Performance PerSPECtives

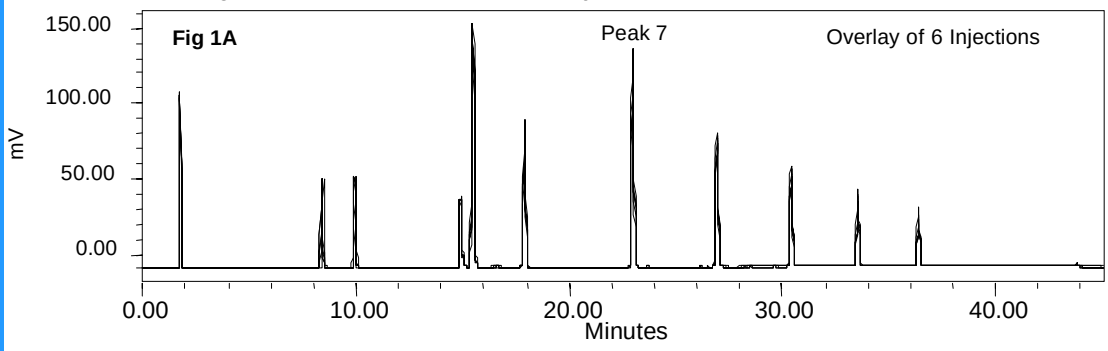
Waters® Breeze™ Systems

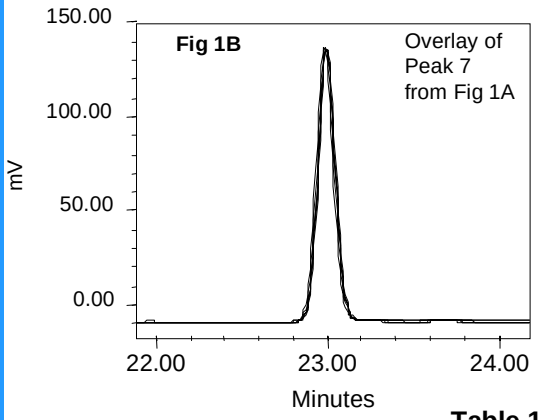
Gradient Chromatography

Gradient Chromatography for Demanding Applications:
The need to separate chemically different compounds contained in complex sample mixtures is a challenge faced by many chromatographers. While isocratic methods are favored for less demanding separations, gradient chromatography provides the means to resolve complex mixtures through precisely programmed variations in solvent strength (e.g., organic for reversed-phase or ionic for ion-exchange). These gradient methods require accuracy and precision in eluent delivery for dependable and reproducible results.

Outstanding Gradient Performance with Breeze HPLC System:
The technology contained in Breeze Systems make them well suited for gradient chromatography at a variety of operating conditions. Each solvent required for the gradient chromatography is delivered to the column via a separate pump. Breeze Software manages solvent flow rate and composition. Solvent blending occurs after the eluent leaves each pump. "High Pressure Mixing" is the term used to describe this technique for non-isocratic solvent delivery. Typically this is the most reliable method of delivering fast and accurate gradients. The ability of Breeze Systems to program, control and deliver highly reproducible gradient separations of a complex test mixture (See WPP34: A Gradient Test Mix for Characterizing Instrument Performance) is shown in Figures 1A and 1B. The retention time reproducibility data shown in Table 1 demonstrate the excellent gradient performance of Breeze Systems at analytical flow rates (1.0 mL/min.).

Figures 1A , 1B and Table 1: Breeze System Gradient Performance at 1.0 mL/min





Breeze HPLC Conditions Used for Figure and Table 1

Pumps: Breeze 1525 (Gradient)
Injector: 717+ Autosampler
Detector: 2487 at 254 nm at 2 pts/sec
Column: Symmetry® C_{18} 5 µm, 3.9 x 150 mm at 40° C
Sample: 24 uL Gradient Test Mix (WPP34)
Solvents: A= HPLC water with 0.015% H3PO4
B= Acetonitrile with 0.015% H3PO4
Flow: 1.0 mL/min
Gradient: 0 - 80%B in 40 minutes
Data: Breeze Software

Table 1

Peak	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11
R.T. Mean (N=10)	1.752	8.392	9.931	14.901	15.491	17.909	22.994	26.955	30.422	33.545	36.391
R.T. Std. Dev.	0.001	0.037	0.029	0.009	0.008	0.006	0.006	0.007	0.008	0.009	0.008

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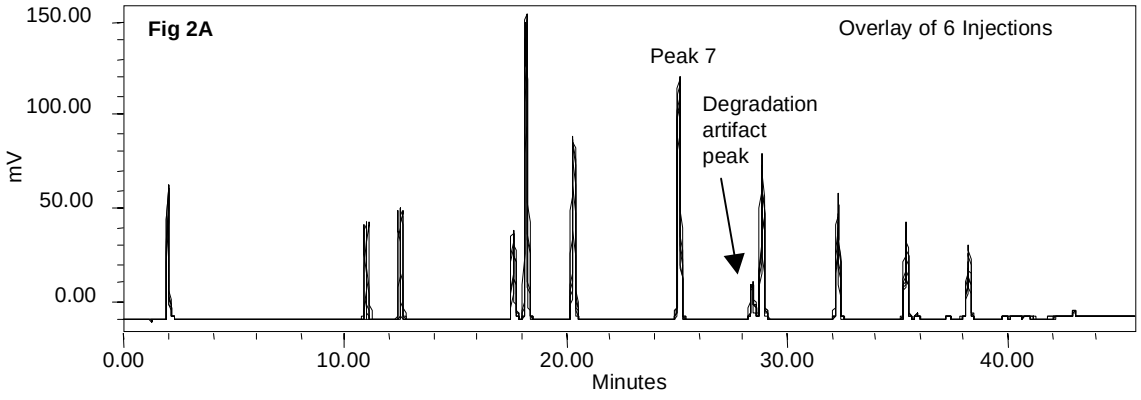
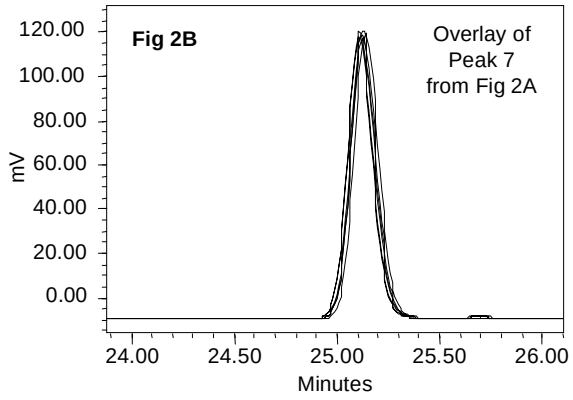
Reduce Solvent Consumption Without Sacrificing Gradient Performance:
The cost of HPLC solvents and/or the cost of solvent disposal as well as the need for increased mass sensitivity has resulted in HPLC gradient methods that use columns of reduced internal diameter (e.g., 2.1 mm vs. 3.9 or 4.6 mm internal diameter columns) and lower flow rates . Effective use of narrow-bore columns for gradient chromatography demands systems capable of delivering eluents reproducibly at lower flow rates (0.29 mL/min.). In addition, HPLC system volumes need to be minimal in order to optimize gradient formation and maximize sample throughput (less re-equilibration time between samples). Breeze Systems are ideally suited for narrow-bore analysis when coupled to the Waters 717+ autosampler configured with the low volume option kit (Waters Part Number WAT055886). The ability of this "high-pressure" HPLC gradient configuration to yield highly reproducible narrow-bore chromatography of a complex test mixture is demonstrated in Figures 2A and 2B. Table 2 below shows retention time reproducibility with Breeze Systems configured for the narrow-bore technique.

Figures 2A , 2B and Table 2: Breeze System Gradient Performance at 0.29 mL/min





Breeze HPLC Conditions Used for Figures 2A & 2B and Table 2

Pumps: Breeze 1525 Gradient
Injector: 717+ Autosampler with Low Volume Kit
Detector: 2487 at 254 nm at 2 pts/sec
Column: Symmetry ® C_{18} 5 µm, 2.1 x 150 mm at 40° C
Sample: 6 uL Gradient Test Mix (WPP34)
Solvents: A= HPLC water with 0.015% H3PO4
B= Acetonitrile with 0.015% H3PO4
Flow: 0.290 mL/min
Gradient: 0 - 80%B in 40 minutes
Data: Breeze Software

Table 2

Peak	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11
R.T. Mean (N=10)	1.975	10.957	12.506	17.607	18.206	20.331	25.122	28.881	32.306	35.395	38.214
R.T. Std. Dev.	0.007	0.067	0.064	0.032	0.031	0.025	0.046	0.022	0.017	0.014	0.014

Summary:

• The ability of an HPLC pump(s) to precisely deliver solvent can be the single-most important factor that affects run-to-run retention time reproducibility for either isocratic or well characterized gradient separations.

• The excellent gradient performance characteristics of the Waters Breeze HPLC Systems make this technology well suited for chemists who prefer using multi-pump systems for analytical or narrow-bore chromatography.