TechnicalNOTE

Waters

2996 PHOTODIODE ARRAY DETECTOR: OPTIMIZATION FOR INTELLIGENT SPEED (*IS*™)COLUMNS

Introduction

The Waters® Intelligent Speed (*IS*™) line of columns have a 20 mm packed bed and optimized hardware that allow for fast, reproducible separations which generate peaks that are often only 2 to 4 seconds wide. This technical note will focus on optimizing the 2996 PDA detector settings for use with the *IS* columns. The effects of sampling rate on reproducibility, peak shape, and resolution are examined along with the effects of digital filtering on signal to noise, resolution, and peak capacity. Additional information on Waters Instruments with *IS* columns can be found in technical note 720000723EN titled: **Alliance 2695 Separations Module: Optimization and Performance with 4.6 mm i.d. Intelligent Speed (IS) Columns.**

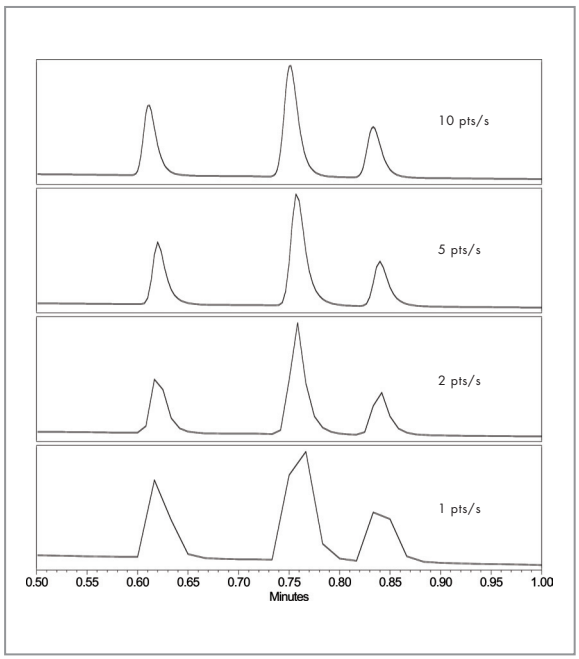

Figure 1: Effect of decreasing the sampling rate

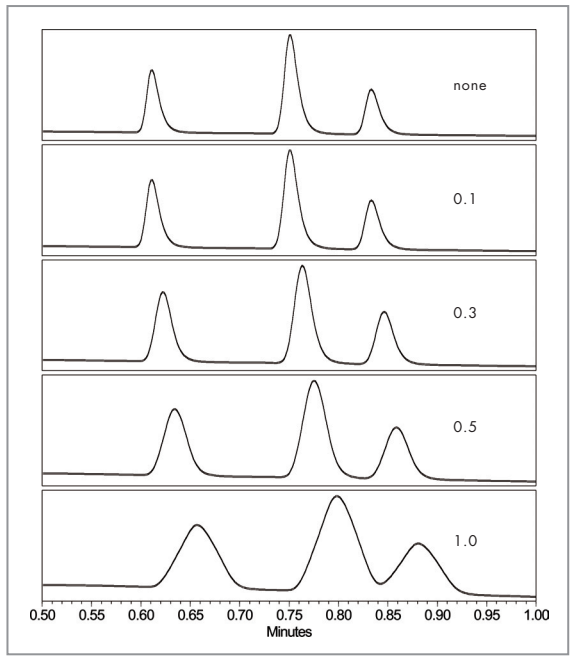

Figure 2: Effect of increasing the filter response of the digital filter

System:	Waters Alliance 2695 Separations Module Waters 2996 PDA Detector Waters Empower™ Software
Column:	Xterra® MS C_{18}, 4.6 x 20 mm, *IS* 2.5 µm at 25 °C
Detection:	Wavelength range 195 – 285 nm Extracted channel at 220 nm
Mobile Phase:	A = 0.1 % TFA in water B = acetonitrile
Flow Rate:	3.0 mL/min
Gradient:	0 % to 50 % over 1 minute
Sample:	10 µL injection of beta blockers (0.1 µg/mL atenolol, 0.1 µg/mL metoprolol, 0.05 µg/mL pindolol)

Narrow Peaks require higher sampling rates

Figure 1 shows the same one minute gradient separation performed at different sampling rates. At sampling rates below 5 pts/s, there is a visible effect on peak shape. Table 1 lists the reproducibility of the peak area and retention times for 10 injections, along with the points across the peaks at the different sampling rates. The reproducibility of the peak area is above 0.5 % RSD (Alliance system specification for injection precision) at sampling rates below 5 pts/s. In addition, when working with peaks which are only 3 seconds wide, the number of points across the peak fall below the minimum number needed for reproducible quantitation (15) at sampling rates below 5 pts/s.

Sampling Rate	% RSD of Retention Time	% RSD of Area	Points Across Peak
10 pts/s	0.440	0.09	39
5 pts/s	0.562	0.20	21
2 pts/s	0.597	0.68	9
1 pt/s	1.034	3.85	5

Table 1: Reproducibility and points across the peak for metoprolol as a function of sampling rate

Digital filtering affects resolution and peak capacity

Digital filtering is performed on data to improve the signal-to-noise ratios. The filter calculates a data point that is a modified rolling average for a wavelength over a number of readings. Figure 2 shows that as the filter response for the digital filter increases (more averaging) the peak width increases. At a filter response of 1.0, baseline resolution is lost. This increased peak width results in reduced peak capacity. Table 2 lists the peak capacities obtained at the different filter response levels along with the signal-to-noise ratios. The data show that there is a minimal increase in the signal to noise for a 2 fold reduction in resolution. At a filter response of 1.0 there is a significant increase in the signal to noise, however at the cost of a loss in resolution and diminished peak capacities.

Summary

Slower sampling rates can dramatically affect peak shape, resulting in poor area and retention time reproducability. For most fast gradients 5-10 pts/s will be required. For very narrow peaks, such as those resulting from separations on the *IS* line of columns, use digital filtering with caution. Digital filtering does not need to be used except in separations where increased sensitivity is much more important than resolution and high peak capacity.

Filter Response	Signal to Noise Increase	Resolution	Peak Width	Peak Capacity
None	--	3.16	3.82s	16
0.1	1.04x	2.99	4.02s	15
0.3	0.94x	2.45	4.56s	14
0.5	1.04x	1.82	5.27s	12
1.0	1.57x	1.08	7.01s	9

Table 2: Effect of filter response level on chromatographic parameters

WATERS CORPORATION
34 Maple St.
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

Waters
RIGHT ON TIME.