Performance PerSPECtives

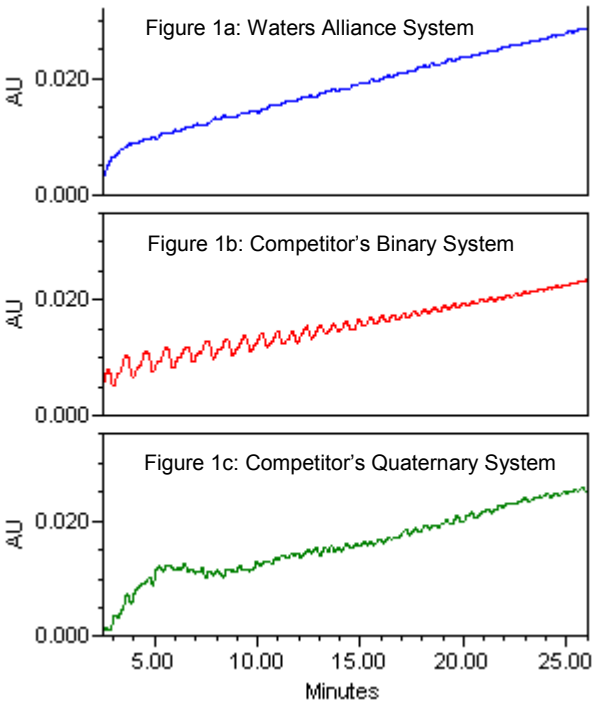
# Waters® Alliance® System
# Effect of Solvent Mixing on Baseline Stability

**Question:** Our company frequently analyzes compounds that exist in very small concentrations (e.g., peptides contained in tryptic digests). These separations are performed using relatively shallow gradients. Don't all HPLC systems do an adequate job of mixing solvents for this variety of gradient chromatography? What is the benefit of Waters Alliance technology compared to another manufacturers' HPLC systems?

**Considerations:** The quality of data generated by an HPLC system is the sum of solvent delivery, chemistry, detection, and data analysis capabilities of the system. In gradient separations, optimum performance is significantly affected by the ability of the solvent delivery system to deliver accurate and precise solvent flow while adequately mixing the blended mobile phase. When one of the mobile phase components has a significant absorbance at the monitored wavelength, minute irregularities in solvent mixing can be problematic. In this situation, a "baseline ripple" can be observed that can affect quantitation accuracy. Unfortunately, it is not always possible to avoid mobile phase components that absorb at low UV wavelengths (e.g., trifluoracetic acid, Triethylamine, biological buffers, etc.) where many HPLC separations are monitored. **This Performance PerSPECtive illustrates how solvent delivery affects the quality of gradient separations in those situations where it is neither feasible nor desirable to avoid low UV absorbing modifiers required for the chromatography.**

**Effect of Solvent Blending on "Baseline Ripple":** To demonstrate how HPLC system design affects baseline performance, a simple gradient of increasing acetonitrile concentration was used employing a mobile phase modifier (0.1% trifluoroacetic acid) which absorbed at low UV wavelengths. Figure 1 clearly demonstrates how baseline stability is affected by HPLC system design. (Note: The increase in baseline from 0 to 26 min is normal since 214 nm absorbance of TFA increases with increased concentrations of acetonitrile.)

**Fig 1: Solvent Mixing Affects Baseline Noise**




**Conditions:**
Column: Symmetry® $C_{18}$, 5 µm, 3.9 x 150 mm at 35° C.
Mobile Phase:
A: 0.1% TFA in Water.
B: 0.1% TFA in Acetonitrile.
Gradient: 5 - 40% B in 35 min.
Flow: 1.0 mL/min.
Sample: Water (10 µL inj. vol.).
Detection: 214 nm at 1pts/sec using Waters 996 Photodiode Array Detector
Data: Waters Millennium®32 Software

Solvent Delivery Systems Tested:
Fig. 1a: Waters Alliance 2690 Separations Module with in-line degasser
Fig. 1b: Competitor's Binary Pump gradient system with in-line degasser
Fig. 1c: Competitor's Quaternary gradient system with in-line degasser.

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**Effect of Solvent Delivery on Signal to Noise:** Compositional ripple, caused by incomplete solvent mixing, significantly affects signal-to-noise performance as indicated in Figure 2 and Table 1. Data in these series of experiments were generated using systems and conditions as detailed in Figure 1 with the injection of 10 µL of sample containing five synthetic peptides (Sigma Peptide HPLC Standard: Part Number H2016). Results clearly indicate how superior signal-to-noise performance was obtained with Waters Alliance technology compared to the competitor's HPLC systems.

**Figure 2: Solvent Mixing Affects Signal-to-Noise**

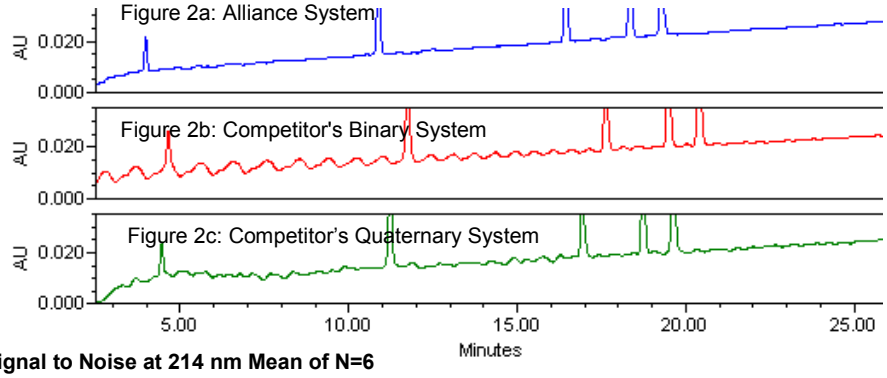



**Table 1: Signal to Noise at 214 nm Mean of N=6**

| | Peptide 1 | Peptide 2 | Peptide 3 | Peptide 4 | Peptide 5 |
|---|---|---|---|---|---|
| **Waters Alliance System** | 22.27 | 58.24 | 48.07 | 75.53 | 68.99 |
| **Competitor's Binary System** | 3.90 | 10.65 | 8.97 | 13.83 | 12.66 |
| **Competitor's Quaternary System** | 11.11 | 31.11 | 22.90 | 39.44 | 36.47 |

**Effect of Solvent Delivery on Limit of Detection:** Limit of detection (LOD) is defined as the lowest analyte concentration that can be detected over baseline noise. It is expressed as a concentration at a specified signal-to-noise ratio (usually 2:1 or 3:1). Using data in Table 1, the expected LOD at two times the signal-to-noise was calculated for each of the evaluated systems. To verify the calculated limits of detection, a 1:10 dilution of the five synthetic peptide test mix was injected onto each of the HPLC systems with results shown in Figure 3. Again, Waters Alliance technology yields superior LOD results compared to the other systems.

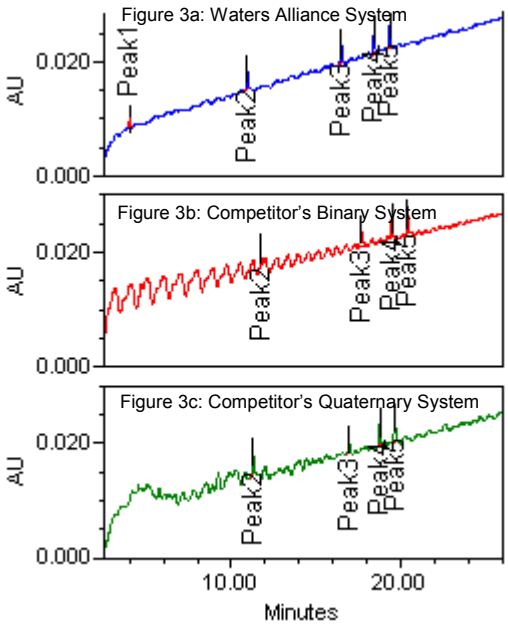
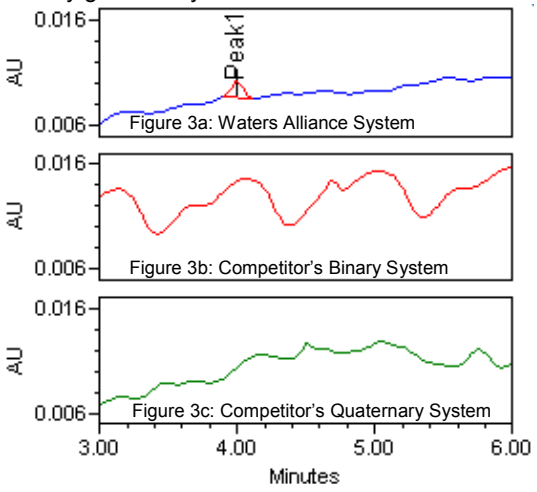



**Figure 3:** 5 ng of the five peptide test mixture on column can be easily distinguished from the baseline in the chromatograms generated on Waters Alliance HPLC System. At 5 ng, Peak 1 is lost in the baseline ripple present in the chromatograms generated on the Major Manufacturer's Binary pump and Quaternary gradient systems.




**Summary:**

▪ Waters Alliance technology results in superior performance for gradient separations that use low UV detection with low UV absorbing modifiers (e.g., peptide separations with acetonitrile / TFA gradients).