

## Waters®Alliance® HT System

### HPLC Performance For High Throughput Chromatography:

#### Challenges in today's R&D laboratories:

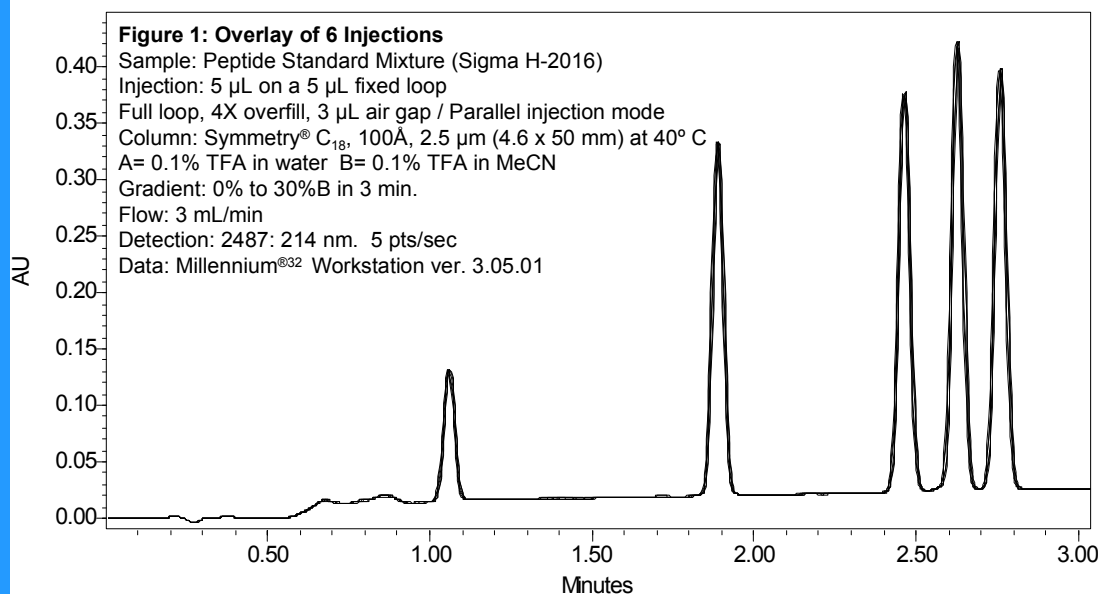
Scientists in many pharmaceutical and industrial laboratories need to obtain high quality results for an ever-increasing number of test samples. The analysis of 10,000 samples or more per week is common. Today, HPLC and LC/MS methods are being created to meet these challenges. High throughput methods, developed on short HPLC columns (e.g., 4.6 x 50 mm) using rapid gradients and high flow rates are helping chemists to overcome analysis bottlenecks.

The purpose of this study was to determine performance characteristics (i.e., peak retention time and area reproducibility) achievable with the Alliance HT System when operated in a high throughput environment. In addition, relative cycle times for high throughput analyses performed on the Alliance HT System and that of a major manufacturer's traditional HPLC system were compared.

#### No-compromise performance in high throughput HPLC:

The Waters Alliance HT System is specifically designed to provide superior isocratic and gradient performance for conventional as well as high throughput applications. As seen in Figure 1, excellent retention time and area reproducibility are obtained when a biological test mixture is separated using "fast gradient" conditions. Retention time precision of  $\leq 0.02$  min. or  $\leq 0.075\%$  RSD is expected at flow rates from 0.200 to 5.000 mL/min. (isocratic premix). Area count %RSDs < 0.3% are typical when using the 50  $\mu$ L fixed loop in the full-loop mode.

When methods are run on the Alliance HT System, scientists no longer need to choose between high quality results or increasing sample throughput.

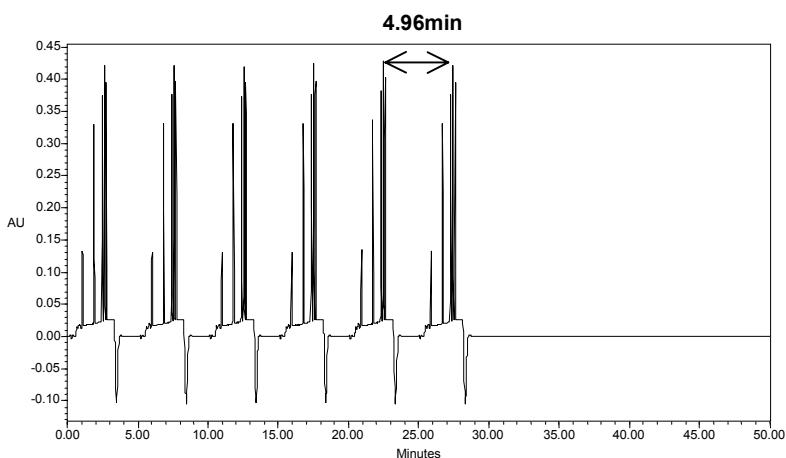


Peak Number	1	2	3	4	5
Average Retention Time (N=6), mins.	1.060	1.888	2.464	2.627	2.760
Retention Time Std. Dev., mins.	0.003	0.003	0.003	0.004	0.003
Average Area x 10 <sup>5</sup> (N=6)	2.8	7.8	9	10	9.6
Area %RSD	0.642	0.519	0.539	0.44	0.499

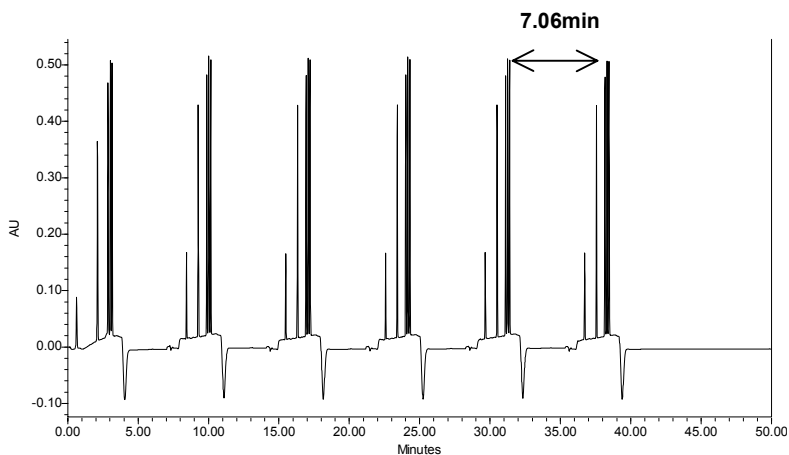
### Cycle time affected by instrument design:

The parallel operation capability of Waters Alliance HT System translates into increased sample throughput. **When programmed for parallel operation mode, the Alliance HT sample manager prepares a sample for injection during the analysis of the prior sample so that it can be immediately injected once the previous sample is complete.** Most traditional HPLC systems do not begin preparing a sample for injection until *after* the current analysis is complete. In high throughput laboratory environments, this delay translates into lost productivity and increased cost of operation. Figures 2 and 3 compare results obtained on an Alliance HT and that of another manufacturer's system using conditions as described in Figure 1. The other manufacturer's traditional HPLC system required an additional 1.25 minutes to prepare sample for the next injection (i.e., sample pickup and needle wash), resulting in significantly fewer analyses completed in the time allotted. In the example shown below, 30% more samples could be run using the Alliance HT compared to chromatography performed on a traditional HPLC system.

**Figure 2:**  
Six repetitive injections on  
Alliance HT System using  
parallel operation mode.  
Cycle time between  
analyses = 4.96min



**Figure 3:**  
Six repetitive injections on  
a major manufacturer's  
HPLC system.  
Cycle time between  
analyses = 7.06min



### Summary:

- Many pharmaceutical and industrial laboratories are challenged to obtain high quality results on an ever increasing number of samples submitted for HPLC or LC/MS analyses.
- Traditional HPLC systems are not specifically designed to reduce analytical bottlenecks by decreasing cycle time for each analysis.
- The Alliance HT System contains technology that increases sample throughput while maintaining the high-quality performance characteristics required for today's demanding applications.