Application Note

2487 Dual Wavelength Absorbance Detector: Optimization for Intelligent Speed *(IS)* Columns

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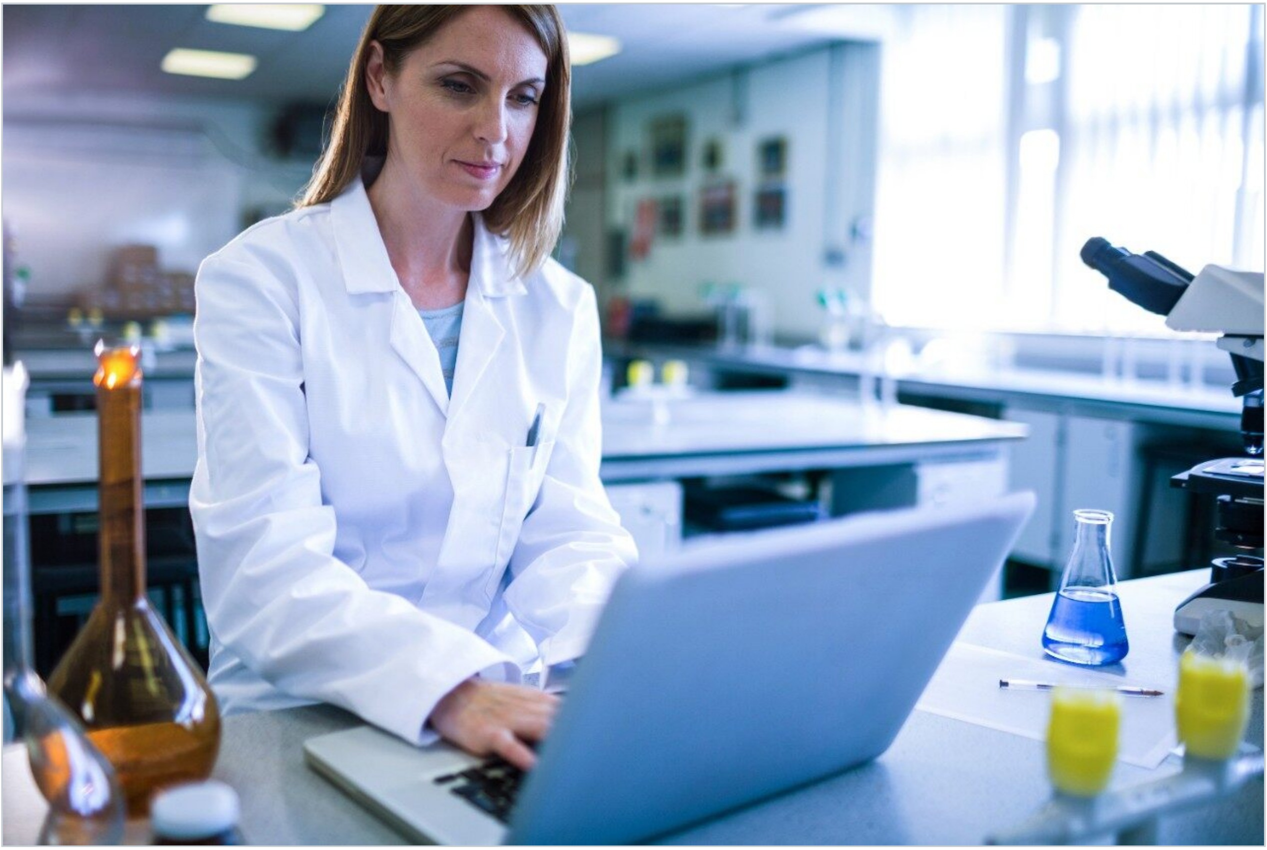

Abstract

Today's labs need faster sample throughput while retaining the reliability of the data that is being generated. To address the need for increased sample throughput, Waters has designed the new Intelligent Speed (*IS)* line of columns. These columns have short packed beds and optimized column hardware. When combined with the Waters Alliance System with 2695 Separations Module, *IS* Columns enable separations with high peak capacities and significantly shorter run times. Peaks generated by Alliance/IS systems are often only a few seconds wide, requiring a detection method with high data sampling rates. The Waters 2487 Dual Wavelength Absorbance Detector can perform with exceptional reproducibility under these demanding conditions.

Benefits

The Waters 2487 Dual Wavelength Absorbance Detector is able to reproducibly detect the narrow peaks generated by the fast chromatography obtained with the Waters *IS* Columns

Introduction

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Waters has designed the new Intelligent Speed (*IS)* line of columns. These columns have short packed beds and optimized column hardware. When combined with the Waters Alliance System with 2695 Separations Module, *IS* Columns enable separations with high peak capacities and significantly shorter run times. Peaks generated by Alliance/IS systems are often only a few seconds wide, requiring a detection method with high data sampling rates. The Waters 2487 Dual Wavelength Absorbance Detector can perform with exceptional reproducibility under these demanding conditions.

Additional information on Waters instruments with *IS* Columns can be found in technical note 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 4.6 mm i.d. Intelligent Speed (*IS)* Columns.

Narrow peaks require higher data sampling rates

Fast gradient separations were performed on a mixture of five analgesics at different detector sampling rates in single channel mode. The results are displayed in Figure 1. There is a definite impact on peak shape when the sampling rate drops below 5 pts/s due to an insufficient number of data points. Table 1 lists the reproducibility (%RSDs) of the peak area and the number of points across the peak as a function of the sampling rate. At 1 pt/s, there is a substantial decrease in peak area reproducibility resulting from the poor peak shape. In order to achieve reproducible peak quantitation, a minimum of 15 points across the peak is required. The data in Table 1 indicates that this criterion is not met at sampling rates below 5 pts/s. When the detector is run in dual wavelength mode, the only sampling rate available is 1 pt/s, which is not adequate for reproducible quantitation. Therefore, dual wavelength mode should not be used with separations on *IS* Columns.

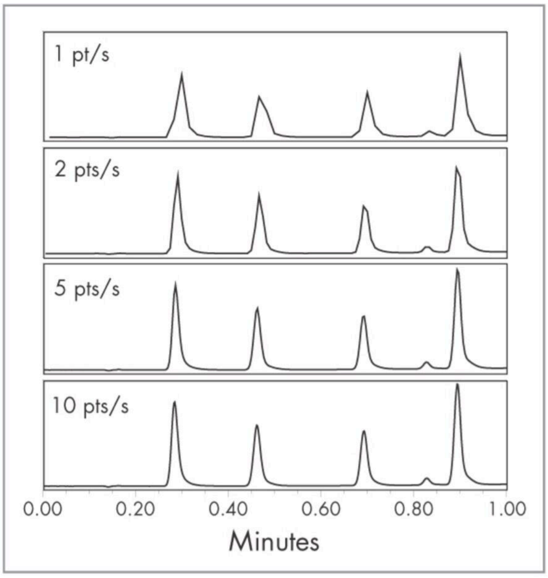

Figure 1. Effect of sampling rate on peak shape (no data filtering).

Sampling rate	Peak area %RSD	Points across peak
10 pts/s	0.38	51
5 pts/s	0.28	26
2 pts/s	0.43	11
1 pts/s	4.65	6

Table 1. Reproducibility and points across peak for the phenacetin peak in the separation of five analgesics (6 replicate injections).

Experimental

LC Conditions

System:	Waters Alliance System with 2695 Separations Module Waters 2487 Dual Wavelength Absorbance Detector Waters Empower Software
Column:	Atlantis dC_{18}, 4.6 x 20 mm IS, 3 f.lm at 25 °C
Deductor settings:	Single channel mode 260 nm
Mobile phase:	A = 0.1 % formic acid in water B = 0.1% formic acid in acetonitrile
Gradient:	10% to 50% B over 1 minute (linear)
Flow rate:	3.0 mL/min
Sample:	10 f.ilinjections of analgesics (20 f.ig/mleach of acetaminophen, caffeine, acetanilide, acetylsalicylic acid, phenacetin)

Results and Discussion

Filtering is performed on data to help reduce noise to enhance signal-to-noise (S/N). There are two types of data filtering available on the 2487 Dual Wavelength Absorbance Detector, the Hamming filter and the RC filter. Selection of the appropriate time constant for filtering is very important. For both of the filters, the higher the time constant, the better the smoothing of the baseline. However, increasing the time constant will also hove the effect of broadening the peak, decreasing signal and peak capacity. Figure 2 demonstrates the effect of using different time constants with the Hamming filter. As the time constant is increased, there are noticeable broadening effects on the peaks and the peak height is reduced. Table 2 lists the noise improvement and S/N increase as the time constant is increased, along with the effect on peak width, resolution and peak capacity. The best time constant, depending on the resolution needed, will generally be 0.1–0.3. This allows for some filtering to smooth the baseline for integration while not having a major impact on peak capacity. There is also a small improvement in S/N at these filtering levels. The level of filtering should be determined for each method to ensure that the impact on resolution is not significant.

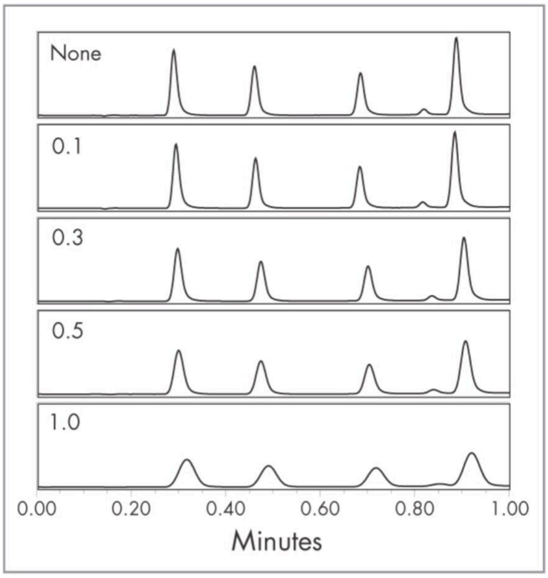

Figure 2. Effect of increasing the time constant of the hamming filter (sampling rate is 10 pts/s).

Time constant	Improvement in peak-to-peak noise	Signal-to-noise increase	Resolution	Peak width	Peak capacity
No filtering	—	—	2.65	4.52	14
0.1	16%	1.15x	2.60	4.59	14
0.3	20%	1.04x	2.21	4.98	13
0.5	27%	0.94x	1.74	5.59	11
1.0	39%	0.73x	1.03	8.01	8

Table 2. Effect using the Hamming filter on signal-to-noise, peak width and resolution of the phenacetin peak in the separation of 5 analgesics (6 replicate injections).

Figure 3 demonstrates the effect of the RC filter. Even with the lowest time constant (1.0), the RC filter has a dramatic effect on peak shape. Because of this, RC filtering should not be used when the 2487 Dual Wavelength Absorbance Detector is used with *IS* Columns.

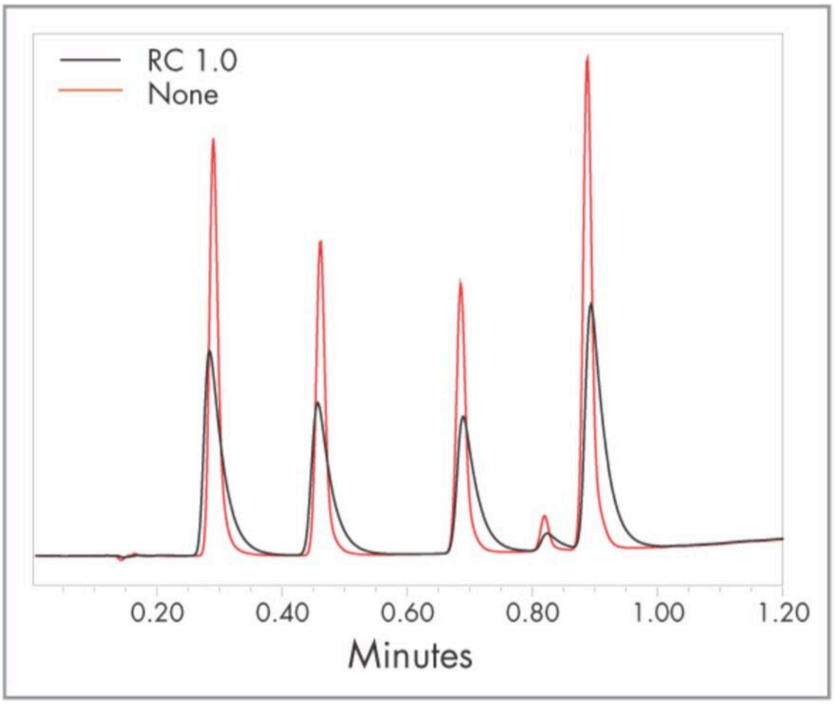

Figure 3. Effect of RC filter at the lowest time constant setting (1.0) on peak shape.

Conclusion

The Waters 2487 Dual Wavelength Absorbance Detector is able to reproducibly detect the narrow peaks generated by the fast chromatography obtained with the Waters *IS* Columns. Sampling rate is very important and should be at least 5 pts/s to ensure reproducible quantitation. Due to the high sampling rates necessary for fast chromatography, only single channel mode can be used. Data filtering should be used with caution. The hamming filter should be used with time constants of 0.3 or less, and this value should be determined for each method to give the best noise reduction while not sacrificing resolution. The RC filter should not be used with the narrow peaks generated on the *IS* line of columns.

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