Performance PerSPECtives

Waters® 515 HPLC Pump

Isocratic Chromatography: Superior Performance with a Traditional Pump

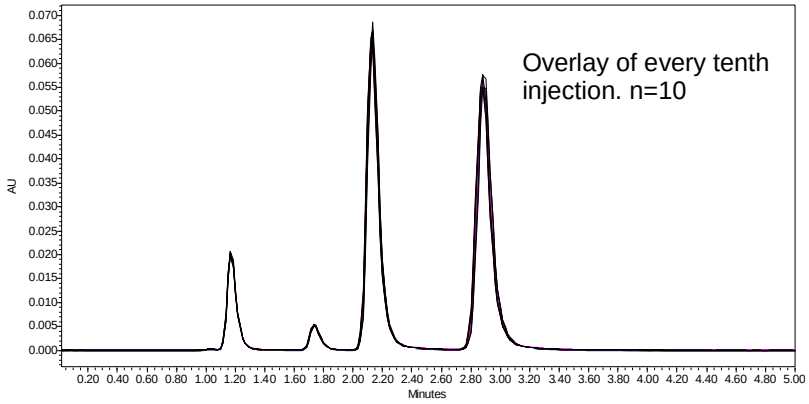
ISOCRATIC SEPARATIONS

Many separation problems can be solved without use of gradient elution protocols if the mobile phase composition is optimized for the separation. Isocratic chromatography continues to be very popular for routine analysis due to the simplicity inherent in the technique. Operation in the isocratic mode allows the scientist to recycle system mobile phase which reduces solvent and labor costs associated with the analysis. In addition, the time associated with reconditioning the column between injections, a necessity for gradient chromatography, is eliminated when operating in the isocratic mode, which creates a very efficient system for routine high throughput testing.

When operating in the isocratic mode, precise flow delivery by the HPLC pump can be the single most important factor that affects run to run reproducibility. This Performance PerSPECtive will demonstrate the excellent flow precision characteristics of the Waters 515 HPLC Pump when used for an isocratic separation.

One hundred consecutive injections of a test mixture (System Startup Mixture, Waters P/N 34544) were made over a period of twelve hours. The flow reproducibility of the Waters 515 Pump is demonstrated in Figure 1 where every tenth injection has been overlaid to demonstrate the extremely consistent retention time performance delivered by the Waters 515 HPLC Pump.

Figure 1

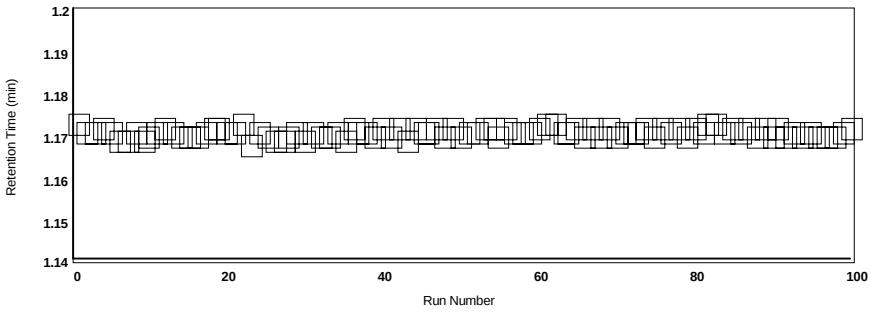


Waters 515 HPLC Pump
Waters 717 plus Autosampler
Eluent: 70% Methanol,30% Water
Flow: 1.0 mL/min.
Sample: System Startup Mixture
Column: Symmerty® C18, 3.9 x150mm.
Waters 486 Detector: 254nm

FLOW RATE PRECISION

Figure 2 is a control chart, processed using Millennium® ^{32} System Suitability Software, for the first peak, Vo, which is not retained on the column. The retention time of this peak is a chromatographic measure of flow rate precision. **The standard deviation of retention time for 100 chromatographic runs over 12 hours is 0.001 minutes (0.06 seconds) . This data attests to the excellent flow rate precision of the Waters 515 HPLC Pump.**

Figure 2



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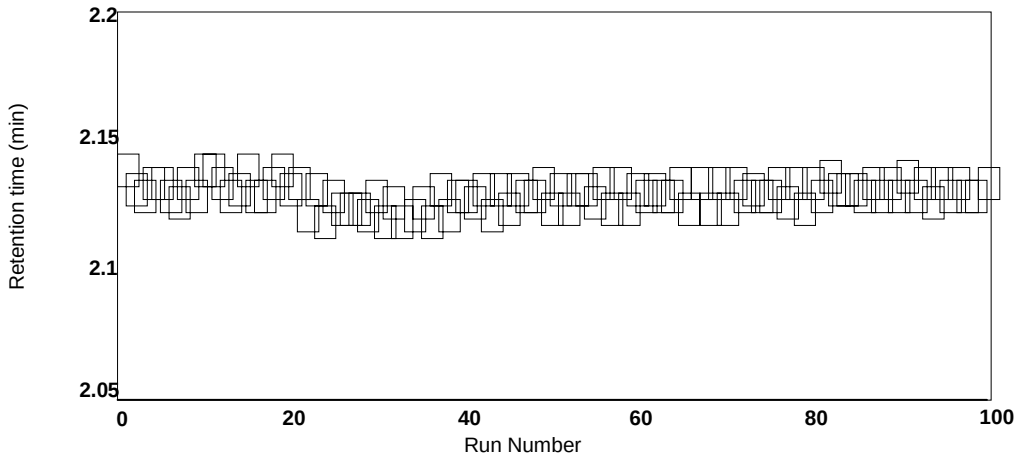
REPRODUCIBILITY OF RETENTION TIME

This excellent reproducibility of flow rate as measured with the Vo peak can be carried over to the retained compounds as indicated by the retention time reproducibility of peak 3 in the test mixture (Figure 3). **The standard deviation of Peak 3 retention time is 0.005 minutes or 0.3 seconds which is superior retention time reproducibility for a retained peak.** Excellent retention time reproducibility provides greater confidence in peak identification as well as more reproducible peak areas for quantitation. The direct link between retention time reproducibility and peak area reproducibility is discussed in Performance PerSPECtive WPP19, Retention Time Vs Peak Area.

Figure 3



SEPARATIONS WITH DIFFERENT I.D. COLUMNS

Standard columns for analytical separations range from 3.9 to 4.6 mm internal diameter and are run at flow rates of 1.0 to 2.0 mL/min. Advances in column technology allow the scientist to maximize assay sensitivity and reduce sample and solvent consumption through the use of smaller diameter columns (WPP207). Conventional HPLC pumps are typically challenged to provide acceptable flow reproducibility at the low flow rates associated with small diameter columns. However, the Waters 515 HPLC pump is able to provide excellent flow reproducibility at standard *and* narrow bore flow rates. The table below illustrates the retention time reproducibility for 100 consecutive injections on a 3.9 x150 mm Symmetry® C18 column at a flow rate of 1.0 mL/min. and on a 2.1x150 mm Symmetry C18 column at a flow rate of 300 uL/min. using a Waters 515 HPLC pump.

Retention Time Summary of 100 Injections

Symmetry C18	**3.9x150 mm** RT (Std dev) min.	**2.1x150mm** RT (Std dev) min.
Peak 1	1.171 (0.001)	1.314 (0.002)
Peak 2	1.739 (0.003)	1.887 (0.004)
Peak 3	2.131 (0.005)	2.282 (0.006)
Peak 4	2.885 (0.008)	3.046 (0.009)

The Waters 515 HPLC Pump delivers exceptional flow rate precision at both analytical and narrow bore flow rates, allowing the scientist to fully capitalize upon the solvent, labor and time saving advantages inherent in isocratic chromatography while enhancing assay reliability and precision.

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