Waters™

應用手冊

Alliance 2695 Separations Module: Optimization and Performance with 4.6 mm i.d. Intelligent Speed (*IS*) Columns

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief focuses on optimization and performance of the Alliance 2695 Separations Module for use with *IS* Columns.

Introduction

The Waters Intelligent Speed (*IS*) line of columns have a 20 mm packed bed and optimized hardware that can be run at higher flow rates and lower backpressures without sacrificing resolution, reducing run times by up to 90%. This technical note focuses on optimization and performance of the Alliance 2695 Separations Module for use with *IS* Columns. A series of fast gradients were run at different flow rates to determine the effect on the reproducibility of retention times and peak areas. Peak capacities for the columns at the different flow rates were also calculated. Additional information on Waters instruments with *IS* Columns can be found in Technical Note 720000722EN <https://www.waters.com/nextgen/us/en/library/application-notes/2003/2996-photodiode-array-detector-optimization-for-intelligent-speed-is-columns.html> titled: Waters 2996 Photodiode Array Detector: Optimization for Intelligent Speed Columns.

Experimental

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 Sampling rate 10 pts/s No digital filtering
Mobile phase:	A = 0.1% TFA in water

	B = acetonitrile
Sample:	10 µL injection of beta blockers (0.1 µg/mL atenolol, 0.1 µg/mL metoprolol, 0.05 µg/mL pindolol)

Results and Discussion

Excellent run to run reproducibility achieved for fast gradients with no modifications to instrument configuration

A series of gradients varying from 0.6 to 4.0 minutes going from 0 to 50% ACN at flow rates varying from 1.0 to 4.0 mL/minute were run. Figure 1 shows the retention time reproducibility that was achieved for six replicate injections under each gradient condition. Excellent retention time reproducibility was achieved for a wide range of fast, steep gradients. Only at 4 mL/min with 1 minute and faster gradients did the retention time reproducibility exceed 0.5 %RSD. Figure 2 shows the peak area reproducibility for the same conditions. All of the gradients resulted in %RSD values for the peak area that were well below instrument specification (<0.5 %RSD), indicating no compromise in system performance when using fast, steep gradients.

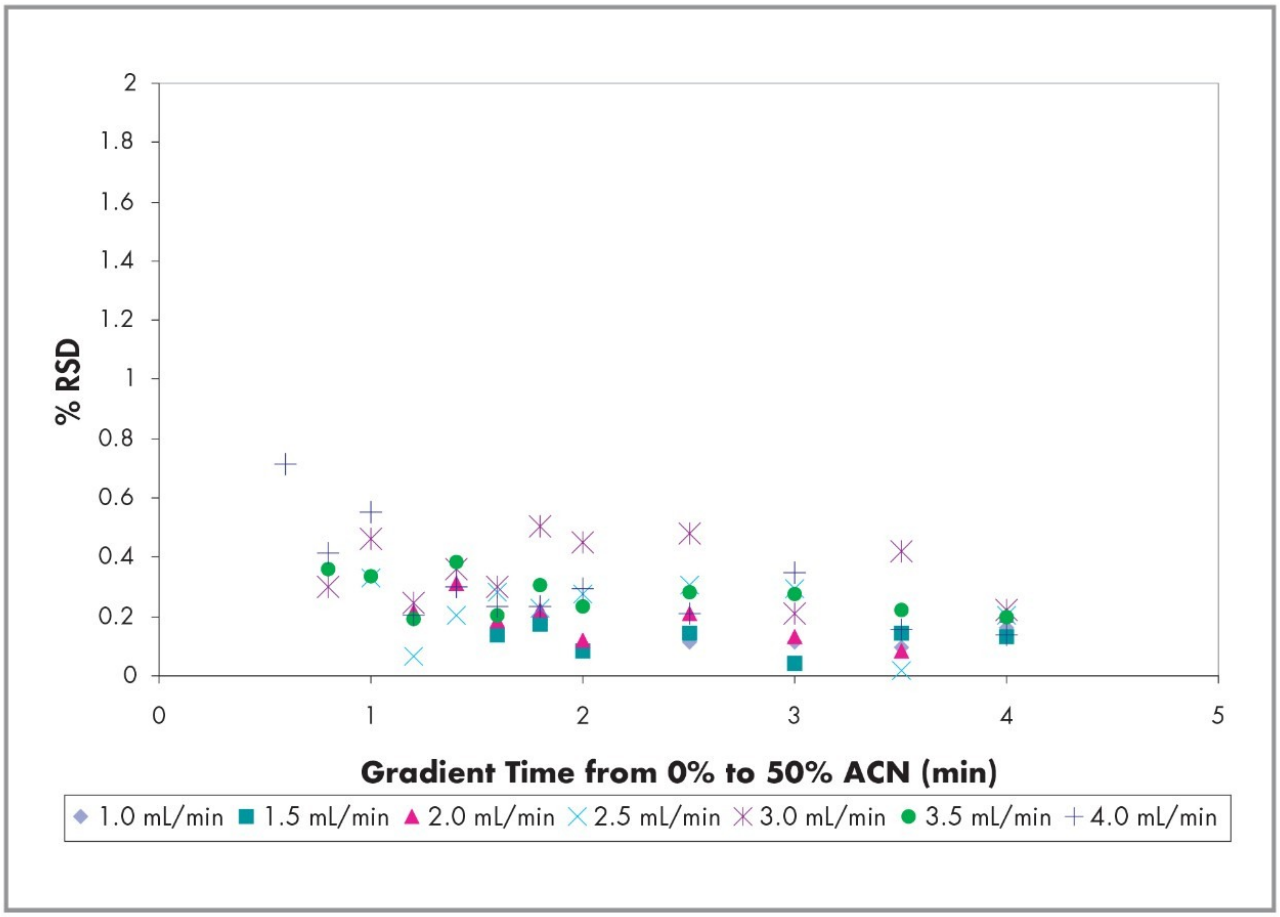

Figure 1. Retention time %RSD as a function of gradient time and flow rate.

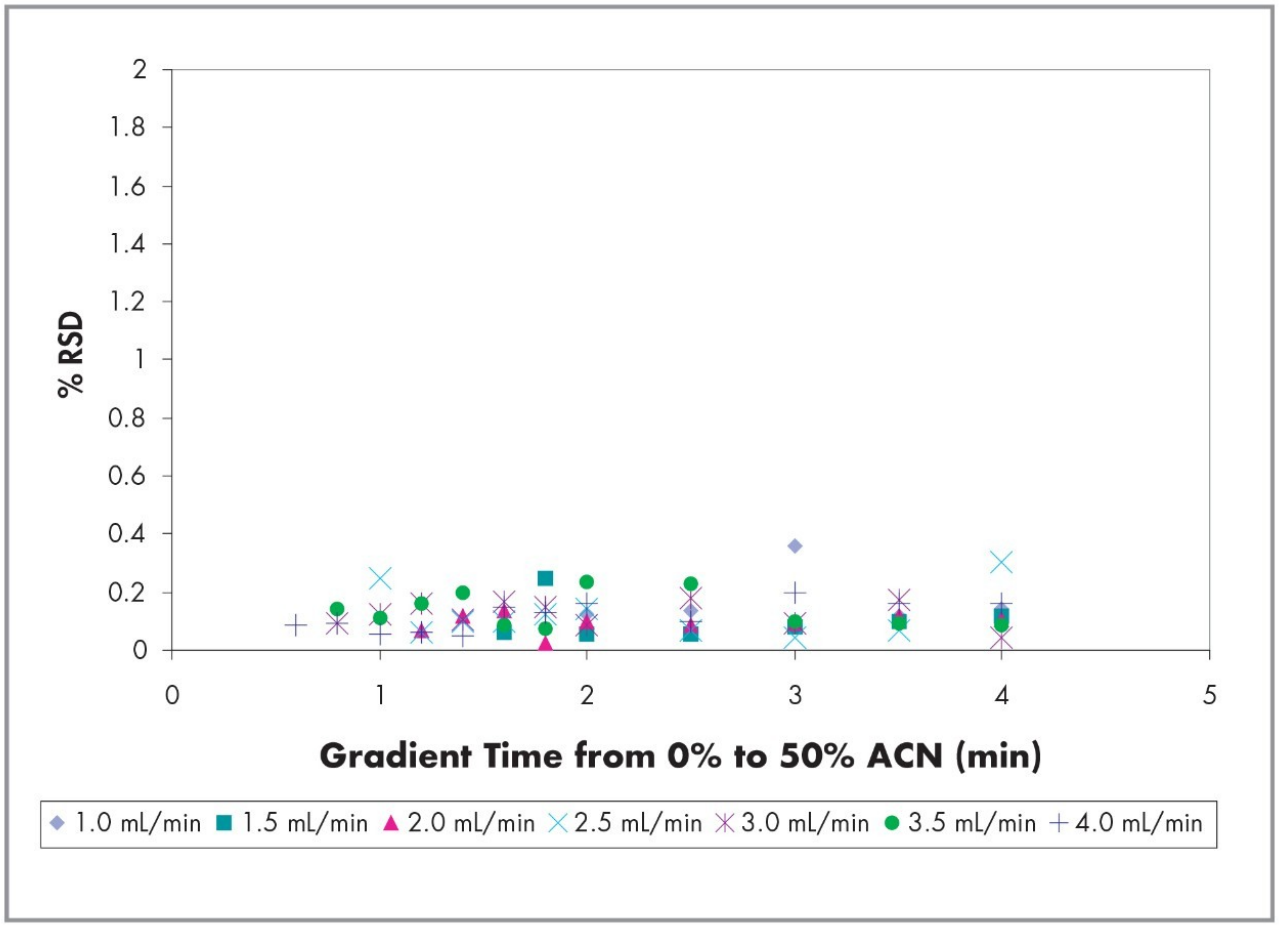

Figure 2. Peak area %RSD as a function of gradient time and flow rate.

Higher flow rates produce the best peak capacities

Peak capacity is a function of the peak width, which is in turn a function of the analyte's linear velocity, and band broadening effects. According to the van Deemter equation, as the flow rate increases, there will reach a point where band broadening effects are minimized and the narrowest peaks will be achieved. For *IS* 4.6 mm i.d. columns, this point is between 3 and 4 mL/min, under most conditions. Table 1 lists the peak capacities achieved for various gradients. The optimal peak capacity for each gradient is listed in bold.

Flow Rate (mL/min) \ Gradient Time (min)	1.0	2.0	3.0	4.0
1.0	--	--	17	24
1.5	--	16	21	28
2.0	--	20	26	34
2.5	14	22	28	38
3.0	16	**29**	37	42
3.5	18	28	**38**	45
4.0	**19**	28	**38**	**46**

Table 1. Peak capacities as a function of gradient time and flow rate.

Shorter run and re-equilibration times

IS Columns allow for much faster separations, Figure 3, increasing sample throughput and decreasing solvent consumption per analysis. Additionally, the higher flow rates and shorter columns allow for much faster post gradient re-equilibration times. Recommended reequilibration times are 3 times system volume and 5 times column volume. For a standard 4.6 x 100 mm column on the Alliance 2695 Separations Module at 1 mL/min, re-equilibration time is 10.3 minutes. For an *IS* Column, 4.6 x 20 mm at 3 mL/min, re-equilibration time is only 1.2 minutes, resulting in much faster injection to injection cycle times.

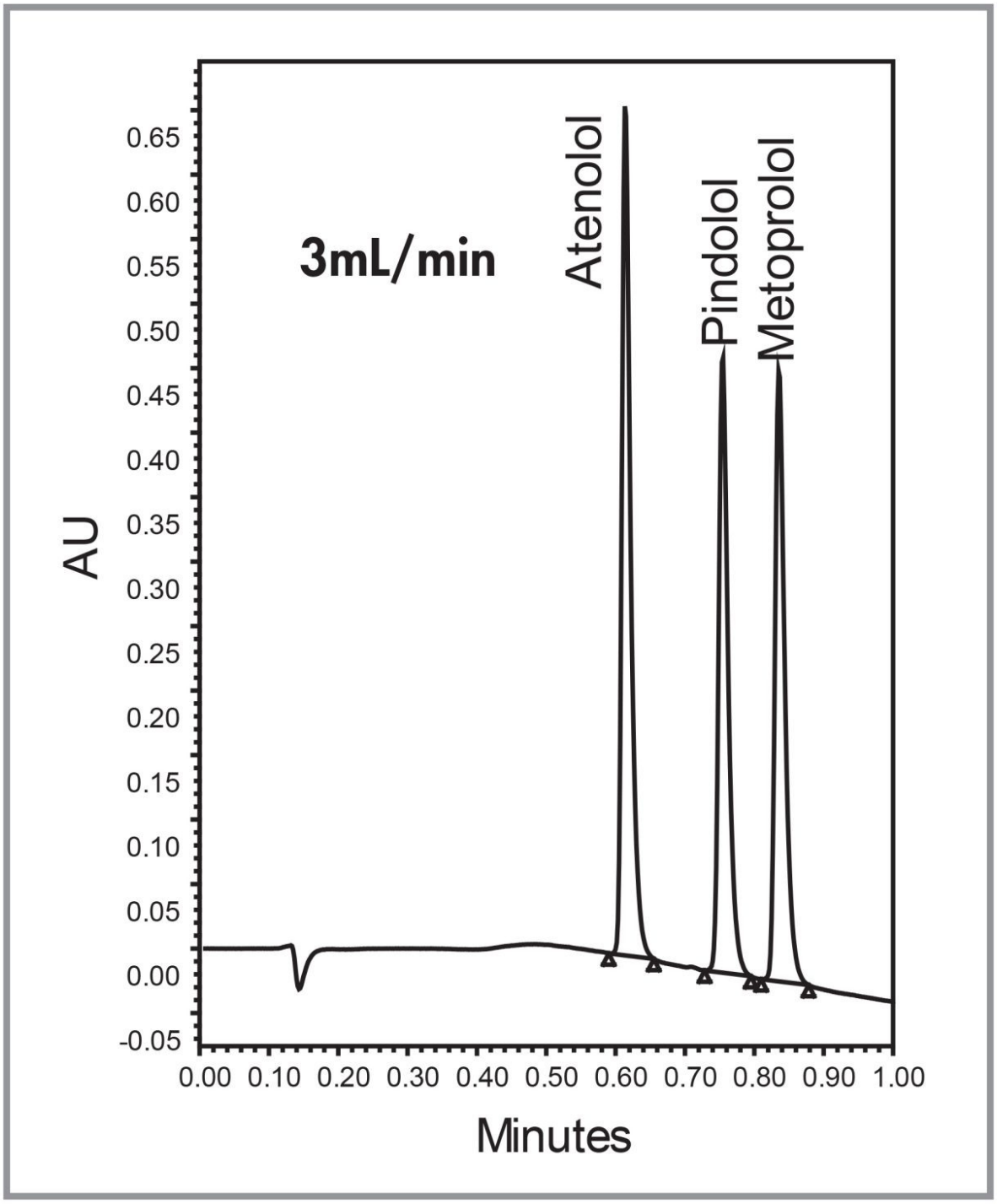

Figure 3. Fast gradient separation of 3 beta blockers.

Conclusion

The Alliance 2695 Separations Module delivers excellent retention time and area reproducibility for fast gradient separations (0 to 50% ACN over 0.6 to 4.0 minutes) without instrument modifications. The best peak capacities for the 4.6 mm i.d. *IS* Columns are achieved between 3 and 4 mL/minute. Run times and the reequilibration times are greatly reduced with *IS* Columns (re-equilibration times reduced 8.6 times as compared to 100 mm columns), allowing for increased sample throughput and increased productivity.

Featured Products

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720000723, August 2003