Waters Alliance[®] System Gradient Reproducibility Peptide Separations

The Waters Alliance System consisting of the Waters Alliance Separations Module and the Waters 996 Photodiode Array Detector has proven excellent gradient reproducibility for mixtures of small molecules. This has been demonstrated previously (WPP202) for flow rates of 1 mL/min, 200 and 50 μ L/min on Waters Symmetry[®] C₁₈ columns of 3.9, 2.1 and 1.0 mm i.d.

Peptides are larger molecules containing a mixture of hydrophobic, acidic and basic side chains. To achieve a separation on reversed phase columns a shallow gradient is generated with 0.1% trifluoroacetic acid (TFA) in water and acetonitrile. The TFA serves several functions. It is a pH modifier and an ion pairing reagent. TFA has significant low-UV absorbance which causes baseline ripple when monitoring 214 nm or lower. As a result, baseline ripple can be observed as noise if the eluent mixing is incomplete. Excellent performance of an HPLC system is required to obtain good retention time reproducibility with TFA gradients. Below is an overlay of ten injections of peptides from a cytochrome c tryptic digest on an Alliance System. Note the excellent retention time reproducibility at 200 μ L/min and the lack of baseline ripple.



Narrowbore and Microbore

A greater chromatographic challenge is the separation of peptides from a bovine serum albumin (BSA) tryptic digest because this peptide mixture has at least one hundred peaks. A shallow gradient from 0 to 40% acetonitrile (0.3%/min) in 120 min is required. The gradient reproducibility of a Waters Alliance System with a 996 PDA (standard 10 mm flow cell) was done at 200 and 50 μ L/min using Symmetry C₁₈ columns. Chromatograms and overlays are shown. The standard deviation of retention times are summarized in the table for the Waters Alliance and traditional systems.



The Alliance system has excellent gradient performance for narrowbore (2 mm) and microbore (1 mm) chromatography.

0.012 - 0.099

0.052

0.055 - 0.219

0.116

0.019 - 0.126

0.070

0.0080 - 0.063

0.041

Range of Std Dev

Mean of Std Dev

(Min)

(Min)