2996 Photodiode Array and 2487 Dual Wavelength Absorbance Detectors: Detector Flow Cell Choices For Use With 2.1 mm I.D. Intelligent Speed (*IS*) Columns

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

Abstract

This application brief examines the effects of using the microbore flow cells for narrow bore *IS* separations.

Introduction

Extra-column band broadening effects are generally more important when running narrow bore chromatography than in analytical scale separations. When using Waters Intelligent Speed (*IS*) narrow bore columns, these effects are of particular importance due to the extremely narrow peaks that are generated. Extra-column band broadening can result from the use of improper fittings, mis-seated ferrules, and post-injector tubing volumes. Another source of extra-column band broadening can be the detector flow cell itself. This can result from the tubing used in the flow cell (often there as a heat exchanger), the volume of the flow cell, and even the physical design of the flow cell. The Waters 2996 Photodiode Array and 2487 Dual Wavelength Absorbance detectors both have optional microbore flow cells which are designed for narrow bore chromatography. They have reduced tubing and flow cell volumes which minimize post-column band broadening effects. This application brief examines the effects of using the microbore flow cells for narrow bore *IS* separations. Additional information on the use ofWaters instruments with narrow bore *IS* columns can be found in application brief 720000723EN <https://www.waters.com/nextgen/us/en/library/application-notes/2003/alliance-2695-separations-module-optimization-and-performance-with-4-6-mm-i-d-intelligent-speed-is-columns.html> titled: Alliance 2695 Separations Module: Optimization and Performance with 2.1 mm i.d. Intelligent Speed (*IS*) Columns.

Experimental

2996 Photodiode Array Detector: Analytical and Microbore Flow Cells

A mixture of five analgesics was separated on a 2.1 mm i.d. column using a 2 minute, linear gradient at five different flow rates and was detected using the 2996 PDA detector with either the analytical or microbore flow cells. The average peak widths and peak heights achieved with each flow cell are listed in Table 1. There is an improvement in peak widths of approximately 30% when the microbore cell is used. This results from the reduced cell volume (8.0 µL to 2.7 µL) and the reduced tubing volume (0.009" tubing replaced with 0.005" tubing). However, the reduced cell volume comes at the cost of a decreased flow cell path length (10 mm to 3 mm) resulting in a reduction in signal. The dependence of absorbance signal on flow cell path length is described by Beer's Law (A=εbc where A is absorbance, ε is molar absorptivity, b is path length, and c is concentration). Table 1 shows that the average drop in signal due to the decreased path length of the microbore flow cell is approximately 60%. The choice of whether to use the microbore cell will depend on the purpose of the method. If increased peak capacity is required, the microbore flow cell should be used since the decreased peak width will increase resolution. However, if sensitivity is more important, the analytical flow cell should be used to achieve the maximum signal.

Flow Rate (mL/min)	Analytical Peak Width (sec)	Microbore Peak Width (sec)	Peak Width % Improvement	Analytical Peak Height (AU)	Microbore Peak Height (AU)	% Drop in Peak Height
0.4	10.75	7.72	**28%**	0.1063	0.0413	**61%**
0.5	9.52	7.07	**26%**	0.0922	0.0375	**60%**
0.6	8.94	6.06	**32%**	0.0785	0.0334	**57%**
0.7	8.35	5.60	**33%**	0.0707	0.0297	**58%**
0.8	8.37	5.83	**30%**	0.0628	0.0259	**59%**

Table 1. Effect of 2996 flow cell on peak width and height.

2487 Dual Wavelength Absorbance Detector: Analytical and Microbore Flow Cells

The same separation was performed with the 2487 UV/Vis detector to compare the analytical and microbore flow cells. The average peak widths and peak heights are displayed in Table 2. As with the 2996 PDA detector, there is an improvement of approximately 30% in peak width when the analytical flow cell is replaced by the microbore flow cell. This results from the reduced flow cell volume (16.7 µL to 3.9 µL) and the reduced tubing volume (0.009" tubing replaced with 0.005" tubing). Figure 1 is an example of the separation with the analytical and microbore flow cells. The improvement in resolution of the first two peaks results from the reduced extra-column band broadening of the microbore flow cell. There is an approximately 55% drop in signal due to the decreased path length (10 mm to 3 mm). Ultimately, the choice of flow cell will depend on the purpose of the method (sensitivity or peak capacity).

Flow Rate (mL/min)	Analytical Peak Width (sec)	Microbore Peak Width (sec)	Peak Width % Improvement	Analytical Peak Height (AU)	Microbore Peak Height (AU)	% Drop in Peak Height
0.4	13.93	8.33	**40%**	0.1134	0.0552	**51%**
0.5	11.87	8.03	**32%**	0.1012	0.0471	**53%**
0.6	10.81	7.60	**30%**	0.0899	0.0412	**54%**
0.7	10.02	7.13	**29%**	0.0822	0.0366	**55%**
0.8	9.43	7.07	**25%**	0.0742	0.0327	**56%**

Table 2. Effect of 2487 flow cell on peak width and height.

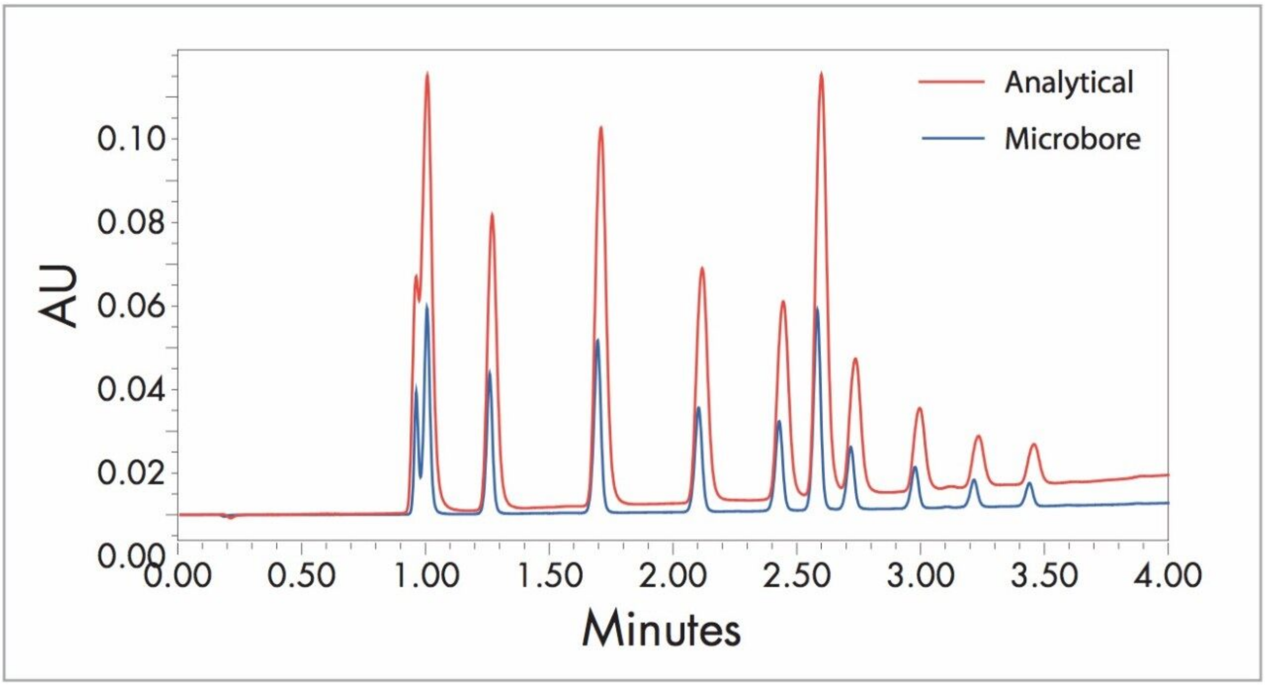

Figure 1. Overlay of separation using the analytical flow cell and the microbore flow cell. This demonstrates the impact of the decreased peak width and decreased peak height when using a microbore flow cell.

System: Waters 2695 Separations Module

Waters 2996 PDA Detector or 2487 UV/Vis Detector

Waters Empower Software

Column:	Atlantis dC_{18}, 2.1 x 20 mm, *IS* 3 µm at 25 °C
Detection:	10 pts/s No digital filtering 2996: Range 200–300 nm, extracted at 260 nm 2487: 260 nm
Mobile phase:	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile
Gradient:	10% to 50% B (linear) over 2 minutes
Flow rate:	0.6 mL/min
Pre-column volume:	550 µL
Sample:	5 µL injection (20 µg/L of each – 2-acetylfuran, theophylline, acetanilide, acetophenone, propiophenone, butyrophenone, benzophenone, valerophenone, hexanophenone, heptanophenone, octanophenone)

Conclusion

The Waters UV/Vis detectors can easily manage the narrow peaks generated by the fast chromatography of the Intelligent Speed (*IS*) line of columns. Extra-column band broadening can be significant with narrow bore *IS* columns. Both the 2487 Dual Wavelength Absorbance and 2996 Photodiode Array detectors have optional reduced volume microbore flow cells which minimize extra-column band broadening effects due to the flow cell. Peak width improvement with the microbore cell is ~30%. The microbore flow cell should only be used for methods in which increased peak capacity is required since the reduced path length leads to lower peak heights.

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