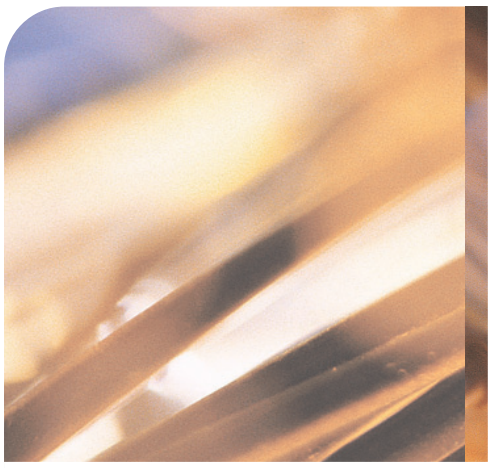

Chromatography Focus

DO SUB 2µm PARTICLES OFFER THE BEST PERFORMANCE FOR SHORT FAST GRADIENTS?

Ken Butchart, Tim Potter, Andy Wright, Alex Brien

Since the introduction of high performance liquid chromatography in the mid 1970's there has been a continuing gradual shift towards the use of smaller particle size supports. The driving force being the reduction of analysis time through increased plate count and resolution.

Recent developments in LC instrumentation capable of operating at elevated pressures have facilitated the development and introduction of small particle (2µm or smaller) stationary phases for HPLC. This trend towards using high pressure in LC is well-documented: high efficiencies, good resolution and fast throughput being the goal that has driven the move towards the use of sub 2µm particles.

> ***"THE MOVE TOWARDS USING SUB 2µm PARTICLES HAS BEEN DRIVEN BY THE THEORY THAT THE RESULTING JUMP IN EFFICIENCY WILL LEAD TO SIGNIFICANT IMPROVEMENTS IN RESOLUTION"***

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In this article we show that analysts can achieve much if not all of these variables by correct use of current systems. By making use of a well-packed column bed and optimised column hardware design (a Fortis™ C18 column was used in these experiments), sharp peak widths, excellent separation, speed, efficiency and resolution can all be achieved. Sub 1min run times can be achieved by the correct use of 3um particles without the associated pressure issues, allowing analysts high throughput on regular systems.

IMPROVING RESOLUTION

Approaches to improving resolution involve making changes to one or more of three variables: efficiency, retention or selectivity. Recent advances in HPLC instrumentation have been driven by the requirement to run with high backpressure whilst using sub 2µm particles as the stationary phase. The move towards using sub 2µm particles has been driven by the theory that the resulting jump in efficiency will lead to significant improvements in resolution.

The fundamental resolution equation (also known as the Purnell equation) shown in *Figure 1* shows that efficiency (N) does play a significant part in improving resolution, however an important consideration is the fact that resolution (Rs) is not directly proportional to N, but to the square root of N. By far the greatest factor influencing resolution is column selectivity, which can be altered by altering the stationary and mobile phases.

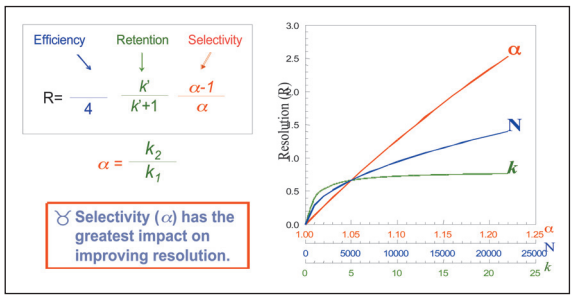

Figure 1. Selectivity Diagram

Although the majority of chemistries produced and sold in column format by manufacturers are octadecylsilane chemistries (ODS/C18), it should not be assumed that they have the same selectivity.

At time of this article going to print the USP L1 listing (Octadecylsilane chemically bonded to porous silica or ceramic micro particles, 1.5 – 10µm in diameter, or a monolithic rod) contains 346 products supplied by approximately 53 manufacturers.

It is obvious that all these supports will not offer the same selectivity for the analyst, indeed due to the multiple phases on offer there have been many studies made and methodologies developed to categorise more accurately C18 chemistries that behave in a similar manner, such as the Euerby [1] method or the work of Snyder and co workers [2,3].

Therefore rather than switching to sub 2µm particles and the expense of an instrument capable of running at extreme pressures it might be wise to test some alternative C18's or alternative chemistries to make use of the selectivity changes available and also to improve resolution by improving peak widths.

INFLUENCE OF COLUMN DIMENSION AND PACKED BED

The degree to which resolution is improved by the use of sub 2µm particles is dependent on the efficiency gained by switching to the smaller particle columns. Two factors affect the degree to which a column is well packed and thus the column plate count; (1. the particle size) and (2. the column dimensions).

Typically the smaller the particle diameter the greater the difficulty in preparing a well-packed column bed, particle aggregation, frit blockage, particle fracture are all issues when using the high pressure required to pack sub 2µm particles into the column hardware.

As column geometry is reduced the quality of the packed bed also suffers. Shorter columns have a higher proportion of poor bed quality at the bottom of the column, whilst the narrower columns suffer from a decrease in performance due to column wall effects.

A third factor to consider is the affect of heat of friction caused by the high pressures required to force the mobile phase through a packed bed of sub 2µm particles. The mechanical energy required to force the mobile phase through the column is converted to thermal energy leading to radial temperature gradients and in turn viscosity changes in the centre of the column, which causes distortion to the chromatographic band.

Halasa [4] and co workers described in detail the effects of heat of friction in the 1970's when working with 5µm particles. It can therefore be assumed that today's sub 2µm particles will generate greater heats of friction and as a consequence the theoretically expected gain in column performance by moving to sub 2µm particles cannot be fully realised.

When we then compare the actual column efficiency versus what would in theory* be possible to achieve we see large deviations in performance.

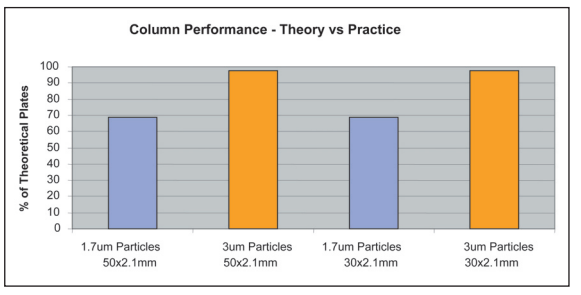

Figure 2. Column potential Diagram

**Snyder, L. R.; Kirkland, J. J.; Glajch, J. L. Practical HPLC method development. 2nd Edition, Wiley, New York, 1997. N=3500 x L / dp*

In *Figure 2* we can see the efficiency for two different column formats both containing 3µm Fortis C18 particles and a competitor sub 2µm particle. All four columns were tested under isocratic conditions using Naphthalene as a test probe. The results show that the sub 2µm particle column falls considerably short of its theoretical potential, whereas the columns packed with 3µm particles achieve very near the theoretical maximum expected. When studying the factors affecting resolution shown in *Figure 1* we can see that selecting efficiency as the factor to improve resolution means

that relative resolution gain can be interpreted as the ratio of the square root of any efficiency gained. The fact is that in certain column geometries the packed bed deficiency seen for sub 2µm particles means that resolution improvement gained by moving to sub 2µm particles can be less than 10%.

Experiment 1: Resolution Gain versus Theory

Column: 50x2.1mm

Mobile phase: Acetonitrile/H_20 60/40 0.5ml/min

Test Compound: Naphthalene

Instrument: Agilent 1200

Results:

Table 1. Resolution gain

	3um Fortis™ C18	Sub 2um C18	Sub 2um Theory
Column Plates	6368	7640	10294
√N/4	19.95	21.85	25.36
Resolution Gain >3u	-	9.5%	27.0%
Back pressure	122 Bar	347 Bar	-

The results of experiment 1 show that, for the 50x2.1mm geometry tested, the gain in resolution achieved by the sub 2µm particles is in fact only 9.5%. This is a very low gain considering the backpressure increase generated by the small particles and the associated issues that come with running at increased pressure. Furthermore when the use of gradient conditions is applied to the columns this small resolution gain will be further diminished.

Figure 3 shows a comparison of backpressure values for a range of particle sizes in a Water/Acetonitrile mobile phase, it is seen how the sub 2µm particle generates a threefold increase in backpressure.

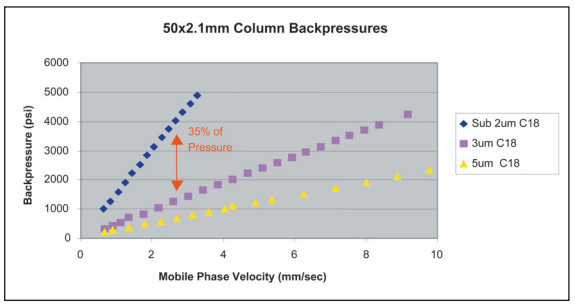

Figure 3. Column Backpressure vs dp

REAL LIFE VAN DEEMTER PLOTS

Van Deemter curves have been extensively published in the promotional data put forward for the application of sub 2µm particles. These plots are generated under isocratic conditions with the column diameter typically being 4.6mm so as to provide a better packed bed, whereas a much more typical column dimension to use in high throughput analysis would be the short 2.1mm diameter columns.

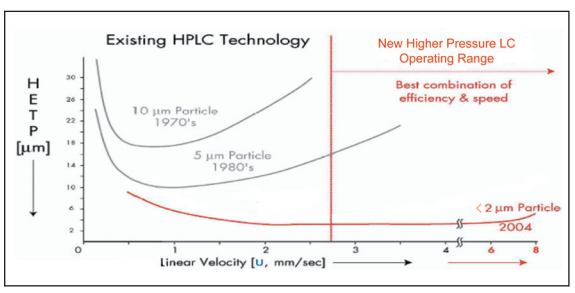

Figure 4. Typical Van Deemter Plot for Sub 2µm Products

This presentation of data (as seen in *Figure 4*) can mislead the chromatographer into believing that they themselves will see large improvements in their chromatography simply by switching to a sub 2µm column. Whereas in reality each Van Deemter plot is specific to the set of conditions, column geometry and instrument used to generate the data, yet no mention of this is made by those presenting the information.

Experiment 2:

Two 50x2.1mm columns containing different particle size C18 phases were tested under identical conditions on two separate HPLC systems, one designed to run at high pressures (up to 600 Bar) and the other designed for standard pressures (up to 400 Bar only).

Results:

As can be seen in *Figure 5a*, when using standard pressure ranges on a system that has not been optimised to reduce system volume, apart from backpressures generated, there is very little if any difference in the optimum performance of the 3µm and sub 2µm materials.

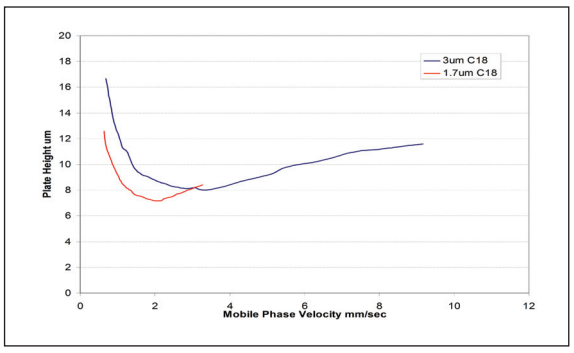

Figure 5a. Van Deemter Agilent 1100 System Result

However when the same columns are tested on a system that has had the system volume reduced in order to run small particles there is a minor improvement seen in the performance of the sub 2µm column as seen in *Figure 5b.*

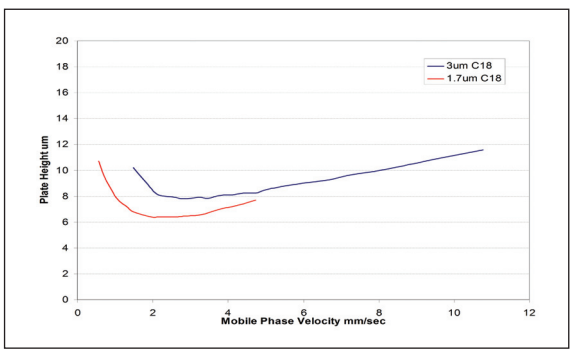

Figure 5b. Van Deemter Agilent 1200 System Result

This shows how the extra system volume is detrimental to the performance seen for small particle columns and that the use of these columns on standard HPLC systems is unlikely to offer the chromatographer any improvement in performance.

It also appears that even when sub 2µm columns are used on optimised instrumentation the increase in efficiency (decrease in plate height) is far less than is routinely shown in the marketing of these products.

PEAK CAPACITY AS A MEASURE OF PERFORMANCE

Peak capacity is often used as a measurement of performance for a HPLC column, by calculating the theoretical number of peaks that could fit within a gradient based on the average peak widths of a set of compounds run across that gradient. For sub 2µm particles to offer an increase in peak capacity they would need to provide a significant decrease in mean peak width.

Experiment 3:

Using a short Acetonitrile/TFA gradient on an Agilent 1200, a range of 20 pharmaceutical purity samples and 10 basic compounds were analysed. Five analytical columns containing sub 2um and 3um particles were compared, and the peak widths at half height were measured for comparison.

Column A: Sub 2um, 50x2.1mm (Manufacturer 1)
Column B: Sub 2um, 30x2.1mm (Manufacturer 2)
Column C: Sub 2um, 50x2.1mm (Manufacturer 3)
Column D: 3.5um, 50x2.1mm (Manufacturer 2)
Fortis C18 3um, 50x2.1mm (Fortis Technologies)

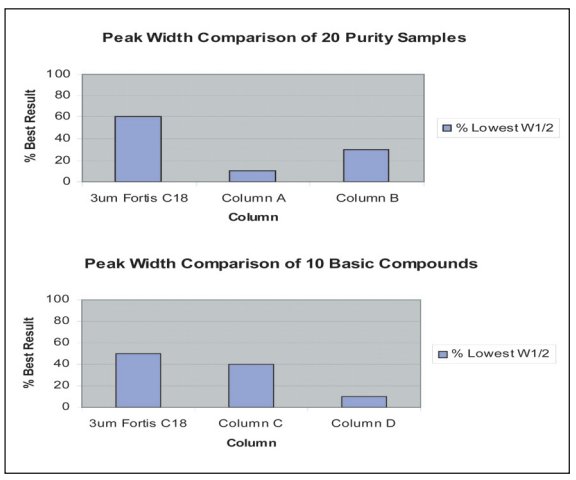

Figure 6. Peak Widths Results

As *Figure 6* shows, when compared against three different manufacturers' sub 2µm products for the analysis of 20 purity samples the 3µm C18 column gave the narrowest peak width for the majority of samples. If a comparison is done purely with the 10 basic compounds the 3µm C18 column still gave the narrowest peak width for 50% of the samples. The narrower peak width obtained when using the 3µm column can be clearly seen when the traces are overlaid as shown in *Figure 7*.

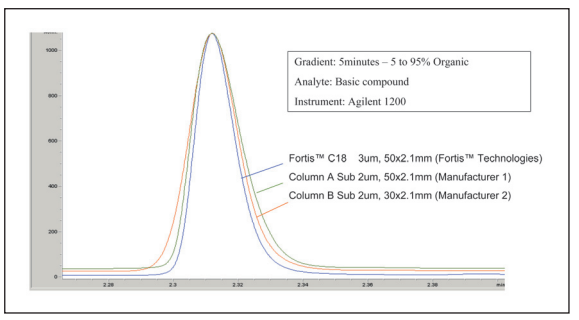

Figure 7. Peak Widths Comparison

SHORT FAST GRADIENTS

Figure 8 shows how little or no advantage is gained by using a sub 2µm particle C18 for the analysis of alkylphenones in 1.5mins, the pressure increase of 50% in order to reduce peak width by 0.1 seconds would seem to be unjustified.

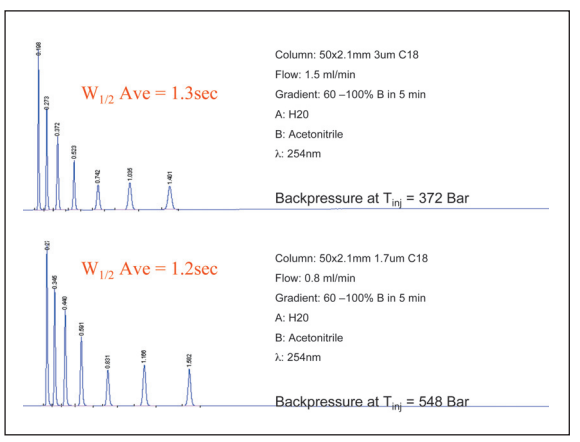

Figure 8. Alkylphenones Application C2-C8

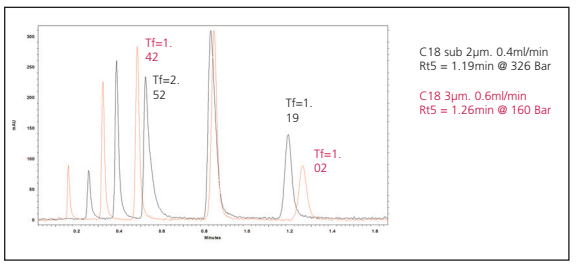

Figure 9a. Resolution - Lower pressure

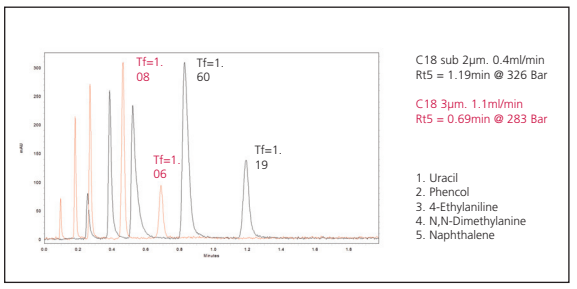

Figure 9b. Speed - Equivalent Pressures

Short well-packed 3µm columns offer the opportunity to obtain equivalent resolution to the current sub 2µm particles (*Figure 9a*) at greatly reduced backpressure, alternatively this reduced backpressure can be used to increase flow and speed up analysis. (*Figure 9b*)

CONCLUSION

Theoretically, columns packed with small particles should provide extremely high plate counts which in turn should lead to extra resolution and peak capacity. However we have shown that currently the use of small particles for fast chromatography in short column formats offers no improvement over 3µm columns when run on standard HPLC systems or even the newer systems designed to run at the elevated pressures. Under isocratic conditions it is possible to achieve much of the efficiency of sub 2µm particle columns with only 1/3rd of the backpressure by using a well-packed 3µm column. When run under fast gradient conditions it is difficult to see any performance benefit in moving to sub 2µm particles, even on optimised systems, gradient conditions negate any small efficiency gains seen from sub 2µm particles.

Therefore any analyst considering the move towards using sub 2µm particle columns for high throughput screening (HTS) should consider carefully whether they would achieve any benefits by doing so. Certainly if they are continuing to use standard HPLC instrumentation then they will gain nothing under gradient conditions. So do sub 2µm particles offer any benefits? Well the answer would be yes to those perhaps looking for some increased efficiency under isocratic conditions or for some gain in sensitivity. However do not suddenly expect large gains in resolution, more likely a scenario is getting baseline resolution of peaks where previously resolution was nearly baseline.

Another possible benefit of small particles is in the application of long gradient analysis in longer column formats (100mm and above) using extreme pressure conditions. Should current manufacturers improve their column packing techniques to the point where these columns reach their theoretical potential then it is possible that they may be suitable for fast gradient work. However the analyst must remember that for each analysis the Van Deemter curve is unique and therefore the thought that ultra high pressure chromatography will answer all questions is ambiguous.

Note: Fortis™ C18 is a trademark of Fortis Technologies Ltd, Fortis Technologies Ltd recognises the trademarks of all other manufacturers. All columns are original manufacturers packed columns

References:

1. Euerby MR, Peterson P, J.Chrom. A 994 (2003) 13-36
2. L.R. Snyder et al., J.Chrom. A (2004) 49-57
3. L.R. Snyder et al., J.Chrom. A (2004) 59-74
4. I. Halása, R. Endele, and J.Asshauer, J.Chrom. 112, 37 (1975)
5. Snyder, L. R.; Kirkland, J. J.; Glajch, J. L. Practical HPLC method development. 2nd Edition, Wiley, New York, 1997

New Innovative Ultra-Bright X-ray Source for Structural Biology is Announced

Bruker AXS introduces its Microstar Ultra™, an ultra-bright X-ray source for structural biology. Comparable in brightness to many second-generation synchrotron beamlines, the Microstar Ultra is the most intense home laboratory X-ray source available.

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Despite its performance, the Microstar Ultra is a remarkably compact and low-maintenance X-ray source, which features long operating times and low maintenance costs. There is an affordable upgrade path for existing Microstar generators to the Microstar Ultra. Initial Microstar Ultra generators have been installed in Bruker demonstration laboratories and at pharmaceutical customers and are performing exceedingly well in the field.