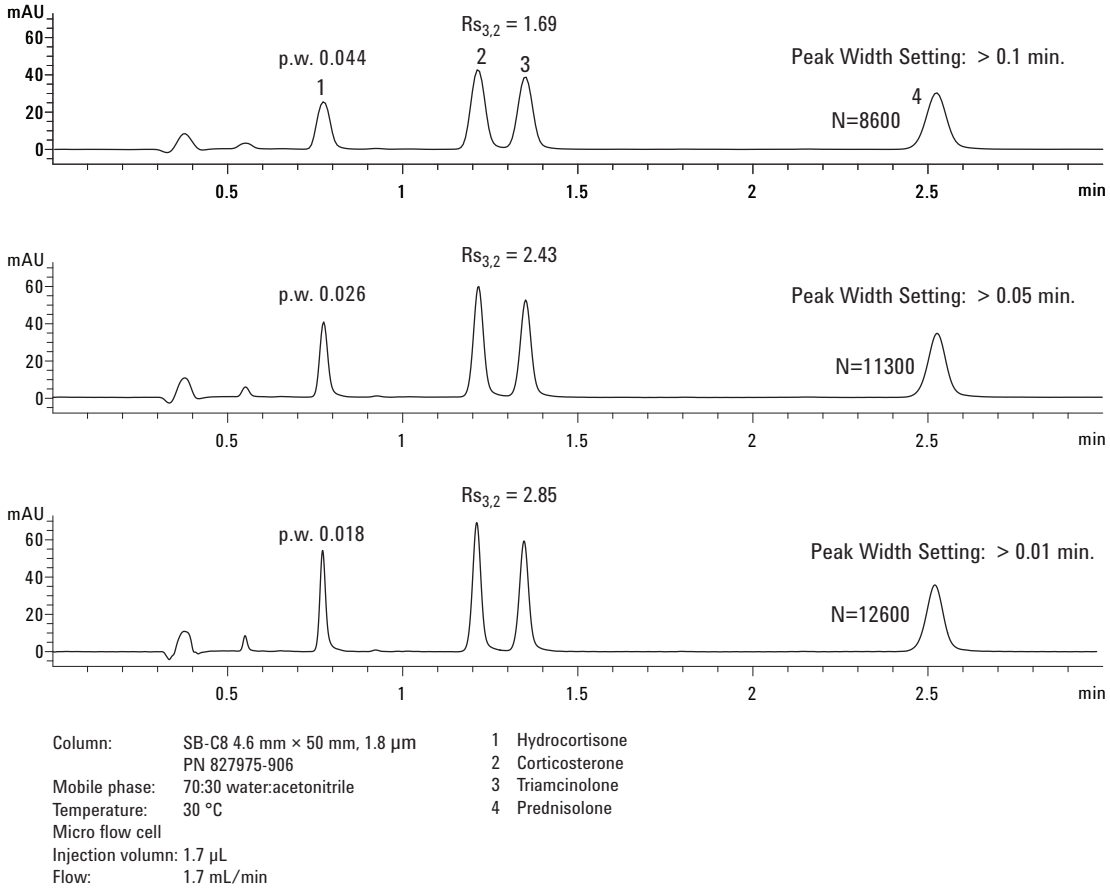


Figure 1. Transferred method without peak width setting optimization.

the slower data sampling rate. Therefore, the DAD peak width setting is incorrect for the RRHT column, and optimization should be carried out. The sub-standard chromatogram (Figure 1B) has nothing to do with the column's condition. Figure 2 shows repeated chromatographic runs, with faster data sampling rates. In each run the artificially broadened peaks narrow and efficiency and resolution improve. If the data rate is arbitrarily set to the maximum, the data files can be unnecessarily large and also can produce an unnecessarily noisy baseline. The best choice for maximum performance of the RRHT column in Figure 2 would be a 0.01 min peak width setting (bottom chromatogram).The importance of data rate optimization is not just limited to smaller particles. Every time a column with a volume less than the original column is used, the data rate should be examined. This is especially true for early eluting peaks. Note in Figure 2 how the peak width of the early eluting hydrocortisone peak narrows significantly as the peak width setting is optimized.



Column: SB-C8 4.6 mm × 50 mm, 1.8 µm
PN 827975-906
Mobile phase: 70:30 water:acetonitrile
Temperature: 30 °C
Micro flow cell
Injection volumn: 1.7 µL
Flow: 1.7 mL/min

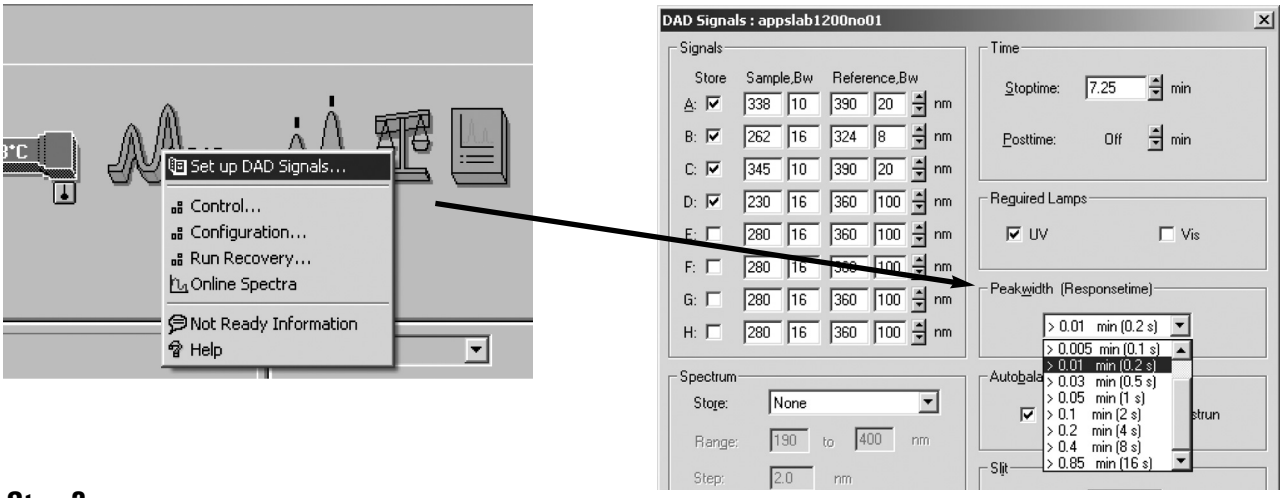
1 Hydrocortisone
2 Corticosterone
3 Triamcinolone
4 Prednisolone

Figure 2. Effect of data sampling rate on chromatographic performance.

Optimizing the peak width setting for a specific method is easy. One way is presented in Figure 3. The control of the data sampling rate for Agilent 1200 Series UV detectors is accessed through the handheld control module or ChemStation under the "Set up Detector Signals" menu in the "Method and Run Control" view. For ultra-fast RRHT analyses of less than one minute and peak widths of less than one second, the faster (80 Hz) SL Series detector is recommended for best resolution.

The flexibility of the RRHT Agilent 1200 Series technology, consisting of more then 90 HPLC column configurations, including eight different bonded phases, lengths from 15 to 150 mm, and inner diameters of 1 to 4.6 mm, and a variety 1200 Series detectors, is a robust option to transfer existing methods to RRHT separations. The ease of method transferability may lead to overlooking some method parameters; data sampling rate (peak width) is one parameter that merits close consideration.

Step 1



Step 2

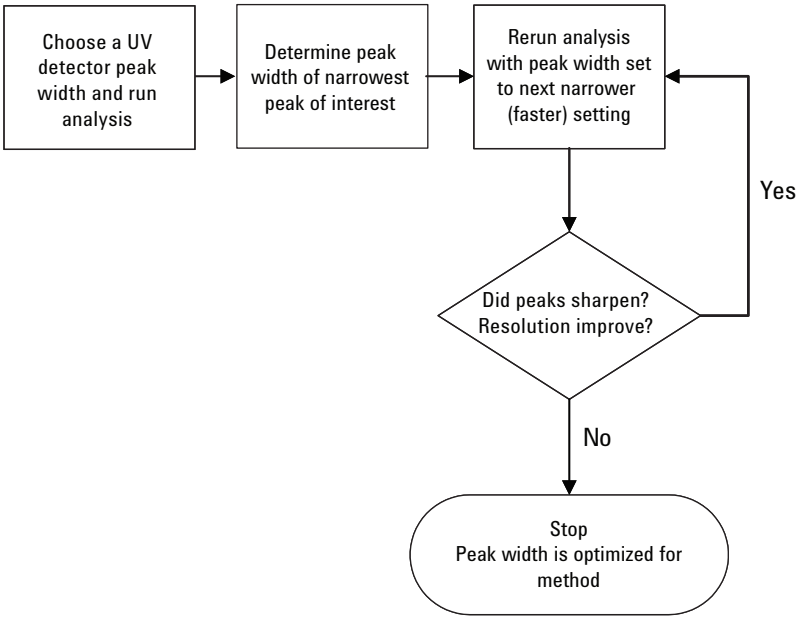


Figure 3. Setting the peak width for a DAD method.

Reference

"Agilent 1200 Series Diode Array and Multiple Wavelength Detectors Reference Manual," Chapter 2, p/n G1315-90004

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