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INTELLIGENT SPEED: THEORY AND PRACTICE FOR INCREASING THROUGHPUT

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OVERVIEW-

Pharmaceutical companies have been driven to cut costs by increasing sample throughput. Because of the mandate to run faster separations, researchers have tried to move away from hour-long separations on 15 or 25 cm columns. We show that by using column hardware in 20 mm lengths, packed with a variety of spherical particle packing materials, one can increase sample throughput. Columns packed with spherical particles can be used to run separations in under 5 minutes with flow rates of 3 mL/min or less without any compromise in chromatographic performance. We also present how an application run on a long column can be transferred to these shorter columns resulting in optimum chromatographic performance in a much shorter run time, as well as resulting in cost savings. The short columns can be used for quick method scouting runs, as well as for LC/MS applications without flow splitting. Hundreds of injections can also be made on the 20 mm length columns.

INTRODUCTION-

Using current HPLC technology, we know that by optimizing the column length, particle size and the flow rate/ temperature of the separation, we can achieve faster separations. One means of achieving high-throughput separations was to use guard cartridges — i.e. very short columns. However, these cartridges were never intended to be used for chromatography and therefore can suffer from poor peak shape. Therefore, we designed columns in 20 mm lengths in 2.1, 3.0, 3.9 and 4.6 mm inner diameters for optimum chromatographic performance and fast separations. These columns are available in the chemistries listed below. We have used these columns to scale down separations, to develop new separations for both LC/UV and LC/MS applications. We will show examples of a scaled-down separations and the cost savings that you can achieve.

The Intelligent Speed (*IS*™) columns are available in the following chemistries:

XTerra® MS C_{18}
XTerra® RP_{18}
XTerra® MS C_8
XTerra® RP_8
Atlantis™ dC_{18}
Symmetry® C_{18}
SymmetryShield™ RP_{18}
Symmetry® C_8
SymmetryShield™ RP_8

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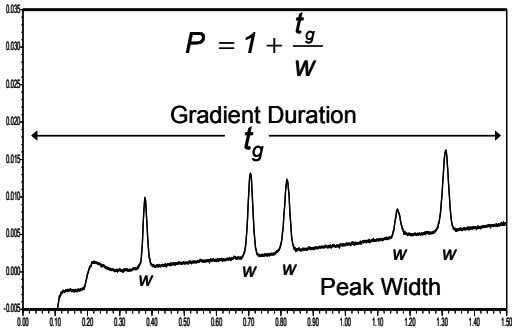
MAXIMIZING PEAK CAPACITY-

Peak capacity is a measure of the separation power of a gradient on a particular column.



Peak capacity is affected by:
–Gradient duration (tg)
–Flow rates (F)
–Column length (L)
–Particle size (dp)

Start with the simple equation for peak capacity

$$P = 1 + \frac{t_g}{w}$$

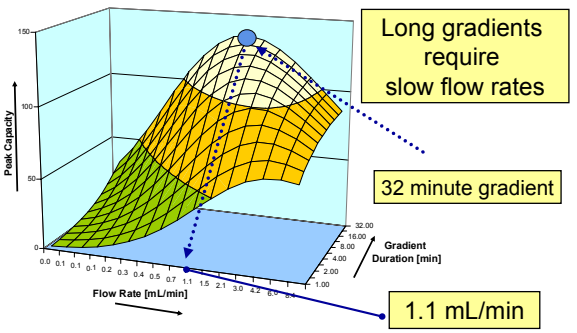
Make a few substitutions

$$P = 1 + \frac{\sqrt{\frac{L}{a \cdot d_p + b \cdot D_M \cdot \frac{t_0}{L} + \frac{d_p^2}{c \cdot D_M} \cdot \frac{L}{t_0}}}}{4} \cdot \frac{B \cdot \Delta c}{B \cdot \Delta c \cdot \frac{t_0}{t_g} + 1}$$

We can now generate a 3-dimensional plot to examine how the gradient time and flow rate effect the peak capacity of a separation.

Neue, U. D., Mazzeo, J. R. J. Sep. Sci. 2001, *24*, 921-929.
Cheng, Y-F., Lu, Z., Neue, U. Rapid Commun. Mass Spectrom. 2001, 15, 141-151.

4.6 x 20 mm, 3.5 µm Column

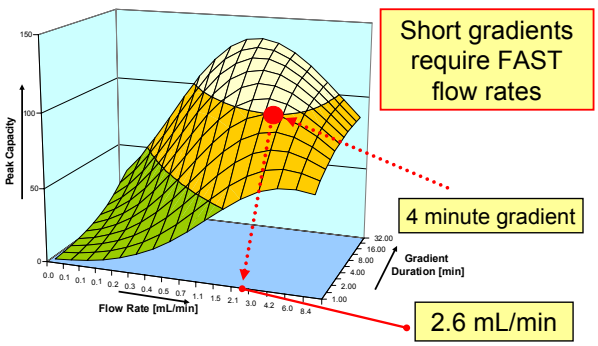


Long gradients require slow flow rates

32 minute gradient

1.1 mL/min

4.6 x 20 mm, 3.5 µm Column



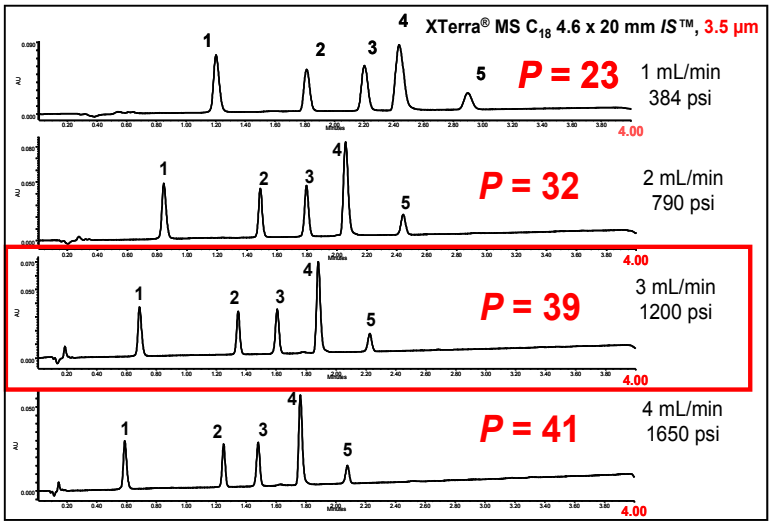
Short gradients require FAST flow rates

4 minute gradient

2.6 mL/min

We see above that for a 32 minute gradient run on a 4.6 x 20 mm *IS*™ column, a flow rate of 1.1 mL/min is predicted to maximize the peak capacity. But, for a 4 minutes gradient, a fast flow rate of 2.6 mL/min is predicted for maximum peak capacity.

We can now run experiments to test the theory. We observe that a flow rate of 3 mL/min offers the best solution as far as peak capacity, solvent consumption and fast separations.



Recommended starting flow rates, to be adjusted according to the separation.

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	0.63 mL/min

SCALING SEPARATIONS

To successfully scale separations run on long columns to the 20 mm length columns, we use the following equation to scale the gradient:

- To scale a gradient

$$\frac{L_2}{L_1} \times t_{g1} = t_{g2}$$

L_1 = Long column length
L_2 = Short column length
t_{g1} = Gradient time on long column
t_{g2} = Gradient time on short column

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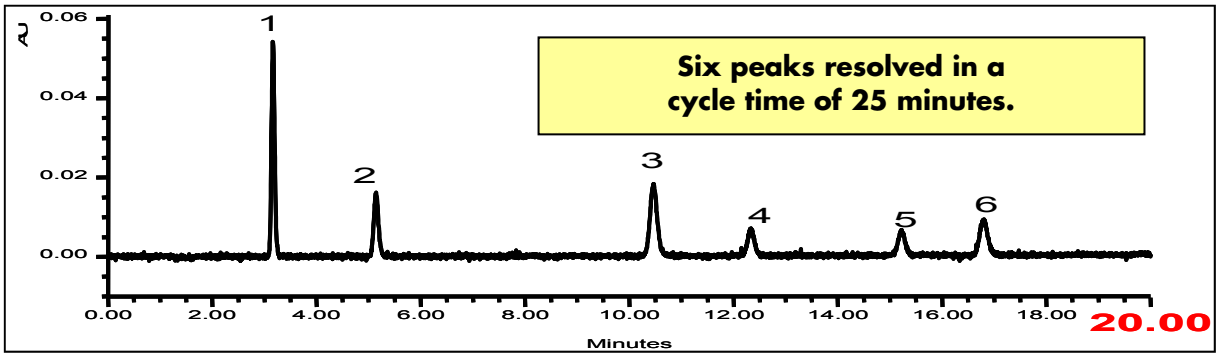
Conditions
Column: XTerra® MS C_{18}, 4.6 x 150 mm, 5 µm
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Mobile Phase C: 100 mM NH_4HCO_3, pH 10
Flow Rate: 1.4 mL/min
Gradient:

Time (min)	%A	%B	%C
0.0	80	10	10
20.0	50	40	10
21.0	80	10	10
25.0	80	10	10

Injection Volume: 10 µL
Sample concentration: 20 µg/mL
Temperature: 30 ° C
Detection: UV @ 254 nm
Sampling Rate: 5 pts/sec
Filter: 0 (no filter)
Instrument: Alliance® 2695 with 996 PDA

Analytes:

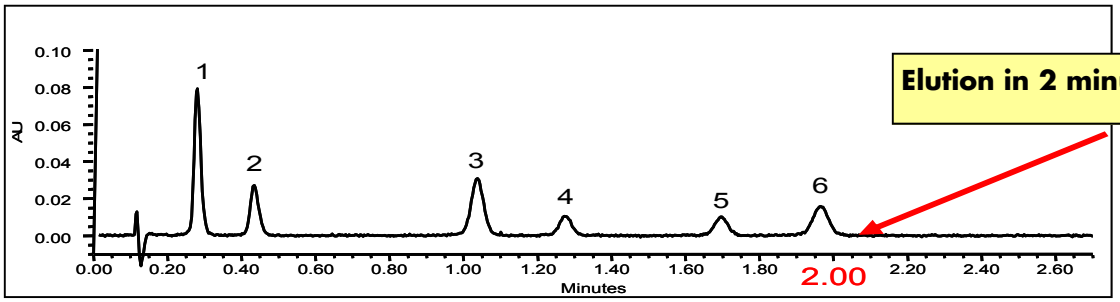
1. Caffeine
2. Aniline
3. N-Methylaniline
4. 2-Ethylaniline
5. 4-Nitroanisole
6. N-N-Dimethylaniline



To scale the separation to a 20 mm column, we first scale the gradient using the previous equation:

$$\frac{20\ mm}{150\ mm} \times 20\ \text{min} = 2.7\ \text{min}$$

We ran a 2.7 minute gradient on a 4.6 x 20 mm IS™, 3.5 µm column at 3 mL/min, for a total cycle time of 4 minutes – down from 25 minutes! We further optimized the method to a cycle time of 3 min.



COST SAVINGS: CALCULATIONS

Assume that an HPLC is running about 67% of the year, or 4000 hr. We used the previous separation as an example:

	4.6 x 150 mm	4.6 x 20 mm *IS*™
Cycle time (min)	25	3
# of Samples Run per Year	**9600**	**80,000**

This is 8.3x as many samples run in one year, on ONE HPLC by using an intelligently designed column!

We can now calculate the solvent cost savings assuming that 5000 samples will be analyzed for the study.

	4.6 x 150 mm	4.6 x 20 mm *IS*™
Cycle time (min)	25	3
Total time for 5000 samples (hours)	**2083 (87 days)**	**250 (10 days)**
Flow rate (mL/min)	1.4	3
Total solvent consumption (L)	**175**	**45**
Amount ACN Consumed (L) (~ 25% is ACN)	43.75	11.25
Cost for ACN ($42.50/L)	$1860	$480
Cost for waste disposal (~$2.50/L)	$438	$113
Total solvent costs	**$2298**	**$593**

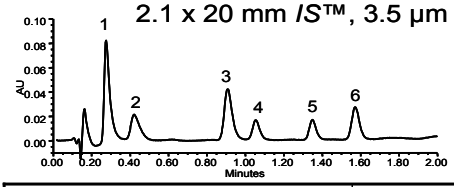
For even further cost savings, we can scale the inner diameter from a 4.6 to a 2.1 mm and calculate the savings:

- To scale a flow rate for different internal diameters

$$\frac{(d_2)^2}{(d_1)^2} \times F_{1=}F_2$$

d_1 = Diameter of original column
d_2 = Diameter of second column
F_1 = Flow rate on original column
F_2 = Flow rate on second column

2.1 x 20 mm *IS*™, 3.5 µm



- Scale from a 4.6 mm i.d. to a 2.1 mm i.d. column.
- Flow rate scales from 3 mL/min to **0.6 mL/min.**

	4.6 x 150 mm	2.1 x 20 mm *IS*™
Flow rate (mL/min)	1.4	0.6
Total solvent consumption (L)	**175**	**9**
Amount ACN Consumed (L) (~ 25% is ACN)	43.75	2.25
Cost for ACN ($42.50/L)	$1860	$96
Cost for waste disposal (~$2.50/L)	$438	$23
Total solvent costs	**$2298**	**$119**

Cost savings of $2179!

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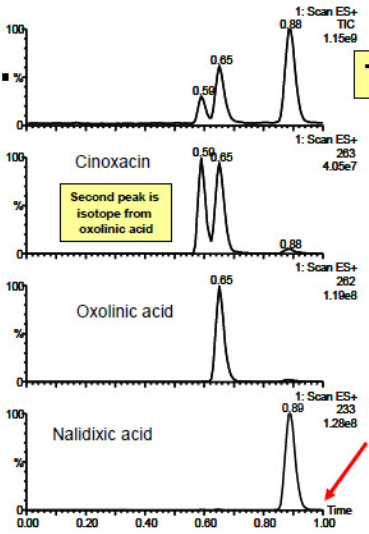
LC/MS SEPARATIONS-



Conditions
Column: Atlantis™ dC_{18}, 2.1x 20 mm *IS*™, 3 µm
Mobile Phase A: H_2O
Mobile Phase B: MeOH
Mobile Phase C: 1% HCOOH in H_2O
Flow Rate: 0.4 mL/min
Gradient:

Time (min)	%A	%B	%C
0.0	50	40	10
1.0	30	60	10

Injection Volume: 2 µL
Sample Concentration: 10 µg/mL
Temperature: 30 °C
Instruments: Alliance® 2795 and Waters ZQ™

Analytes	MW
Cinoxacin	262.2
Oxolinic Acid	261.2
Nalidixic Acid	232.2

With a 2.1 mm i.d. column, lower flow rates enable DIRECT flow into the MS and the separation is only 1 minute long!

No flow splitting!

CONCLUSIONS-

- Shorter analysis times can be achieved with shorter particulate columns with excellent results
  - –Significant cost savings
  - –Good column lifetimes
- New 20 mm length Intelligent Speed (IS™) columns are available
  - –Scale-down longer methods
  - –Perform Method Development
  - –Simplify LC/MS
- Waters can assist with a complete solution:
  - –IS™ columns
- Choose from a wide range of chemistries and configurations
  - –Method development technical support
  - –Waters Instrumentation and Service

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