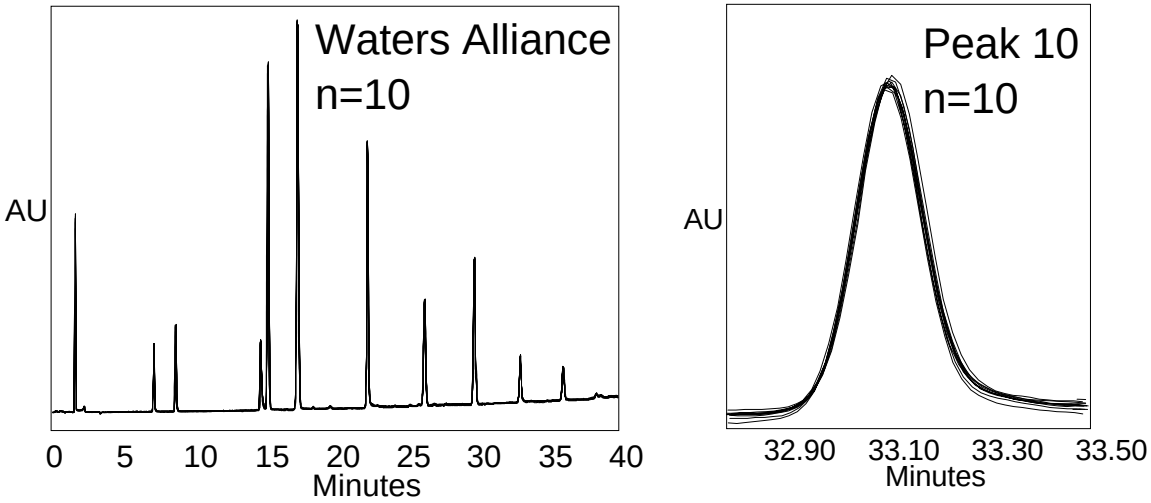
Performance PerSPECtives

Testing Gradient Performance

A Gradient Test Mix for Characterizing Instrument Performance

A gradient proportioning valve test can be performed on any multisolvent delivery system to characterize expected performance for both gradient and automated solvent blending modes (see WPP33). But the smallest compositional changes (steps) we typically make with these tests is 1%. But the length of these tests is not the only challenge to their value. Gradient solvent delivery in practice consists of infinitesimally small changes in the solvent proportioning over a prescribed period of time. Can the proportioning valve test with 1% steps really define our instruments performance when taken to these gradient extremes? Perhaps then the best evaluation of instrument gradient performance lies in running a series of a gradient separations and observing the results. In order for this approach to provide a high level of gradient performance assurance, a mixture of compounds must be injected that challenge the solvent delivery across the range of gradient performance while at the same time not introducing complex separation chemistries that will provide added variables and inhibit our ability to attribute the observed results to instrument gradient performance. Figure 1 shows the separation of a gradient mixture which goes a long way towards meeting these criteria. These results are from a Waters® Alliance™ System.



Conditions: Column: Symmetry® C18, 3.9 x150mm, 30°C; Flow rate: 1 mL/min; Mobile Phase: A=water, B=acetonitrile, both with 0.015% H3PO4; Gradient 0-80%B, 40 min, linear, vacuum degasser on normal; Detection: 254 nm; Sample: Gradient test mix

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

Peak retention time reproducibility depends on the instruments gradient performance. The overlay of 10 consecutive injections seen in Figure 1 gives a good indication of the gradient performance. Enlarging the view around peak 10 gives us a better understanding of peak retention time reproducibility. We can statistically reduce the data to meaningful information as seen in Table 1 below. The gradient mix used here contained ionic compounds (acidic and basic) as well as a series of neutrals. The ability of Alliance to achieve excellent retention time reproducibility throughout the gradient separation is predicated by the instruments ability to accurately and reproducibly deliver buffer at the beginning of the gradient as well as introduce acetonitrile to elute the neutral alkylphenones, towards the end of the separation.

Table 1

Standard Deviation, min n = 10	Pk 1	Pk 2	Pk 3	Pk 4	Pk 5	Pk 6	Pk 7	Pk 8	Pk 9	Pk 10	Pk 11
Waters Alliance System	**0.004**	**0.012**	**0.014**	**0.013**	**0.013**	**0.011**	**0.009**	**0.006**	**0.006**	**0.006**	**0.007**
% Relative Std Dev (%RSD) n = 10											
Waters Alliance System	**0.21**	**0.16**	**0.16**	**0.09**	**0.08**	**0.06**	**0.04**	**0.02**	**0.02**	**0.02**	**0.02**

The test mixture detailed below is useful in helping evaluate the gradient HPLC performance. The compounds, solvents and column are easily obtained and the results will indicate the level of gradient performance for any given HPLC system. The test mix is easily formulated from individual standards which are made at a 1.0 mg/mL stock concentration in acetonitrile. They are diluted to the working standard concentrations listed below. For example, 2mL uracil stock + 5mL theophylline stock + 5mL caffeine stock + 20mL benzoic acid stock + 5mL methylparaben stock etc. diluted to a total volume of 100mL with 30% acetonitrile in water to produce the working standard levels listed below. This also represents the elution order of the compounds with peaks 6 and 7 coeluting.

Compound	Concentration
1. Uracil	2 ug/mL
2. Theophylline	5
3. Caffeine	5
4. Benzoic acid	20
5. Methylparaben	5
6. Acetophenone	5
7. Ethyl-para-aminobenzoate	10
8. Propylparaben	5
9. Butyrophenone	5
10. Valerophenone	5
11. Hexanophenone	5
12. Heptanophenone	5

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