Method Optimisation

Moving to Fortis SpeedCore particles

Transfer of Methods

- Reduction of current HPLC methods

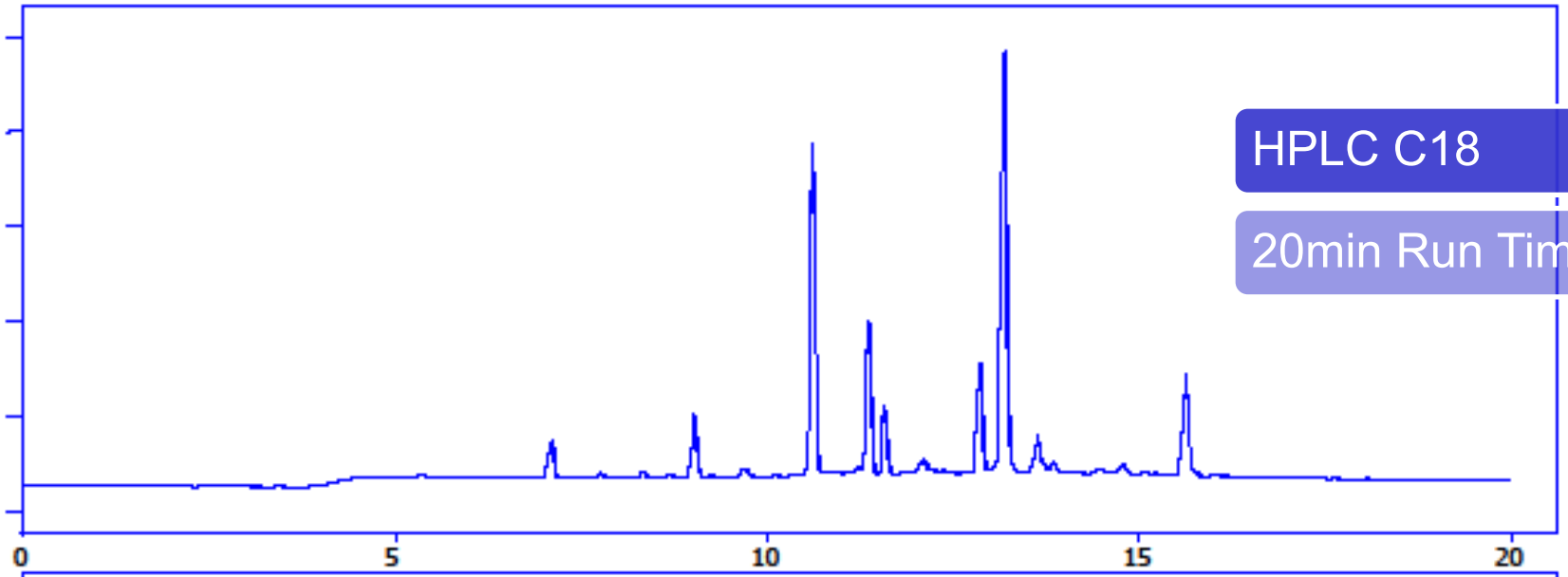
  1 – ‘Legacy’ method transferred to new technology

  2 – ‘New’ method back-scaled to prep or production scale

  3 – Method transfer between differing systems

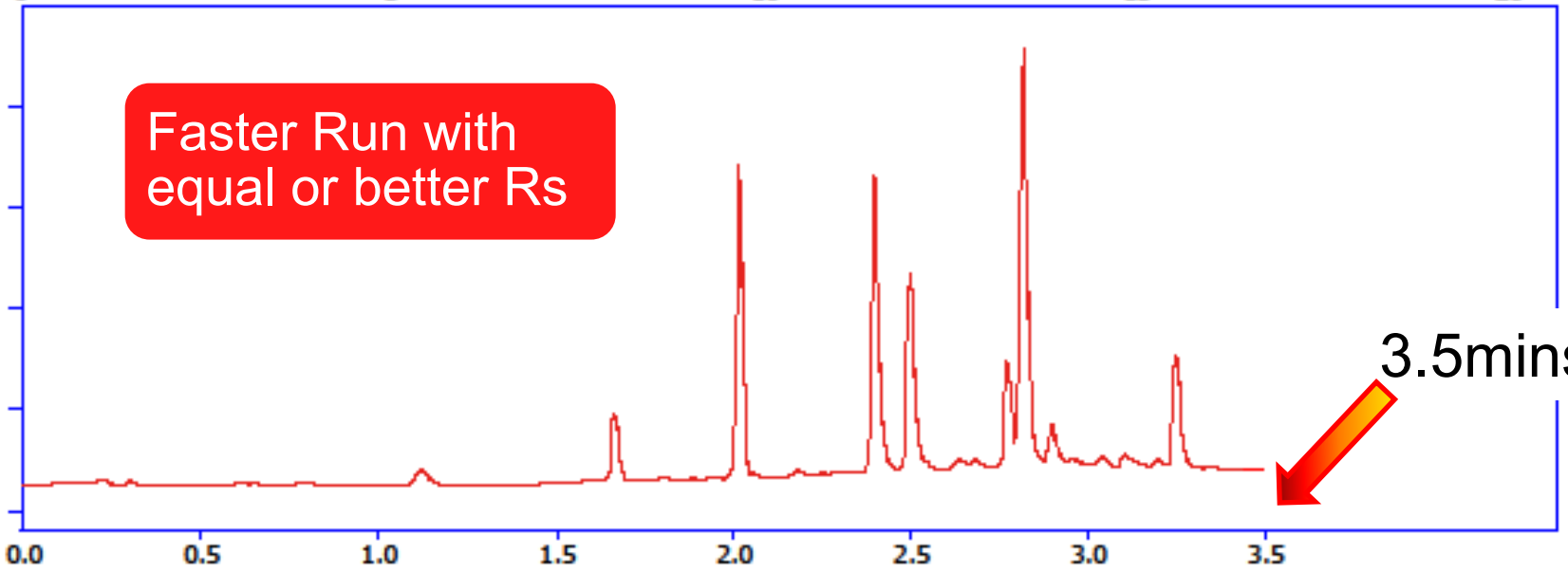
  (Equivalence studies)

Our Goal



HPLC C18

20min Run Time

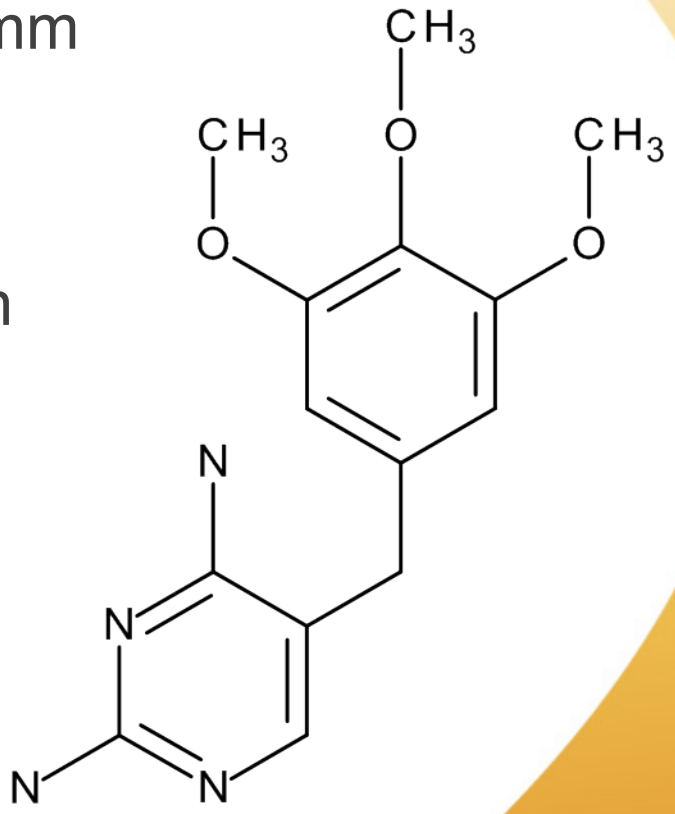


Faster Run with equal or better Rs

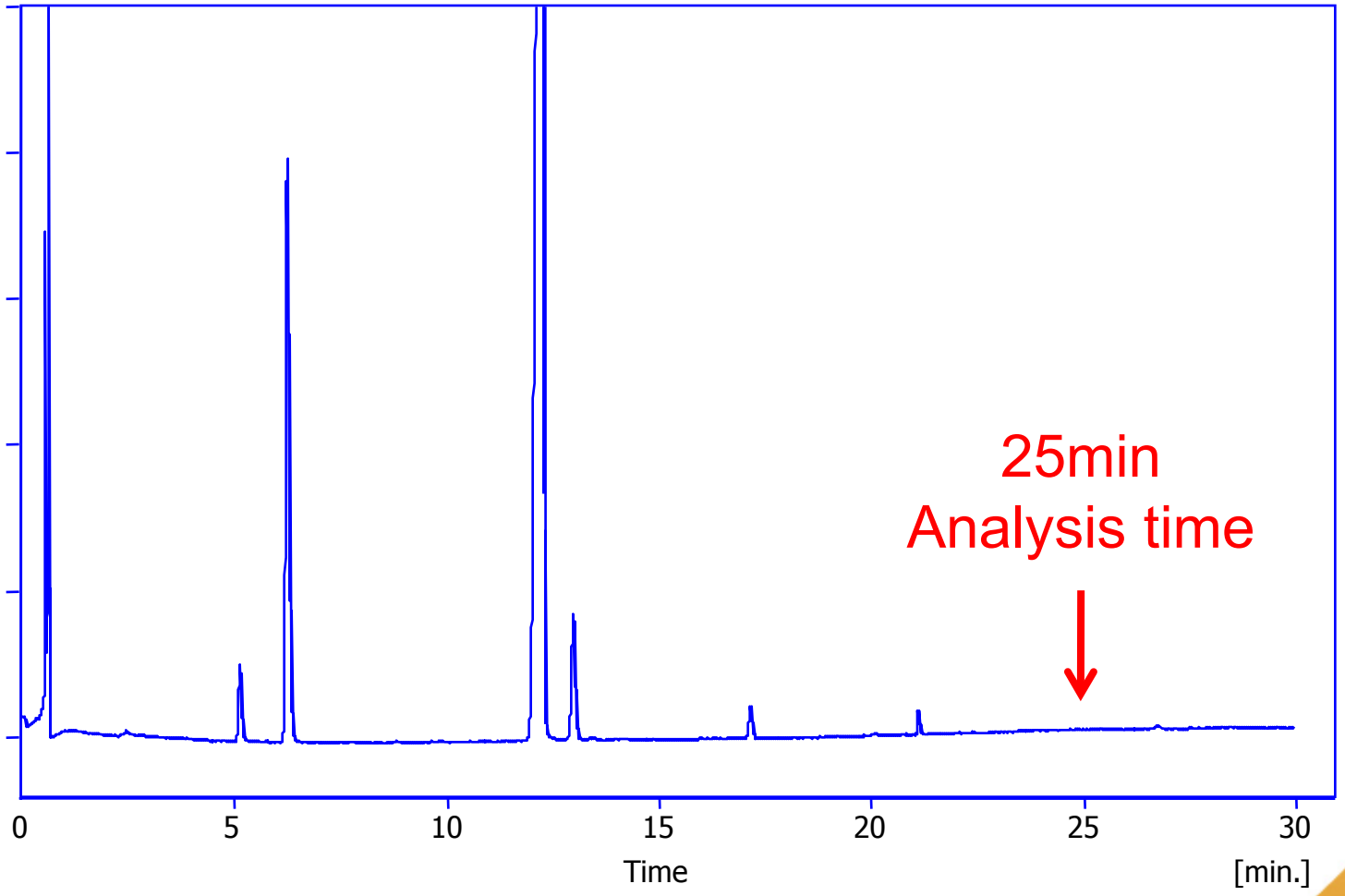
3.5mins

Trimethoprim

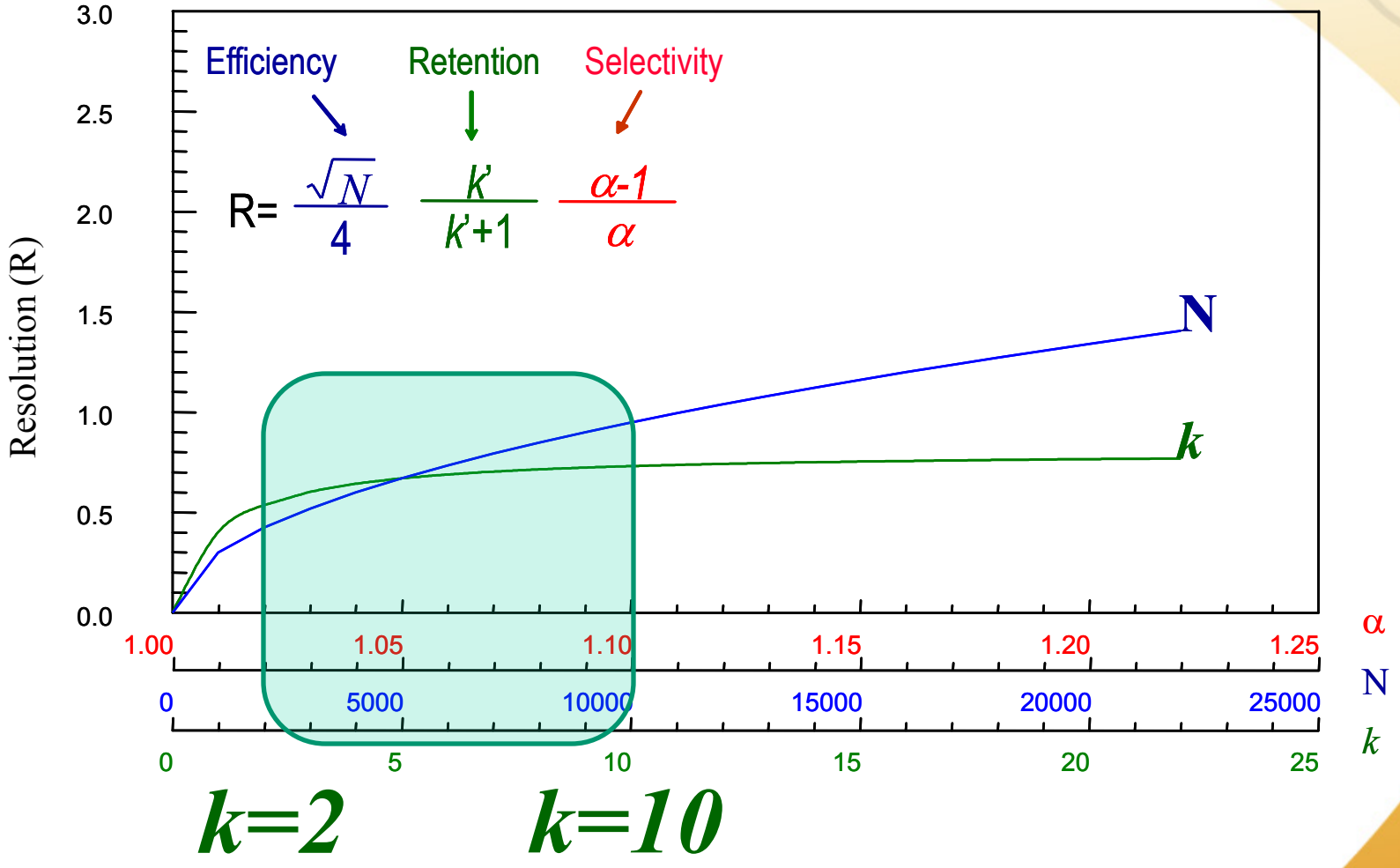
- Original Method:
  - 5µm Fortis C18 250x4.6mm
  - A: Phosphate pH3
  - B: MeOH
  - 70:30 → 100%B in 20min
  - 1ml/min
  - 280nm



250x4.6mm Original Method



Adjust k' for increased Rs



Efficiency HPLC → UHPLC

Column Length	Efficiency of 5µm	Efficiency of 3µm	Efficiency of 2.6µm Core-shell
250	22000		
150	12700	16800	26460
100	8300	10700	21000
50	4000	6000	11200
30		3200	7000
20			3000

Increase Speed

Save Solvent

8 fold

Moving from a 250mm 5µm column to a 150mm 3µm will provide the same efficiency

Therefore equivalent Separating Power

Calculations

- 3 Critical Equations
  - Alter Flow Rate – In line with i.d. change
  - Alter Gradient time
  - Calculate dwell volume

Calculations

Alter Flow Rate

$$F_2 = F_1 \times \frac{(dc_2^2)}{(dc_1^2)}$$

Alter Gradient time

$$t_{g2} = t_{g1} \times \frac{V_{m2} \times F_1}{V_{m1} \times F_2}$$

F = flow rate (ml/min
dc = column diameter (mm)
1 = Original column parameter
2 = New column parameter
T_g = gradient time (min)
Vm = interstitial column of column

Calculations

Interstitial volume

$$V_m = \pi \times r^2 \times L \times w$$

Vm = interstitial column of column
r = column radius (mm)
L = column length (mm)
W = column % interstital porosity

Approximation

W for fully porous material = 68% = 0.68
W for core-shell material = 55% = 0.55

1st Change
250x4.6mm 5µ to 150x4.6mm 3µ

Alter Flow Rate

N/a as both 4.6mm i.d. columns

Alter Gradient time

$$t_{g2} = \frac{t_{g1} \times V_{m2} \times F_1}{V_{m1} \times F_2}$$

$$t_{g2} = \frac{20 \times 1695 \times 1.0}{2825 \times 1.0}$$

$$t_{g2} = 12\text{min}$$

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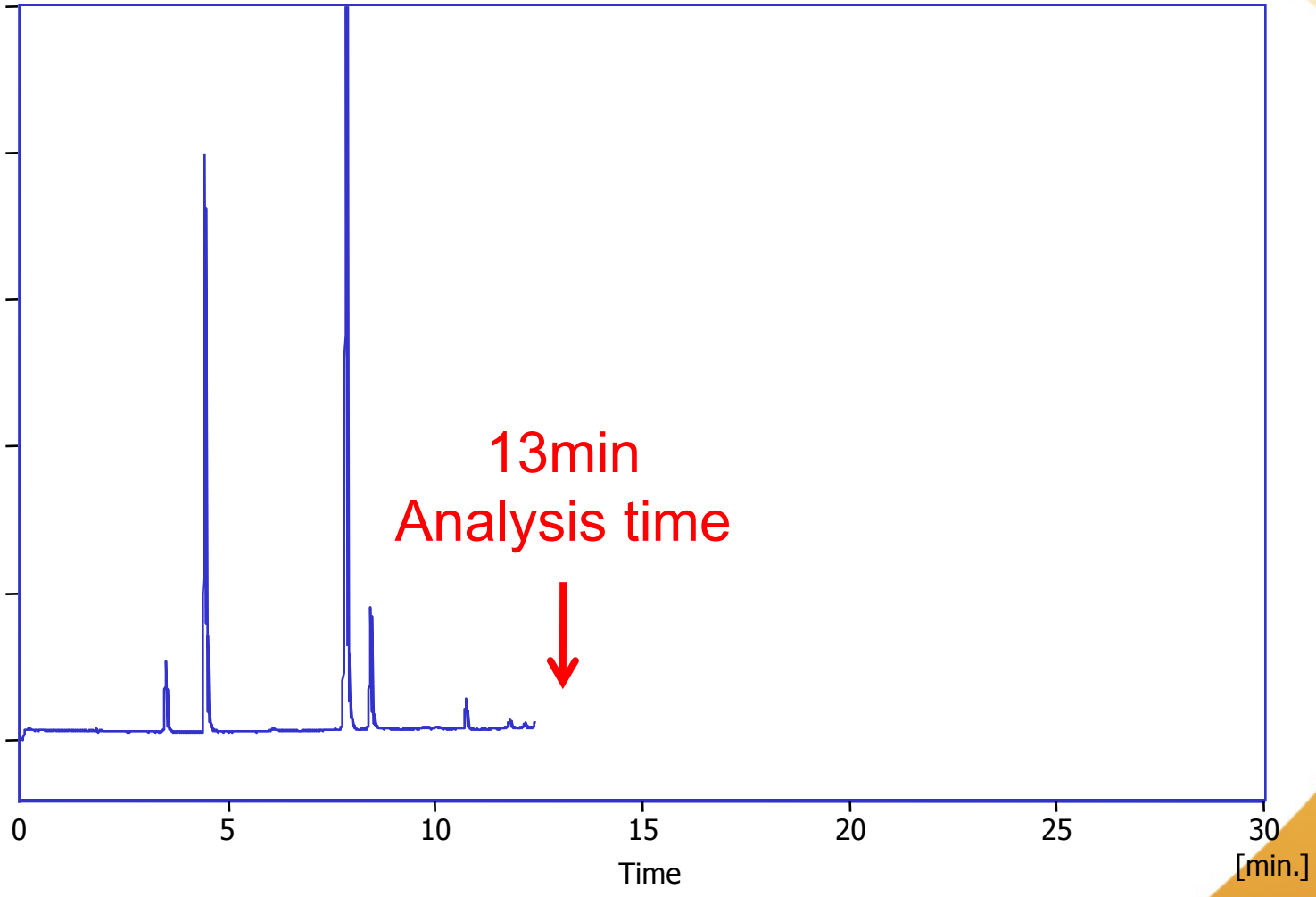
Strength in Technology

150x4.6mm First Method Change



Efficiency HPLC → UHPLC

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250	22000		
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50	4000	6000	11200
30		3200	7000
20			3000

Increase Speed

Save Solvent

8 fold

Moving from a 150mm 3µm column to a 100mm 2.6µm will provide the same efficiency

Therefore equivalent Separating Power

2nd Change
150x4.6mm to 100x4.6mm SpeedCore

Alter Flow Rate

N/a as both 4.6mm i.d. columns

Alter Gradient time

$$t_{g2} = \frac{t_{g1} \times V_{m2} \times F_1}{V_{m1} \times F_2}$$

$$t_{g2} = \frac{20 \times 914 \times 1.0}{2825 \times 1.0}$$

$$t_{g2} = 6.5\text{min}$$

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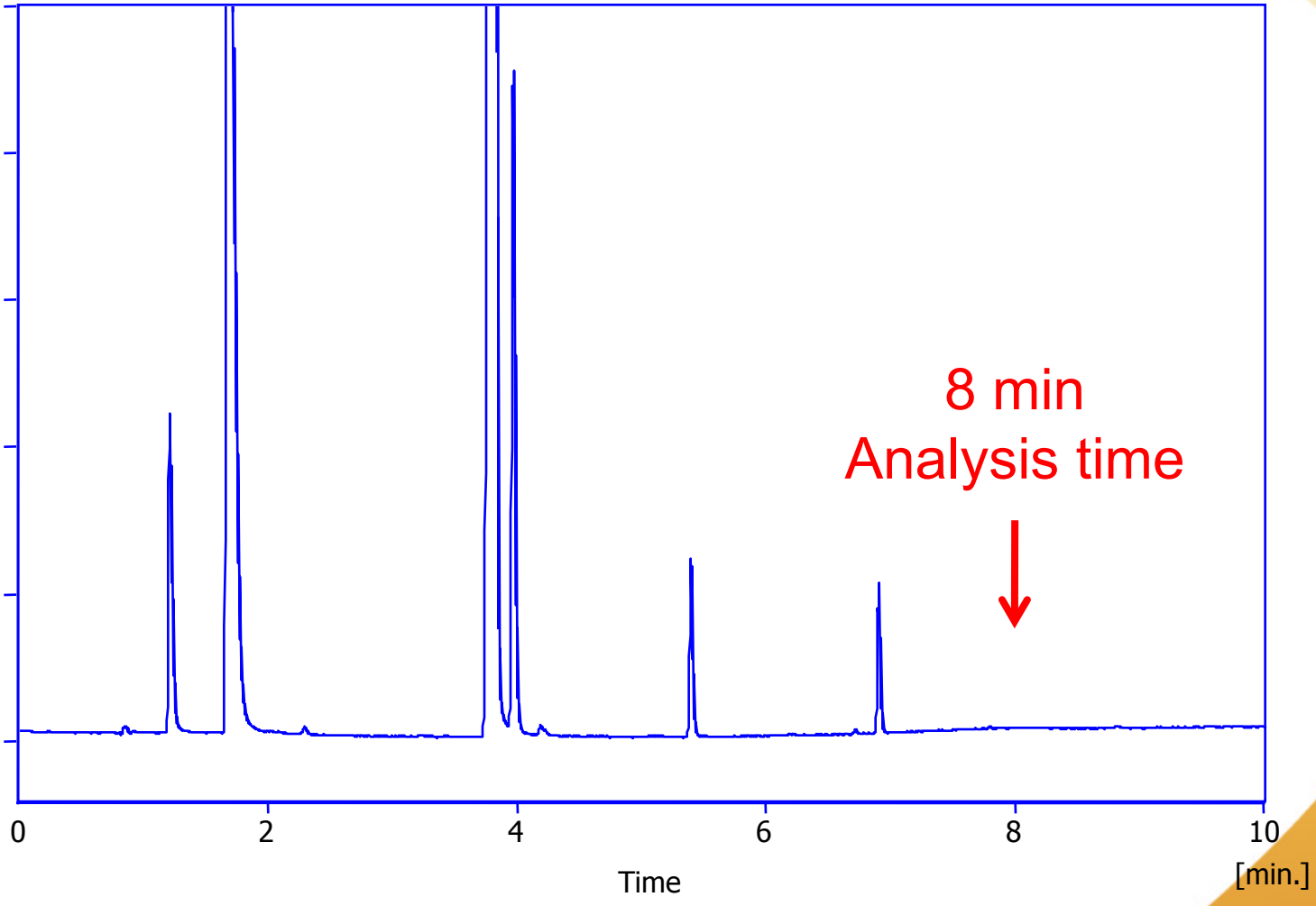
Strength in Technology

100x4.6mm SpeedCore Change



Calculator

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Method Transfer Calculator

Current HPLC Method

Adjust Column Length

Existing Column length	250	mm
Existing Particle Size	5	µm
Existing Column Diameter	4.6	mm

Adjust injection Volume

Existing Injection Volume	20	µl

Adjust Flow Rate

Existing flow rate	1.00	ml/min

Adjust Gradient Program

Existing Gradient Time	20	min

Backpressure

Existing Backpressure	105	bar

Solvent Consumption

Existing Solvent Used	20	ml

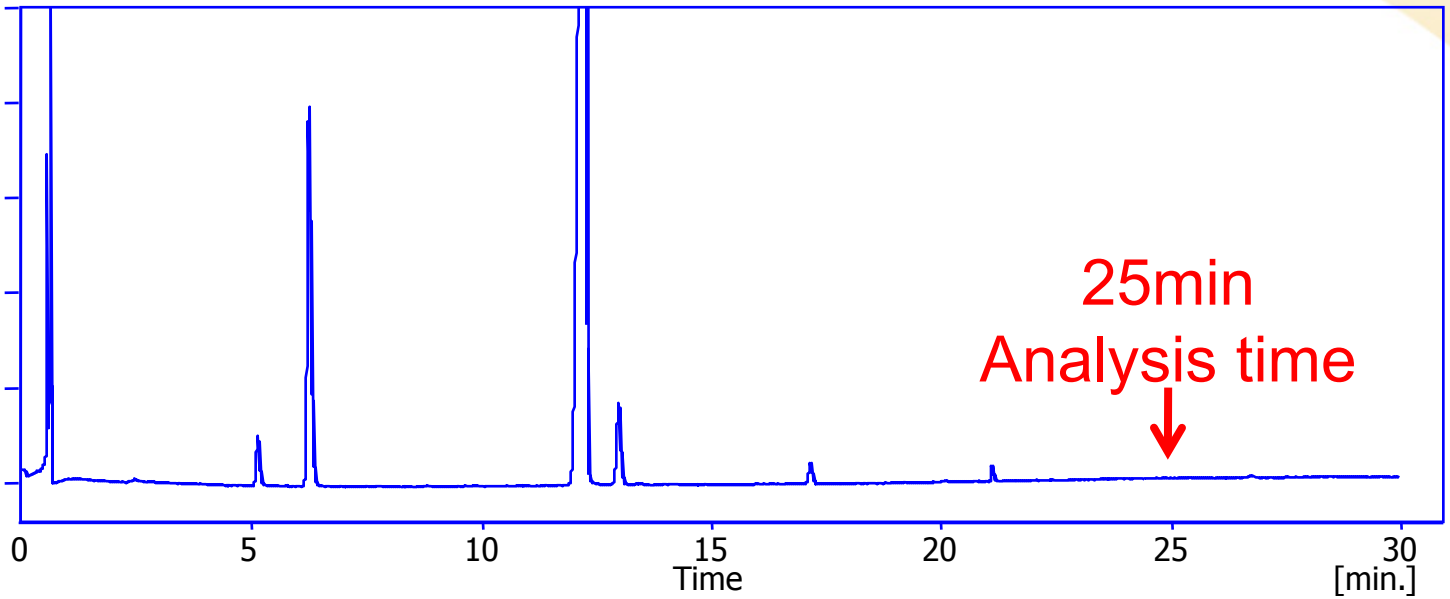
SpeedCore Core-Shell Meth

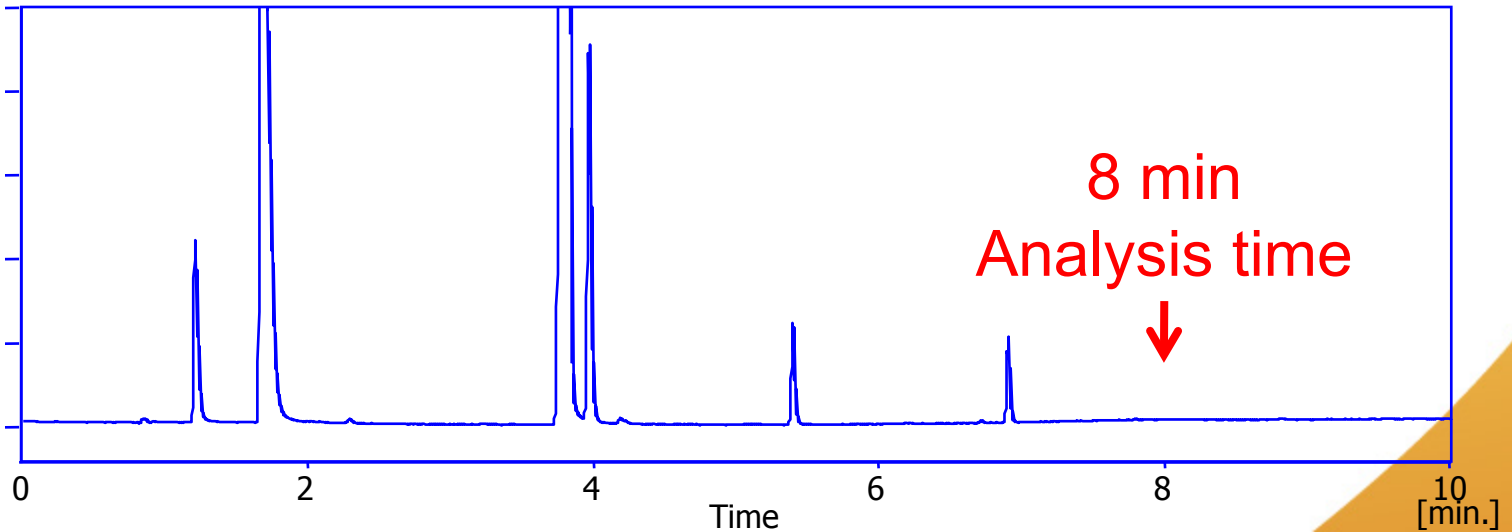
New Column Length	100	mm
New Particle Size	2.6	µm
New Column diameter	4.6	mm
New injection volume	8.00	µl
New flow rate	1.00	ml/min
New Gradient Time	6.5	min
If higher flow rate is required then please enter here	1.50	ml/min
New Gradient Time will be	4.3	min
New Backpressure @ 1.0ml/min	155	bar
New Backpressure @ 1.2ml/min	186	bar
New Backpressure @ 1.5ml/min	233	bar
New Solvent Usage	12	ml

Saving in Time (%) 67.65 %

Saving in Solvent (% 38 %

Final optimised method





Allowable Changes

Type of Change	USP Adjustment	EP Adjustment
Column Length	± 70%	± 70%
Column i.d.	± 25%	± 25%
Particle size	- 50%	- 50%
Column Temperature	± 10%	± 10%
Flow rate	± 50%	± 50%
Mobile phase pH	± 0.2 units	± 0.2 units
Concentration of buffer	± 10%	± 10%
Ratio of components in mobile phase	± 30% of the minor component, but a change in any component cannot exceed ± 10% absolute	± 30% of the minor component, or 2% absolute of that component, whichever is greater
Injection Volume	Reduced as far as consistent with accepted precision and detection limits	Increased by as much as twice the volume specified, providing no adverse effects

Summary

- Moving to a Core-shell particle is simple as long as 3 equations are adhered to:
  - Change Flow rate
  - Alter gradient time
  - Calculate dwell volume

Conclusion

- Moving to Speedcore particles provides gains in:
  - Time
  - Sensitivity
  - Resolution
  - Less solvent usage