Application Note

Method Transfer to Core-Shell Particles

Introduction

'Older ' HPLC methods using 5um 250x4.6mm diamter columns are becoming an outdated option now that UHPLC and core-shell particles allow much faster method development or revaidation of methods to take place. In this application note we show how the correct use of core-shell particles can lead to a 3 fold decrease in analysis time whilst maintaining resolution equivalent to that first achieved in the much longer run time.

Experimental Analysis

We look at the analysis of Trimethoprim an antibiotic used most commonly in the treatment of bladder infections, ear infections and diarrhea. It is present on the World Health Organisation's List of Essential Medicines needed in a basic health system.

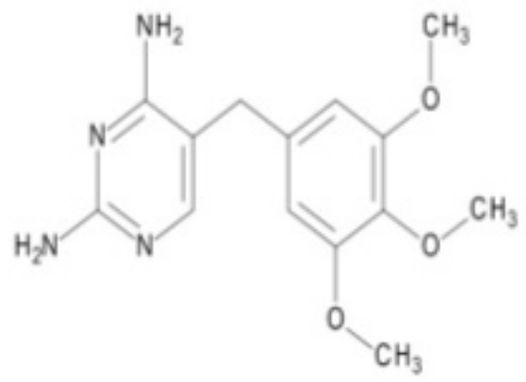

Figure 1. Trimethoprim

In the original analysis of this compound resolution of Trimethoprim and its impurities can be achieved in approx 30mins using the 5µm 250x4.6mm columns. So along with requilibration time this probably represents a 40minute overall method turnaround.

Figure 2 shows the separation of Trimethoprim and its impurities on an older method and run time. Good resolution of several impurites that are present is achieved. If we want to run this method in a quicker timeframe then we need to ensure that this resolution between analytes is not affected or lost. Transfer of methods is only allowed if certain critical aspects are maintained.

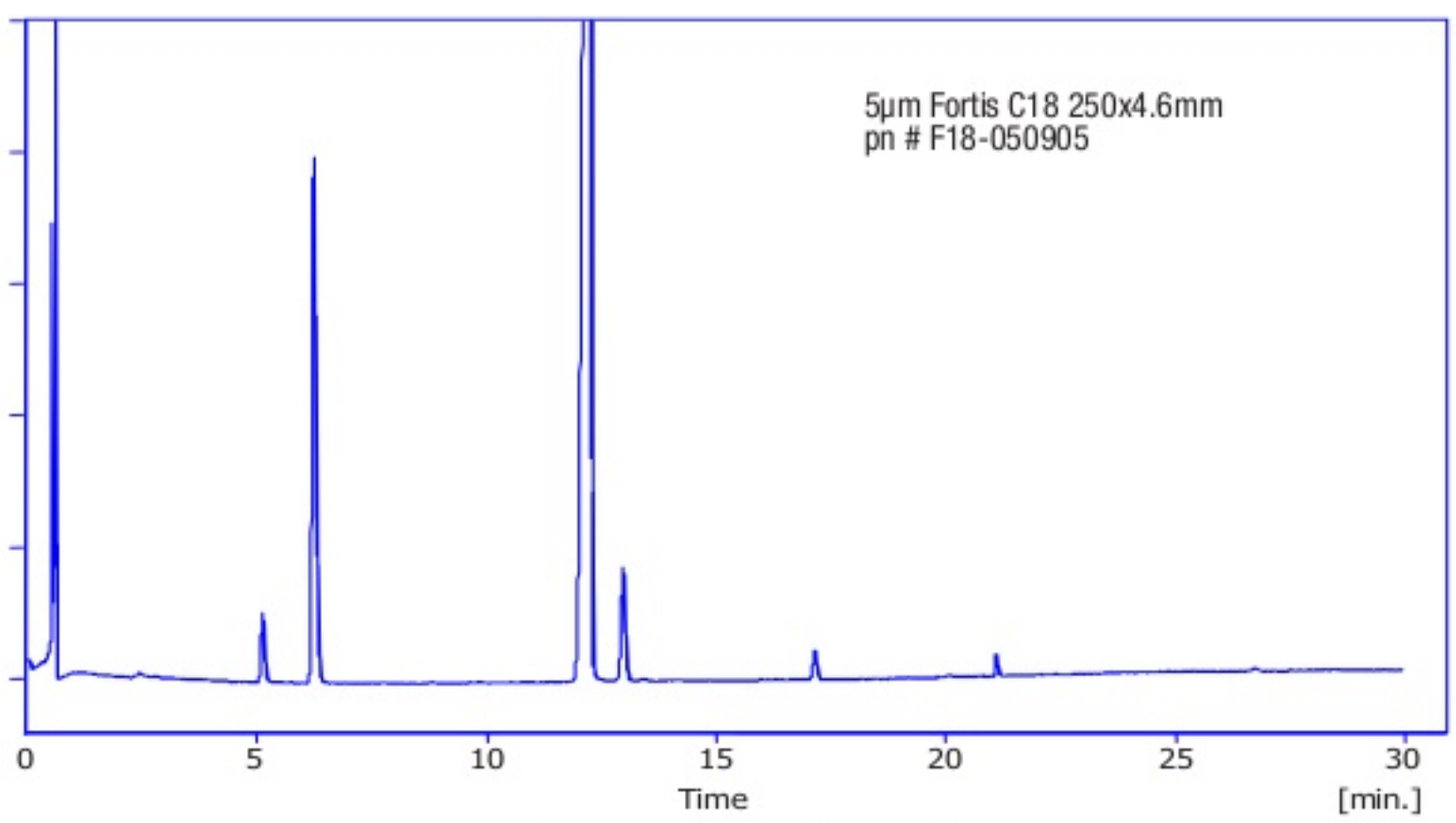

Figure 2. Separation of Trimethoprim

Initial Conditions

Column: 5µm Fortis® C18 250 x 4.6 mm
p/n F18-050905
Mobile phase
A: Phosphate buffer pH3
B: MeOH
30 - 100% B in 20min
Flow Rate: 1.0ml/min
Temp: 25°C
Detection: 280nm

Equations

Figure 3 shows the separation power of equivalant column dimensions/particle size. So from this table we can see that moving to a 150mm 3um fully porous column or moving to a 2.6um 100mm Speedcore particle would offer the same separation power for our critical resolutions within this method.

We need to apply three equations to ensure that the method is equivalent when we make this transfer:

1. Alter flow rate - in line with i.d. change
2. Alter gradient time
3. Calculate dwell volume

If we use these three equations correctly- then our separation selectivity and resolution should not alter. The equations can be done manually, or are available to download in calculator format in the technical section of the website: www.fortis-technologies.com/technical.html
The equations are outlined in Figure 4-6 for

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

Therefore equivalent Separating Power

Figure 3. Separation Power

those wishing to manually calculate the new parameters required:

$$F_2 = F_1 \times (dc_2^2) / (dc_1^2)$$

Figure 4. Alter Flow rate

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

Figure 5. Alter Gradient

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

Figure 6. Calculate dwell volume

Where :
F = flow rate (ml/min)
dc = column diameter (mm)
T_g = gradient time (min)
V_m - interstitial volume of column
r = column radius (mm)
L = column length (mm)
w = column % interstital porosity
(w for fully porous particle = 68% = 0.68)
(w for core-shell particle = 55% = 0.55)

Optimised Method

In this example it is realised that we can move to a 100x4.6mm 2.6um SpeedCore column with the conditions outlined below to achieve the result in Figure 7.

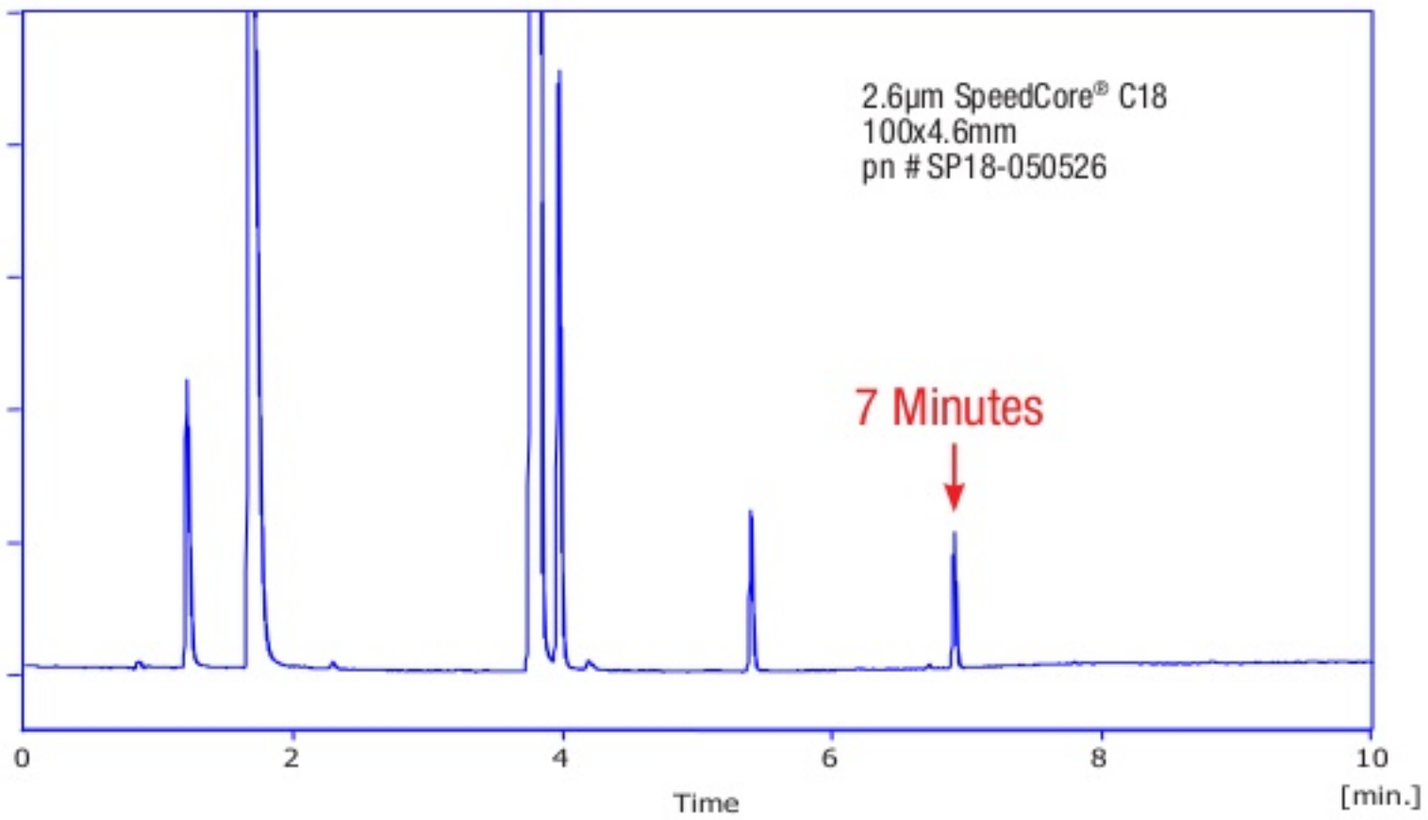

Figure 7. Faster Separation of Trimethoprim

Our gradient profile is now 6.5minutes instead of the original 20minutes, our overall run time is down to less than 8minutes so our turnaround time with re-equilibration will be approximately 10minutes. This is a significant gain on our original method of 40minutes.

Final Conditions

Column: 2.6µm SpeedCore®C18 100x4.6 mm
p/n SP18-050526
Mobile phase
A: Phosphate buffer pH3
B: MeOH
70:30 - 100% B in 6.5min
Flow Rate: 1.0ml/min
Temp: 25ºC
Detection: 280nm

Conclusion

In this application note we have shown how 'older' HPLC methods can be succesfully transferred to core-shell particle columns such as SpeedCore in order to speed up analysis time, gain resolution increase sensitivity whilst cutting solvent cost.
Initial Method development costs and time can also be cut for new LC methods using these SpeedCore particle columns. With a wide range of stationary phase choices now available on core-shell the analyst can potenially move all historical methods to the newer particles and save time and money.
Changes as outlined above are also within the stipulations of allowable changes in the USP monographs[1].

1. *Pharmacopeial Forum* 35(6), 1622-1626, 2009

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