Maximising Core-Shell Performance on Conventional HPLC Systems

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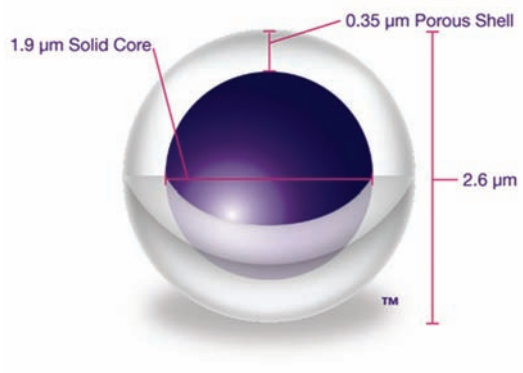
In order to fully realise the performance potential of core-shell columns on conventional HPLC systems, some simple system optimisations maybe necessary to minimise extra-column band broadening effects. In this report, a series of simple measures (adjusting detector acquisition rate; optimising connective tubing; use of a low-volume flow cell) are made to a standard HPLC system in order to achieve UHPLC-comparable performance with sub-3µm core-shell media.



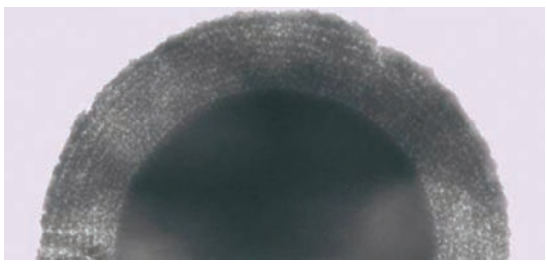


Figure 1: Schematic diagram of a Kinetex 2.6µm core-shell particle (a) and TEM micrograph of a cross-section through a Kinetex 2.6µm core-shell particle (b).

Introduction

The dominant trend in liquid chromatography column technology has been the development of progressively more efficient media in order to increase laboratory productivity while maintaining chromatographic performance. To this end, the use of higher efficiency particles has the promise of allowing analysts reduced run times while maintaining, or even improving, chromatographic resolution.

Core-shell particles provide an elegant solution to the problem of maximising column performance without generating excessive back-pressure. They consist of an impermeable inner core surrounded by a layer of fully-porous silica *(Figure 1)* and thus are morphologically quite distinct from conventional fully-porous silica particles. While a comprehensive review of the findings of these various publications is beyond the scope of this present investigation, it has been widely reported that core-shell particles can provide performance that is on-par or even exceeds that of fully-porous sub-2µm media [1-3].

The pressure generated by HPLC/UHPLC columns is inversely related to the particle size of the media used to pack the column. Thus, columns packed with sub-3µm core-shell particles are able to achieve sub-2µm efficiencies at operating pressures that are much lower than sub-2µm packed columns, and hence it is possible for chromatographers to achieve levels of performance close to that of sub-2µm packed columns without the need of a UHPLC system. However, although core-shell particles do possess the potential of delivering UHPLC-like performance on conventional HPLC systems, the actual capacity of the end-user to fully realise that potential is highly dependent upon the nature of the HPLC systems that they are utilising.

In this study, we present data showing how the performance of a column packed with sub-3µm core-shell particles (Kinetex 2.6®µm C18) performs on a conventional HPLC system, unoptimised and optimised (using a commercially-available optimisation kit). The final optimised performance is then compared with the efficiency of the same Kinetex 2.6µm C18 column on a UHPLC (Agilent® 1290) system.

Materials and Methods

HPLC System

The unoptimised configuration of the Agilent 1100 HPLC consisted of a binary HPLC pump (Agilent model G1312A), autosampler (Agilent model G1329A), and variable wavelength detector (Agilent model G1314A). Peek tubing of 0.010" ID (0.124mm) was used to connect the various components to each other and to the HPLC column. The detector contained a standard 14µL flow cell and the initial detector acquisition rate was set at 1 sec.

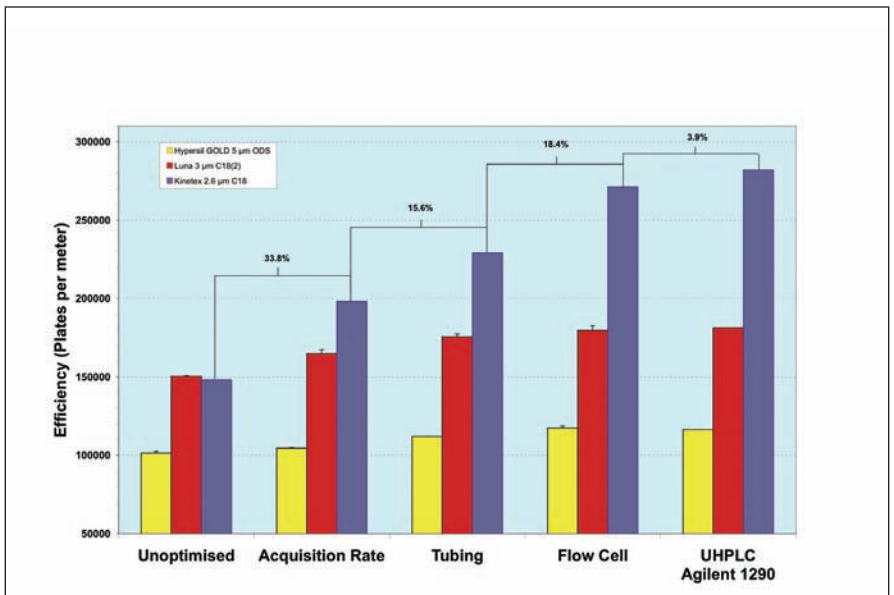


Figure 2. Effect of each optimisation step on column efficiency for the fully-porous 5µm and 3µm columns, and the Kinetex 2.6µm core-shell column. All columns were 100 x 4.6mm. Efficiency was calculated based upon naphthalene using a mobile phase of 65/35 water/acetonitrile, run at the optimal linear velocity for each column. Values depicted are the mean of three replicate injections (error bars =1 standard deviation).

UHPLC System

The UHPLC system used for these evaluations was an Agilent 1290 Infinity UHPLC consisting of an Infinity Binary Pump (Model G4220A), Infinity Sampler (Model G4226A), and Infinity VWD SL+ detector (Model G1314E). Minimal lengths of PEEKsil™ tubing (0.100mm ID) were used to connect the columns to the system. The flow cell was a 2µL micro flow cell.

Determination of Column Efficiency

To determine the efficiency of the columns, three replicate injections of a mixture containing uracil (t0 marker), acetophenone, toluene, and naphthalene were made. The column efficiencies reported represent mean values (±1 standard deviation where indicated) and were obtained from the naphthalene peak, as calculated using the Agilent ChemStation software (version Rev. B. 03.01 (317)). The mobile phase consisted of a pre-mixed solution of acetonitrile and water (65/35 v/v), and the columns tested at ambient temperature.All of the mobile phase components were HPLC grade. Optimisation of the HPLC system components was performed using a Core-Shell Optimization Kit (Phenomenex, Torrance, CA).

The two fully-porous HPLC columns used for evaluation were the Hypersil GOLD® 5µm C18 100 x 4.6mm and Luna® 3µm C18(2) 100 x 4.6mm. The core-shell packed column was Kinetex 2.6µm C18 100 x 4.6mm. All of the columns were all run at flow rates that approximate the optimal linear velocities (1mL/min for Hypersil GOLD 5µm, 1.5mL/min for Luna 3µm C18(2), and 1.8mL/min for the Kinetex 2.6µm C18).

Table 1. Performance (Efficiency in plates per meter) Improvement with System Optimisation

	Unoptimised System	Acquisition Rate	Tubing	Micro Flow Cell	Overall Increase	UHPLC - Agilent 1290
Hypersil GOLD® 5 µm ODS	101,407	104,633	111,947	117,377	16%	116,367
Luna 3 µm C18(2)	150,387	164,943	175,553	179,693	19%	181,347
Kinetex 2.6 µm C18	148,097	198,193	229,207	271,463	83%	282,080

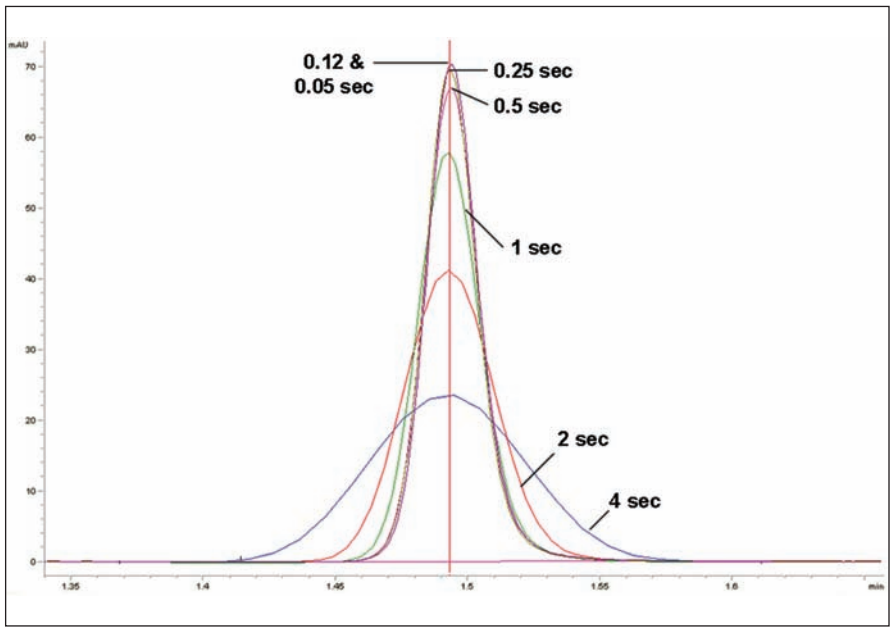


Figure 3: Effect of increasing the detector acquisition rate on peak shape for the naphthalene peak. Chromatograms obtained using the Kinetex 2.6µm core-shell C18 column on the Agilent 1100 system with micro flow-cell.

Pharmaceutical API Forced Degredation Profile

A sample of a proprietary active pharmaceutical ingredient (API) was dissolved in 50/50 methanol/water acidified and exposed to 0.1N HCl for 24 hours to induce acid hydrolysis of the compound and then analysed using both the optimised HPLC system and the UHPLC system (initial 10mg/mL concentration; 5µL injection). Running conditions are included in *Figure 5*.

Results and Discussion

Baseline Performance on an Unoptimised HPLC System

On the unoptimised HPLC system, the Kinetex 2.6µm C18 column generated 148,097 plates per meter *(Figure 2)*, the fully-porous 3µm particles generated 150,387 plates per meter, and the fully-porous 5µm generated 101,406 plates per meter. Thus, on an unoptimised HPLC system, the performance of the sub-3µm core-shell media was essentially identical to that of the fully-porous 3µm media (1.5% difference), and was 46.0% greater than the fully-porous 5µm particles.

Step 1 of 3.
Effect of Increasing Detector Acquisition Rate

The first simple adjustment made to the HPLC system was an incremental increase of the detector acquisition rate from the initial setting of 1 sec to the maximal rate of <0.005 sec. It is clear from the overlayed chromatograms for the naphthalene peak *(Figure 3)* that increasing the acquisition rate has a profound effect on the chromatography, as the peak gets narrower and peak height increases as the acquisition rate increases. This change in peak shape is, in turn, reflected in the increase in measured column efficiency as the acquisition rate is increased (*Figure 4*; data plotted for Kinetex 2.6µm core-shell column). The efficiency of the core-shell column was increased by 33.8% by simply increasing the acquisition rate on the detector to the fastest setting. One thing to note is that extremely high acquisition rates may increase baseline noise.

Step 2 of 3.

Effect of Changing the Tubing Inner Diameter

Next, the 0.010" ID peek tubing was replaced with pre-cut 0.005" PEEKsil tubing (similar lengths in both cases) using a commercially-available kit (Core-Shell Optimization Kit, Phenomenex, Torrance, CA.) containing 3 lengths of pre-cut tubing and high-pressure fingertight fittings. Similar to the results obtained when changing the detector acquisition rate, it was found that changing the tubing had a much greater effect on the performance of the core-shell column than on the two fully-porous columns *(Figure 2)*. The efficiency of the column packed with 2.6µm core-shell particles was increased from 198,193 plates per meter to 229,207 plates per meter, a 15.6% increase in efficiency over optimising the acquisition rate alone *(Table 1)*.

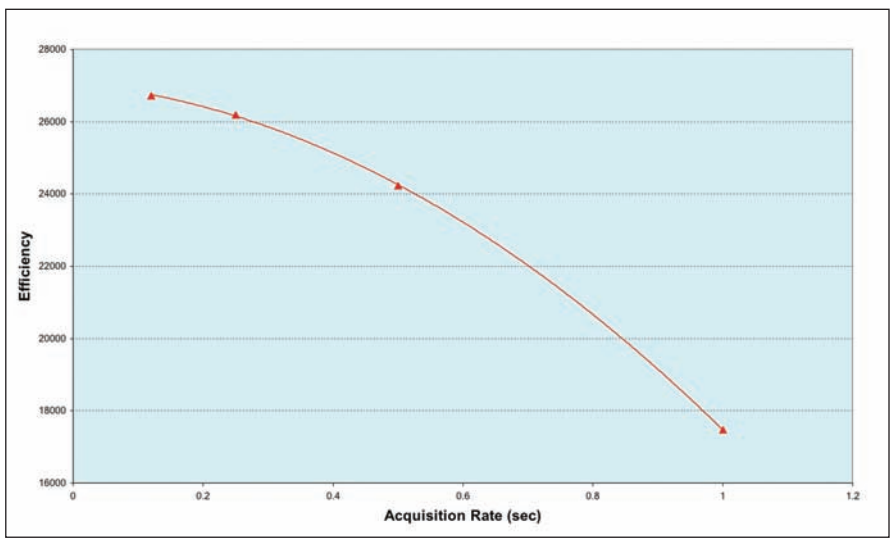


Figure 4: Effect of detector acquisition rate on column efficiency. Efficiency values calculated from chromatograms obtained using the Kinetex 2.6µm core-shell C18 column on the Agilent 1100 system with micro flow-cell.

Step 3 of 3.

Effect of Optimising the Flow Cell

The last optimisation made to the system involved replacing the standard 14µL flow cell with a 2µL micro flow cell. Consistent with the previous sets of data, changing from a standard flow cell to a low-volume micro flow cell had a much more profound effect on the sub-3µm core-shell column than either of the two fully-porous materials. By making this change, the efficiency of the core-shell Kinetex 2.6µm C18 column increased to 271,463 plates per meter, an 18.4% increase in efficiency over the previous optimisations *(Figure 2)*. This increase in efficiency must be attributed to the lower volume of the flow cell, resulting in a reduction in band broadening.

The cumulative effects of these relatively simple system optimisations are shown in *Table 1*. The performance of the fully-porous 5µm media increased by a total of 15.8% with system optimisation, and the efficiency of the 3µm-packed column increased by 19.5%. However, the increase in performance was most significant for the core-shell Kinetex 2.6µm media, which had a net increase in efficiency of over 83% over the unoptimised HPLC system, yielding a final efficiency of 271,463 plates per meter on a standard HPLC system. This efficiency value is only 3.8% less than the efficiency obtained using that same Kinetex 2.6µm column on the Agilent 1290 UHPLC system, indicating that it is indeed possible to achieve UHPLC-level performance on a standard HPLC system by performing simple system optimisations. This efficiency value is also comparable to that obtained using columns packed with fully-porous sub-2µm particles.

Pharmaceutical Forced Degredation Profile

The original tests were all performed using a very simple hydrophobic probe (naphthalene) under unbuffered and isocratic running conditions. To demonstrate that these results could be extrapolated to running conditions that more closely resemble a real-world methodology a further set of samples was analysed.

To this end, a sample of proprietary pharmaceutical API (a moderately hydrophobic, acidic compound) subjected to acid hydrolysis for 24 hours to induce sample degradation was analysed using a gradient method on the HPLC system and on the UHPLC system using the same core-shell Kinetex 2.6µm C18 column (100 x 4.6mm). As is apparent from the chromatograms *(Figure 5)*, optimisation of the HPLC system has a dramatic effect not only on the calculated column efficiency as shown previously, but also on the performance for this impurity profile. For example, resolution between the closely-eluting principle impurities B and C was 2.30 on the optimised Agilent 1100 HPLC system and 2.36 on the UHPLC Agilent 1290 system.

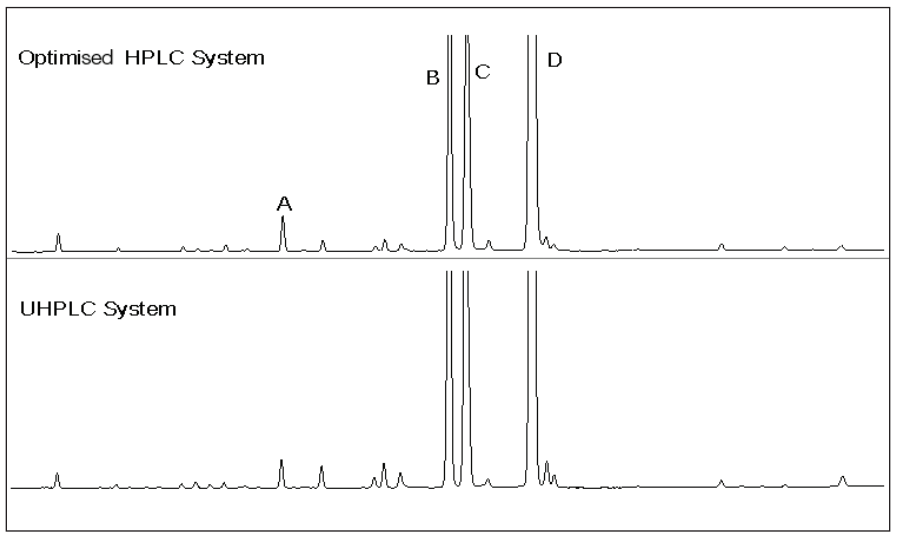


Figure 5: Impurity profile for pharmaceutical API obtained using the Kinetex 2.6µm core-shell C18 column on the optimised Agilent 1100 system (top) and then using the UHPLC Agilent 1290 system (bottom). Please note that the retention times on the two systems was off by ~ 30 sec, presumably due to differences in gradient mixing, so the chromatograms have been aligned relative to the main API peak (D). The method used a linear gradient with mobile phase A = water with 0.1% perchloric acid and mobile phase B = acetonitrile. The gradient went from 10% B to 70% B over 30 minutes, at a flow rate of 1.2mL/min. The same column (Kinetex 2.6µm C18 100 x 4.6mm) was used for both analyses.

Conclusions

The data presented in this report indicates that, with proper optimisation of a standard HPLC system (acquisition rate, tubing, and flow cell), it is indeed possible to achieve UHPLC-equivalent performance from a conventional HPLC system coupled with sub-3µm core-shell media (Kinetex 2.6µm in this case). Using these simple modifications, chromatographers can greatly extend the performance capacity of their current HPLC systems without necessarily having to purchase UHPLC systems.

References

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[2] F. Gritti, I. Leonardis, D. Shock, P. Stevenson, A. Shalliker, G. Guiochon. J. Chromatogr. A, 1217 (2010b) 1589.

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