Gradient Proportioning Valve Test Predicts Performance Definitive Measurement for Gradient Performance

Both Gradient and Automated Solvent Blending performance of an HPLC solvent delivery system can be predicted by a definitive measurement of their accuracy and reproducibility. Step gradients of various solvent changes (1%, 10%, etc.) can be used to measure these important performance criteria. Figure 1 shows the results of such a test on a Waters[®] Alliance[™] System and a quaternary gradient product from Vendor X.



For this experiment, 100% methanol is used as solvent A. Solvent B is 100% methanol containing 9 mg/L of nonvolatile propylparaben to generate a response with UV absorbance at 254 nm. The column is removed from the system and replaced with either a union or a short length of capillary. The flow rate is set at 1.0 mL/min. and a gradient program is used to make 10% step gradient solvent changes at 5 minute intervals from 0% to 100% B. The absorbance provides a linear response to the changes in solvent B and can be used to calculate solvent compositional accuracy of the gradient proportioning valves. This measurement is done by comparing the response at any %B solvent composition to the measured response at 100% B. Since there is only one definition of accuracy, the measurement is either accurate or not. The response for 50% B should be exactly 50% of the response at 100% B. Figure 2 presents the results for the above data in a more informative bar graph format.

Waters Corporation 34 Maple Street Milford, MA 01757 508 478-2000

S

Waters



The graph on the left represents the solvent proportioning accuracy of the two systems. This test provides definitive measurements of the solvent compositional accuracy. The results show that the Alliance System exhibits superior accuracy while the traditional solvent delivery device is challenged to attain this performance. If we repeat the experiment six or more times, we will generate data which can be used to calculate reproducibility results as seen in the graph on the right. Together these results tell us not only if we are accurate, but also how well we can repeat the compositional blend of our solvent from injection to injection. This is important information in predicting the retention time reproducibility of gradient and automated solvent blended methods. The comparison below shows how instrument performance influences chromatography in an automated solvent blended.



For this chromatography, a 40/60 blend of water/methanol was programmed, proportioned and delivered by each instrument. The retention times for the above chromatograms differ and could have been predicted from the gradient test results. Instrument performance in the areas of accuracy and reproducibility for Automated Solvent Blending allows us to minimize solvent handling and variables as well as more easily transfer methods from operator to operator and instrument to instrument.