Waters Alliance[®] System Isocratic Chromatography

ISOCRATIC SEPARATIONS

Isocratic chromatography, the separation of compounds with a single mobile phase, is the simplest form of chromatography and is preferred whenever possible. The example shown below is the PQ (performance qualification) mixture used by Waters in the validation of HPLC systems. It is a good mixture for determining the flow rate precision of a solvent delivery system by measuring peak retention time reproducibility.



Waters Alliance System consisting of: Waters Alliance Separations Module -60% methanol 40% water (automated solvent blending), 1 mL/min Waters 996 PDA at 254 nm Symmetry[®] C₁₈, 3.9x150mm Sample: PQ Mixture (acetone, C2, C3, C4 alkyl phenones)

FLOW RATE PRECISION

Below is a control chart for the first peak, Vo, which is not retained on the column, processed using Millennium^{®32} System Suitability Software. The retention time of this peak is a chromatographic measure of flow rate. The standard deviation of retention time is 0.003 minutes (0.2 seconds) for 100 chromatographic runs over 16 hours. This is excellent flow rate precision.



The reliability of the separation method with the Waters Alliance System is indicated by this consistency over an extended number of runs.



REPRODUCIBILITY OF RETENTION TIME

The excellent reproducibility of flow rate and eluent blending is indicated by the retention time reproducibility of peaks 2, 3 and 4 (figure below). The standard deviation of Peak 3 retention time is 0.010 minutes or 0.6 seconds which is superior to what can be obtained with traditional HPLC systems.



SEPARATIONS WITH DIFFERENT I.D. COLUMNS

Analytical separations on 3.9 to 4.6 mm i.d. columns are typically run at 1.0 to 1.5 mL/min. There are at least two motivations for use of smaller diameter columns: 1) to save solvent; 2) to achieve greater sensitivity. Conventional HPLC pumps are challenged to provide acceptable flow reproducibility at the low flow rates associated with smaller diameter columns. The overlays of two chromatograms shown below are the same separation at 1 mL/min on a 3.9x150 mm Symmetry C_{18} column compared to 300 uL/min with a 2.1x150mm column. The peak elution times are similar because the linear velocities are similar. The peak widths are wider (in minutes) as the flow rate is reduced because extra column effects of the HPLC system are more significant with narrow bore columns.



The separation of the four peaks is good at both flow rates. By reducing the flow rate from 1 mL/min to 300 uL/min, solvent usage can be reduced by 70%. The injection volumes were scaled down for the 2.1 mm column, so there is no apparent increase in sensitivity.

The Waters Alliance System has been designed to provide superior performance at all flow rates. The control chart below shows the excellent retention time reproducibility for peak 3 at 300 μ /min (Std dev = 0.008 min.).



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