UHPLC or Core-Shell Which is the Winner?

Mark Woodruff
Fortis Technologies 45 Coalbrookdale Road, Clayhill Business Park, Neston, Cheshire, CH64 3UG
Tel: +44 (0) 151 336 2266 email: markwoodruff@fortis-technologies.com www.fortis-technologies.com

Commercial UHPLC technology burst onto the scene in 2004. It was the new technology that was going to move chromatography forward, however was it really new? The sceptic in me says no, we had moved from 5µm particles to 3µm particles 10-15 years previously, moving down to the next particle size, i.e. sub 2µm was the next logical step and this is what UHPLC essentially was. An LC system with extreme low dead volume and 'bolt on high pressure'. I say 'bolt on high pressure' because what does the pressure do for our chromatography, it doesn't give us more efficiency, resolution, selectivity or speed, it allows us to use smaller particles, which then does give us greater efficiency and subsequent speed and resolution.

Now in the last couple of years a 'new' technology core-shell (fused-core, solid-core, superficially porous particles, SPP, call it what you will) has now peaked everybody's interest! Again I highlight the word 'new' since these types of particles were actually first discussed and commercialised back in the 1970's by Zorbax technologies.

Core-shell technology uses a solid silica core and a porous outer silica shell to provide the same high efficiency separations as the sub 2µm UHPLC particles, but with potentially lower backpressure. Current theory has the efficiency generated by these particles arising largely from the mono disperse nature of the solid core coupled with the reduced void volume of the column and to a lesser extent from the reduced mass transfer that takes place during the separation. As with all technology there will be differing manufacture processes in the production of these core-shells and therefore a potentially wide range of physical characteristics, resulting in a range of pressures, hydrophobicities and peak shapes (Figure 1).

Figure 1.

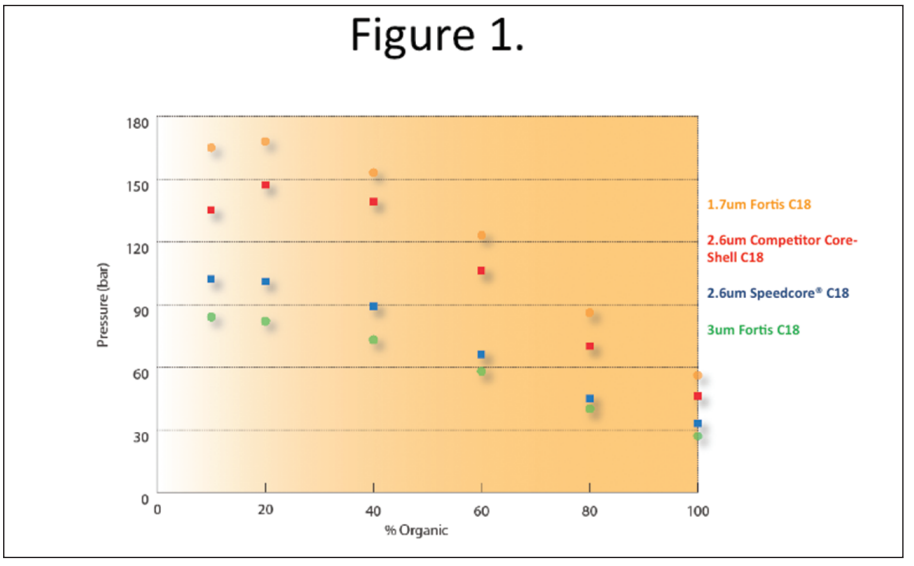

Figure 1. (Backpressure of 2 commercial core-shells vs fully porous particles)

Lifetime

Obviously characteristics such as backpressure then have a big impact on the lifetime of columns, along with the linear velocities that can be achieved (Figure 2).

Does backpressure work totally against UHPLC particles, not really, if the manufacturer has done a good job then the sub 2µm particle should withstand the elevated pressures. As an example, at Fortis Technologies we pack our 1.7µm particles at >1400bar (approx.20,000 psi pressure) ensuring that the packed bed is

Figure 2

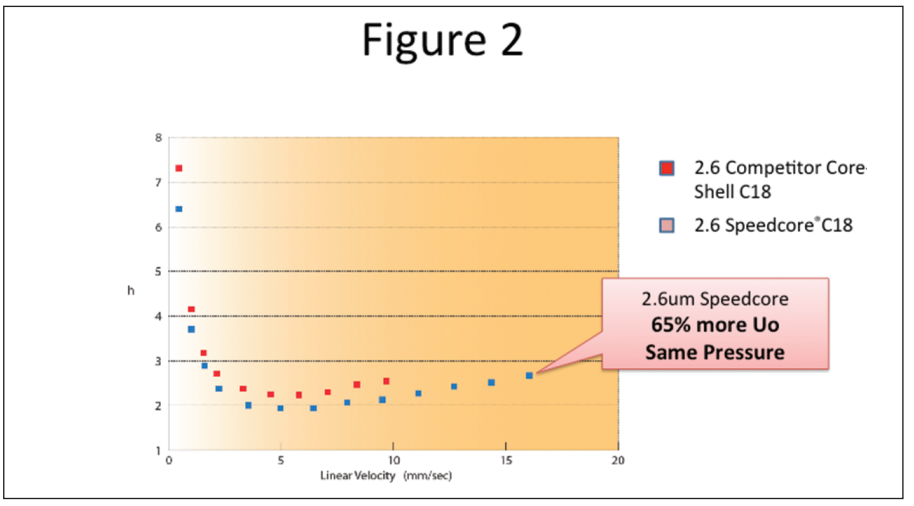

Figure 2. Van-Deemter curve for 2 core-shell, highlights how 65% greater linear velocity (Uo)can be achieved for the same backpressure, giving a greater usable flow rate range.

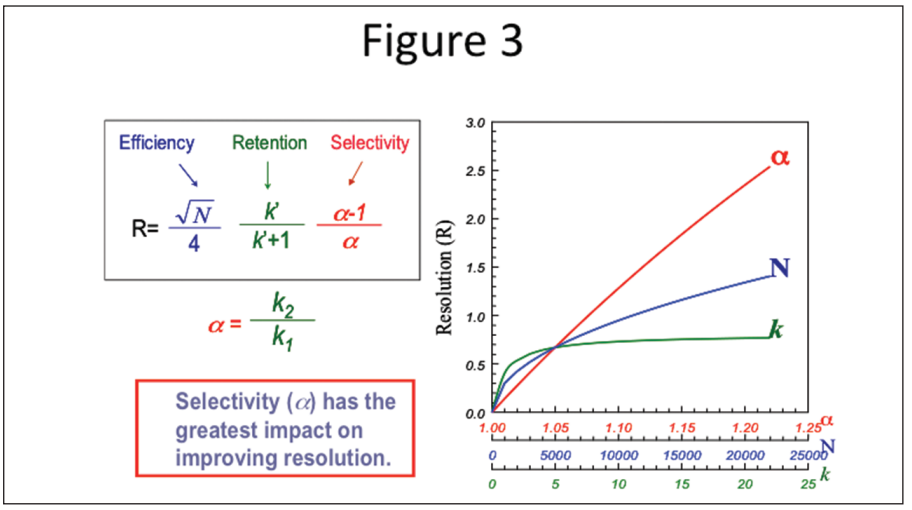

Figure 3. Selectivity (α) is a function of resolution, particle size (dp) and retention factor (k').

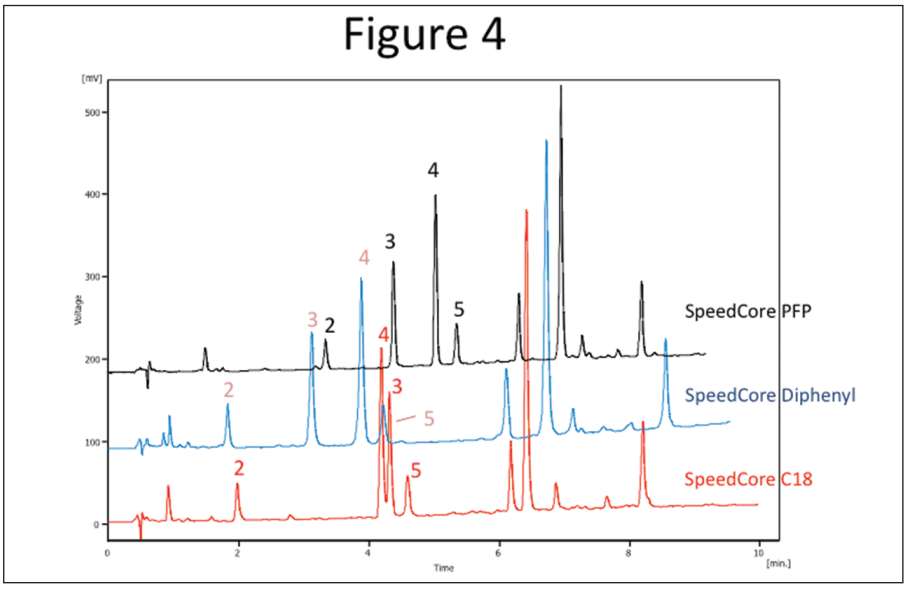

Figure 4

stable well above the usual working limits of the commercial UHPLC systems on the lab bench.

Lifetime is also an interesting issue from a manufacturers and end-users point of view. In HPLC we would expect a lifetime of 1000-2000 injections to be reasonable, sometimes much longer. With a 60minute run time and 5minute equilibration time, 2000 samples would take us approximately 90days working constantly 24hrs a day, 7 day a week. So approximately one column per assay per quarter.

However now if we have a UHPLC or core-shell column and our assay takes 10minutes with a 1 minute equilibration, then our 2000 samples take approximately 15days. Yet the number of times I have a customer say that the UHPLC column now doesn't last 90days like their HPLC column did is strange. Why should it? Column lifetime is more related to sample throughput than to pure 'calendar months'. If the UHPLC column did last 90 days that would mean by the same logic that we would have analysed (90/15 x 2000) 12,000 samples instead on the same column.

So we have to compare apples with apples. If I have a core-shell particle column then I need to compare lifetime with another core-shell, not with my previous HPLC column and I need to use number of samples as the measure, not calendar days.

Selectivity

This leads me onto peak capacity (unit resolution, peaks per unit time), one of the variables that will change heavily in the move from HPLC to UHPLC or core-shell. As we improve N through reduced peak width we can go faster and faster with our analysis, however at a certain point we will start to run out of time for our peaks to elute (no matter how narrow they are). Therefore the one variable that does become most important to this high throughput chromatography is that of selectivity (Figure 3).

We need to incorporate selectivity into our equation for both core-shell and UHPLC: this variable will allow optimum resolution to be found for the wide diversity of compounds types encountered in the pharmaceutical pipeline. Figure 4 shows the separation of a complex mixture of analytes using a C18, Diphenyl and PFP core-shell particle. The orthogonal selectivity offered by the 3 different stationary phases allows a suitable starting point to be made with scope for a highly qualitative method design. Peaks 2-5 not only show differing selectivity but also switches in elution order from phase to phase.

Do UHPLC and core-shell particles offer enough selectivity? It is ultimately not down to the particle technology but to the stationary phases that are bonded to the particles. With both particles being contemporary offerings they are not currently as diverse in stationary phase choice as traditional particles, although this is something that should catch up fairly rapidly.

Analysis Requirements

What does the analyst require? In terms of LC columns the list includes, lifetime, loading, scaling reproducibility, selectivity, robustness, and peak shapes (efficiency, been taken as a given here in context of the article). All of these variables have made silica popular over the years, even with the advent of carbon, zirconium, polymeric, hybrid and even diamond phases, nothing has replaced silica's overall outstanding qualities. Core-shell and UHPLC particles both build on this and now increase substantially the efficiency term.

One of the main areas of interest for UHPLC when it emerged was a speeding-up of method development screening. Different columns and different mobile phases conditions could be quickly evaluated to assess the most suitable starting conditions. Once a suitable starting point was established, optimisation of the method could take place. This approach significantly impacted on development time, potentially bringing down method development time to days instead of weeks. However the first downside of this approach became evident when methods passed to other departments where scaling up to larger particles was required but not always possible.

Core-shell particles potentially have this same issue ahead. It will aid in the method development screening process, but at this time will have a scaling issue for those that wish to move from low quantitative methods to preparative scale chromatography. There are two factors that may limit the application of core-shell technology for method scale up. One is the cost of this new particle technology, with few manufacturers offering a preparative column format. The

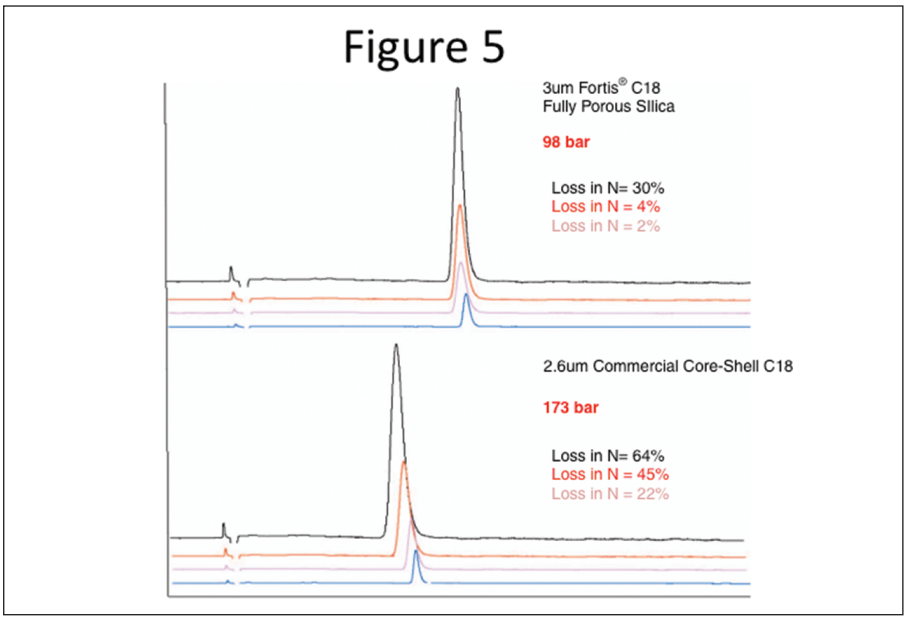

Figure 5
Diphenydramine 0.02mg/ml, 0.2mg/ml, 0.5mg/ml, 1mg/ml
0.6ml/min

second is loadability/loading capacity. Generally core-shell particles have a surface area (S.A.) between 170-210m^2/g and whilst not a small surface area this is relatively low in today's terms of >300m^2/g silica's. If your method cannot scale to preparative level then will QC and production wish to take this on board? Yes in theory multiple methods can be run, one for preparative scale and one for analytical scale, however this is not as productive as having one scaleable method for both departments.

In terms of loading, the smaller surface area of the core-shells can lead to a much quicker overload situation, which can compromise throughput of purification, so maybe becoming a (trade off) compromise on speeding up our analysis Figure 5 (Silica matrix overload). In Figure 5 we see the loading ability of 2 silica based columns, both 50x3mm, one a traditional fully porous 3µm particle with a S.A. of 380m^2/g and one a 200m^2/g core-shell.

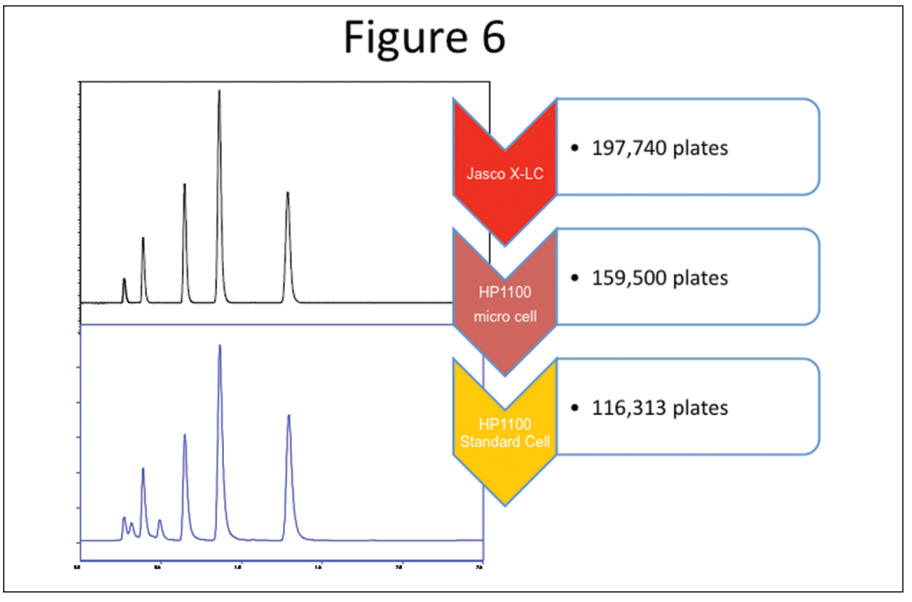

Figure 6

It can be seen that as we increase the mass loaded onto the columns the core-shell losses efficiency much more rapidly than the higher surface area fully porous particle. Losses are in relation to the 0.02mg/ml sample, so by 0.5mg/ml core-shell has lost 45% efficiency whilst the porous particle has lost only 4%. Core shell stability is still largely unproven over time as this is a 'new' technology and will undoubtedly be the first of many iterations of the technique. Over time this should not be an issue as manufacture of these particles becomes robust, historical data will be built up to prove robustness and reproducibility.

System Requirements

UHPLC systems were designed specifically to be low dwell volume, in the region of 60-150µl. If you use sub 2µm particles on a traditional LC system then the efficiency is soon compromised (Figure 6) providing only a small increase over using a normal 3µm particle.

Core-Shell however whilst also affected by system dwell volume, don't drop as significantly as UHPLC if wider bore geometries are used on traditional HPLC systems (Table 1).

Table 1

Column	System	Efficiency
2.6um SpeedCore 150x4.6mm	HP1100 with micro flow cell	230,000
2.6um SpeedCore 150x4.6mm	HP1100 with standard flow cell	191,000
2.6um SpeedCore 150x4.6mm	HP1050	170,000

Conclusion

Core-Shell technology and UHPLC technology both offer high efficiency, fast separations so is one better than the other? I am not sure that there is going to be any clear winner. Both technologies require low dispersion LC systems to provide the much vaunted efficiencies discussed. Both technologies are not as progressed as HPLC columns, in terms of the selectivity's available and scaleability, although on the latter UHPLC definitely offers an advantage if you can have the same surface area and carbon load across 1.7µm, 3µm and 5µm particles.

Both these particles will find use in laboratories in future. I think that personal preference will come into play, UHPLC particles are robust, but the instrumentation may be prone to blockage and is still an expert user tool. Core-shell is largely unproven and still requires low dispersion systems to work to its optimum. I think that these variables will likely split opinion in the techniques and cause people to have their personal favourite. There is no doubt that both higher efficiency particles with much reduced run times are the future of chromatography and that they have improved the ability to speed up analysis exponentially. We need to make the most of this as manufacturers by offering a wide range of stationary phases and scaling options to aid the analysts ability to improve productivity.

The future is here and the future is faster…..