INSTRUMENT AND DETECTOR CONSIDERATIONS USING INTELLIGENT SPEED (*IS*™) COLUMNS

OPTIMIZE HPLC INSTRUMENT BY REDUCING THE SYSTEM VOLUME

To reduce system volume from the injector to the column and from the column to the detector.

(for IS™ 4.6 x 20 mm columns, the recommended system volume should be less than 100µL)

- Use small I.D. tubing
- For larger I.D. *IS*™ columns (for example, 4.6 x 20 mm, 0.009 inch I.D. stainless steel tubing or 0.010 inch I.D. PEEK tubing is recommended. (The standard tubing for Waters Alliance® 2695 HPLC system is 0.009 inch stainless steel tubing.
- Minimize all tubing lengths — remove any excess tubing and extra connections (unions, tees).
- Use precise tubing connections (factory pre-cut tubing is recommended).

OPTIMIZE THE DATA COLLECTION SYSTEM

Data Acquisition Rate -- To insure optimal results with narrow peaks, the data acquisition rate may need to be increased in order to insure more reproducible results. It is recommended that for reproducible quantization of chromatographic peaks, a minimum of 10 points is required. Generally, higher acquisition rates will also provide greater sensitivity for detecting and quantitating lower level degradants and impurities.

Filter Response (Time Constant) Setting – Increasing the filter decreases the baseline noise. However, increasing the filter also reduces the sensitivity. Run some experiments with your software and detector to determine the best settings.

Waters Alliance® 2695 HPLC System

The information obtained for this application notebook was completed using Waters Alliance® HPLC systems with Empower™ Software. Only Waters provides you with every element critical for successful separations–columns, instrumentation, software, and proven methodologies.

Please check with your Waters Representative for additional information on Waters complete solution using Intelligent Speed (*IS*™) columns on the Waters Alliance® 2695 HPlC System. *For additional information on the Waters Alliance® HPLC Systems, please see Appendix B & C.*

SET FLOW RATE

Higher flow rates produce the best peak capacities on *IS*™ columns. According to the Van Deemter equation, as flow rate increases, there is a point where the narrowest peaks can be achieved. Based on our experiments conducted in the Waters laboratories, a 4.6 x 20 mm *IS*™, 3.5 µm column, with a 4 minute gradient time, 3mL/min offers us excellent peak capacities, low back pressures, short runtimes and lower solvent consumptions than the higher flow rates. The recommended starting flow rates for a 4 minute gradient using Intelligent Speed (*IS*™) columns are:

RECOMMENDED FLOW RATE FOR *IS*™ COLUMNS

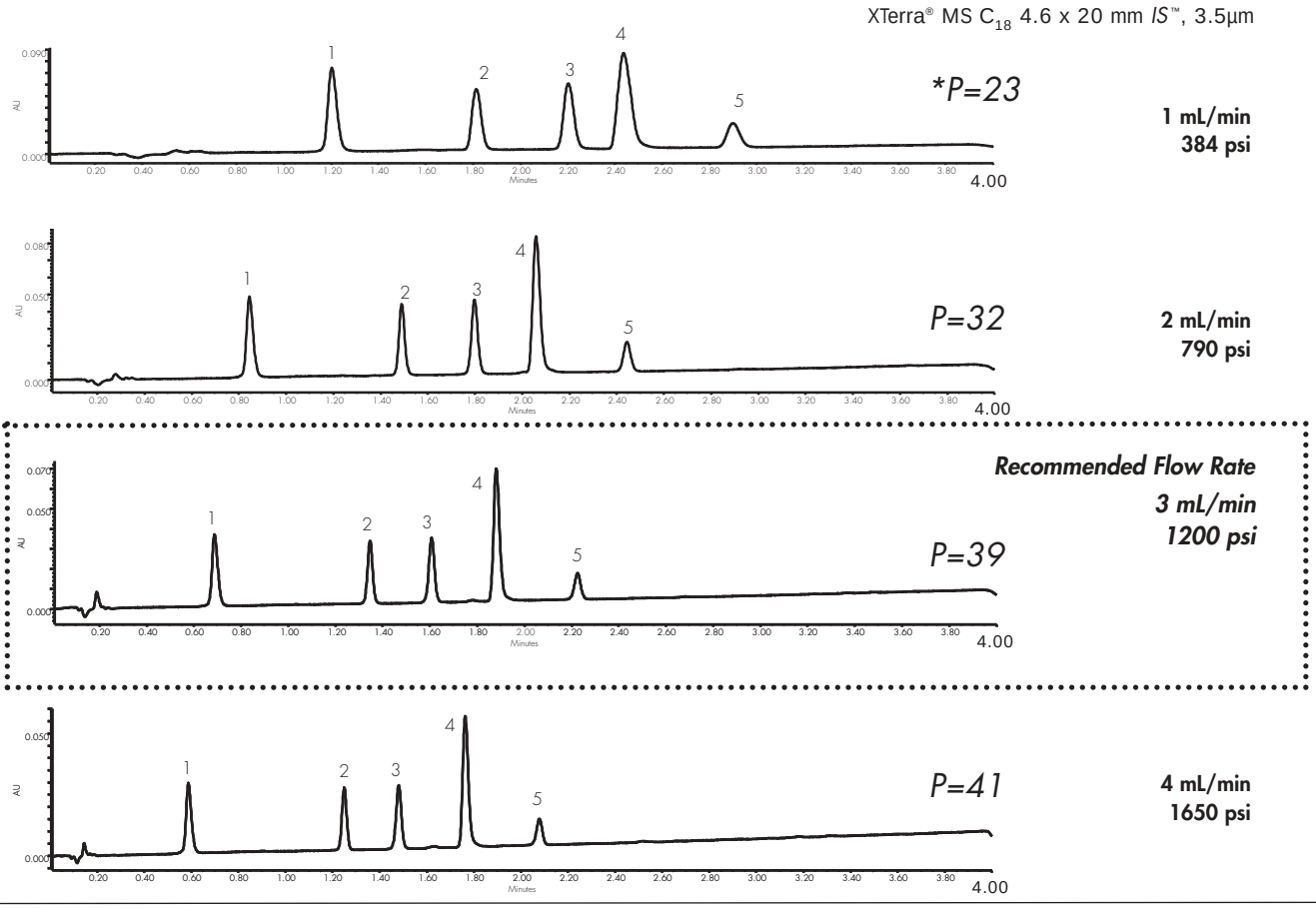
COLUMN DIMENSIONS	FLOW RATE
4.6 x 20 mm	3.0 mL/min
3.9 x 20 mm	2.16 mL/min
3.0 x 20 mm	1.28 mL/min
2.1 x 20 mm	0.68 mL/min

Note: Further optimization of the flow rate may be required depending on your separation.

Waters Alliance® 2695 HPLC System

INTRODUCTION

MAXIMIZING PEAK CAPACITY: EXPERIMENTAL RESULTS



**P= Peak Capacity*

COST ANALYSIS

You can realize significant cost savings when reducing analysis times using *IS*™ columns. With a separation on the 150 mm length column with 25 minute cycle time, it would take nearly 3 months to analyze the samples. Using the *IS*™ columns (20 mm length) the same set of samples would only take 14 days.

The solvent cost includes the cost of Acetonitrile (ACN), plus the cost of disposal of ALL of the solvent waste. The combined total is nearly $2300 in the example using the 150 mm column, but only about $800 for the 20 mm IS™ column. A savings of $1507 for one study!

Additional cost savings can also be determined through improved instrument utilization, labor costs, etc.

CALCULATION OF SOLVENT SAVINGS

Assume that 5000 samples need to be analyzed for a study

	4.6 x 150 mm	4.6 x 20 mm *IS*™
Cycle time	25	4
Total time for 5000 samples (hours)	*2083 (87 days)*	*333 (14 days)*
Flow rate (mL/min)	1.4	3
Total solvent consumption (L)	*175*	*60*
Amount ACN Consumed (L) (~ 25% is ACN)	43.75	15
Cost for ACN ($ 42.50/L)	$1860	$640
Cost for waste disposal (~ $2.50/L)	$438	$151
Total solvent costs	*$2298*	*$791*

Cost savings of $1507 in solvents alone