Performance PerSPECtives

Waters

Waters®Alliance® Systems

Comparative System Performance For µBore Chromatography:

Migration from analytical to µbore chromatography:
Many HPLC and LC/MS applications have migrated from using analytical columns (e.g., 3.9 mm internal diameter) to chromatography performed on 1 mm microbore (µbore) columns used at substantially lower flow rates (50 µL/min vs. 1 mL /min). The reasons for this migration vary. Microbore chromatography is effective in reducing laboratory overhead by minimizing organic solvent usage and the associated cost of procurement and disposal. In other situations, µbore chromatography may offer increased detection sensitivity since peaks elute from the column in reduced volumes. In addition, some LC/MS ionization interfaces (electrospray) perform best when sample from the HPLC "inlet" is delivered to the mass detector at a reduced flow rate (50 µL/min). Studies indicate that the quality of µbore separations, as measured by retention time and area reproducibility, can be significantly affected by the performance characteristics of the HPLC system. The purpose of this report was to obtain comparative information on the Waters® Alliance HPLC System, the Waters Alliance® HT System, and a major manufacturer's traditional HPLC binary pumping system when used for µbore applications.

Experimental Conditions:

Figure 1:
- Waters Alliance HPLC System with solvent vacuum degasser and column heater
- Waters 996 Photodiode Array Detector with µbore flow cell
- Waters Millennium®32 Workstation ver. 3.05.01

Figure 2:
- Waters Alliance HT System with solvent vacuum degasser,column heater and a 50 µl sample loop (Partial loop / sequential injection mode used)
- Waters 996 Photodiode Array Detector with µbore flow cell
- Waters Millennium32 Workstation ver. 3.05.01

Figure 3:
- Major manufacturer's binary pump with vacuum degasser, autosampler, and column heater
- Waters 996 Photodiode Array Detector with µbore flow cell
- Waters Millennium32 Workstation ver. 3.05.01

Column: Waters Symmetry® C_{18}, 5 um, 1.0 x 150 mm at 30°C

Solvents: Eluent A= HPLC grade water with 0.10% formic acid
Eluent B= HPLC acetonitrile with 0.07% formic acid

Gradient: 0 to 40%B in 40 minutes
Note: In keeping with good chromatographic practice, each of the HPLC systems tested was equilibrated at initial conditions for a minimum of 3 system volumes and the HPLC column equilibrated for a minimum of 5 column volumes between consecutive injections.

Sample: HPLC Peptide Standard Mixture (Sigma Cat. Number H-2016)
Note: Five peptides are contained in this standard mixture. With the solvents and conditions used in this study, one peak was unretained while peaks four through five were baseline resolved as indicated in Figures 1 through 3.

Injections: 5 µl per analysis (N=6)
Several sample sets were collected on each system.
The data shown in this report was representative of the performance obtained for a particular system.

System Volume: The "system volume" for each HPLC System evaluated was determined as described in Waters Performance PerSPECtive WPP10 entitled: "Gradient HPLC Solvent Delivery System Volume"

Misc.: Prior to this study, all three HPLC systems underwent routine performance maintenance and were tested to confirm the manufacturer's product performance specifications when used at analytical flow rates (e.g., a gradient separation performed at a flow rate of 1 mL/min).

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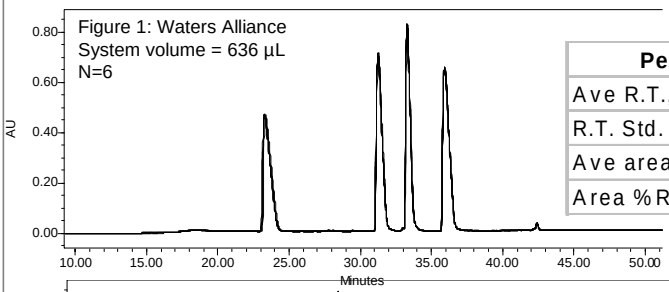
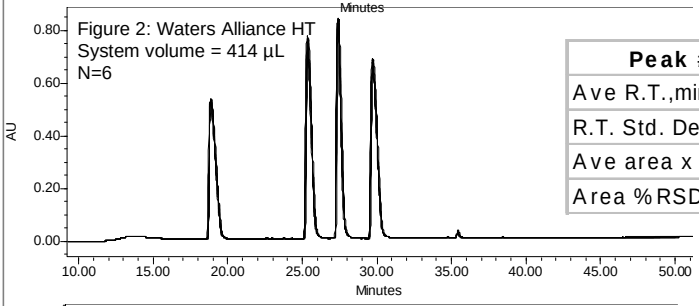
Results and Discussion:

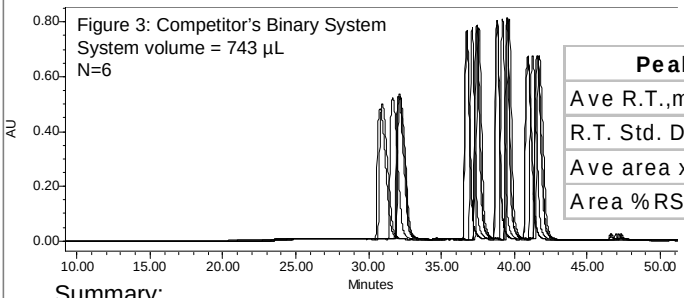
Retention time and area reproducibility continue to be important in many HPLC and LC/MS applications. Many HPLC systems perform well under analytical conditions (with 3.9 mm internal diameter columns at 1mL/min flow or greater). Results can be compromised when some HPLC systems, not designed for µbore applications, are used at µbore flows. The data presented in this report compares system performance for a gradient separation performed on a 1mm µbore HPLC column at a flow of 50 µL/min. Figure 1 represents typical test results obtained using the Waters Alliance HPLC System. Excellent retention time and area reproducibility values were observed. Figure 2 is typical of the results obtained using the Waters Alliance HT system when operated in the partial loop, sequential operation mode. As expected, excellent retention time standard deviations were also obtained. Compared to the Alliance System (2690 Separations Module), peak retention times on the Alliance HT System (2790 Separations Module) were shorter due to the lower system volume of the Alliance HT unit (2690 Separations Module = 636 µL while 2790 Separations Module =414 µL). In addition, area count %RSDs of the HPLC resolved peaks on the Alliance HT System were approximately 1.0% with a 5 µL partial-loop injection made on the 50 µL fixed loop. Area count %RSDs of less than 0.3% are typically obtained on the Alliance HT System using the 50 µL fixed loop in the full-loop mode of injection at 1.0 mL/min. Figure 3 is typical of results obtained on the major manufacturer's binary pump system operated in the standard configuration (mixer and sample loop left on-line throughout the analysis). While good area count %RSD values were obtained, inferior retention time reproducibility were evident compared to the results obtained on the Alliance HPLC System and Alliance HT System.



Peak #	1	2	3	4
Ave R.T.,mins.	23.284	31.247	33.290	35.917
R.T. Std. Dev.,mins.	0.031	0.013	0.010	0.008
Ave area x 10^7	1.70	1.95	1.84	2.00
Area %RSD	0.563	0.454	0.571	0.514



Peak #	1	2	3	4
Ave R.T.,mins.	18.859	25.362	27.391	29.721
R.T. Std. Dev.,mins.	0.024	0.027	0.027	0.023
Ave area x 10^7	1.60	1.89	1.72	1.88
Area %RSD	1.045	0.924	0.883	1.050



Peak #	1	2	3	4
Ave R.T.,mins.	31.622	37.167	39.253	41.322
R.T. Std. Dev.,mins.	0.620	0.369	0.367	0.352
Ave area x 10^7	1.85	1.77	1.68	1.99
Area %RSD	0.515	0.733	0.596	0.433

Summary:

•Many "solvent saving" HPLC as well as LC/MS applications have migrated from using analytical columns (e.g., 3.9 mm internal column diameter used at 1 mL/min) to 1 mm µbore columns used at significantly lower flow rates (e.g., 50 µL /min).

•The quality of µbore separations (i.e., retention time and area reproducibility) can be significantly affected by performance characteristics of the HPLC system. "Compromised data" can lead to decreased confidence in reportable HPLC or LC/MS results. Samples may require retesting which results in decreased laboratory productivity and increased cost of operation.

•The data in this study show the superior performance characteristics of Waters Alliance Systems, compared to a major manufacturer's HPLC binary pumping system, when used for µbore chromatography with shallow gradients at reduced flow rates.