The Use of 1.9 µm Particles to Achieve Enhanced Resolution and Speed

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Introduction

In this poster the merit of moving from traditional particles sizes used in HPLC (5 and 3 µm) to sub-2 µm particle sizes is discussed. Limitations in the current use of 3 and 5 µm particles has lead to interesting theories of how an expanded Van Deemter curve can be utilized with smaller particles. As flow rate is increased, the efficiency of the column which has been gained by the use of small particles, is not reduced with the increased linear velocity.

Using a highly pure silica template and a new technique for creating monodisperse particles, particles ranging anywhere from 0.8 to 2 µm can be produced with excellent yield. This is an issue which has previously caused manufacturing problems. Allying this technology with the latest in bonding and end-capping advances, we can now achieve excellent peak shapes, speed and resolution. Short columns with high flow rates can be used to obtain exceptional peak capacities with very short run times, allowing the analyst to increase productivity, without the loss of the quality of qualitative or quantitative data.

Methods

The use of small particles allows high efficiencies to be achieved. Figure 1 shows the efficiency gains that are realized when moving from a 3 µm particle to a 1.9 µm particle for a given flow rate.

Figure 1. High efficiency provided by 1.9 µm particles

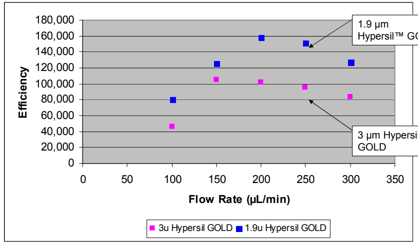


Figure 2. High efficiency

Flow Rate (uL/min)	%Gain from 1.9 µm
100	74%
150	19%
200	56%
250	59%
300	52%

Figure 2 calculates that the gain is on average greater than 50%. This gain can be converted into either a speed or a sensitivity increase by the analyst when trying to optimize their separation.

Utilizing the new proprietary manufacturing procedure, 1.9 µm particles have been produced. This novel technology has two distinct advantages over prior methods: first, it allows particles of a much tighter distribution to be achieved. Second, the particle size can be tailored to make particles in any size from 0.8 to 2 µm, allowing smaller, monodisperse particles to be created if they are deemed necessary to advance separation science.

Both of these will have an impact upon achieving higher resolution and efficiency. Figure 3 shows the d90/d10 distribution for 1.9 µm Hypersil GOLD media compared to a commercially available 1.8 µm packing. It can be seen that the slope of the graph for the 1.9 µm particles is steeper and that this leads to the percentile ratio being much smaller, hence higher efficiency.

FIGURE 3. Comparison of particle size distribution

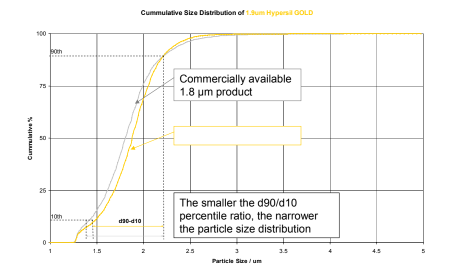
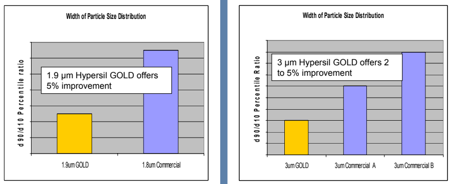


Figure 4 illustrates how the particle size distribution has been improved. Hypersil GOLD in 3 µm particle size improves monodispersity over other commercial 3 µm stationary phases by between 2 and 5%. The 1.9 µm Hypersil GOLD improves monodisperse character over an existing 1.8 µm phase by 5%.

FIGURE 4. D90/10 Ratios for 3 and 1.9 µm Hypersil GOLD compared to commercially available phases.



Results

Hypersil GOLD has already proved itself to be exceptional at producing good peak shapes for basic analytes[1], therefore allowing the chromatographer to gain sensitivity and resolution. This bonding technology has been incorporated into the 1.9 µm particles to ensure that when running high throughput applications, distortion does not appear in results due to co-eluting peaks, which would reduce productivity.

Figure 5 highlights an application of anabolic steroids differing only slightly in their chemical structure (Figure 6); maintaining both resolution and sensitivity while improving throughput of the analysis will be critical. Utilizing 1.9 µm particles allows all of these requirements to be achieved, with analysis under 1.5 minutes.

FIGURE 5. Increase in resolution as particle size is reduced for Anabolic Steriods

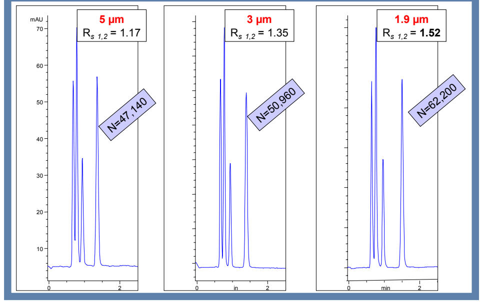
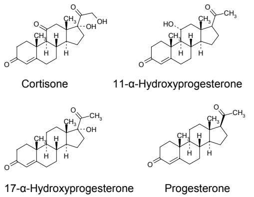


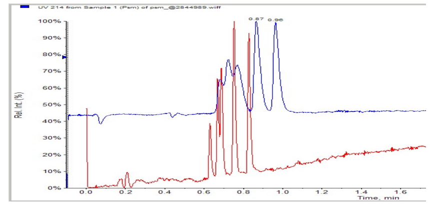
FIGURE 6. Conditions for the anabolic steroid analysis



1.9 µm Hypersil GOLD 50x2.1mm
A: 0.1% Formic acid
B: ACN + 0.1% Formic acid
Isocratic : 25:75 (A:B)

Flow: 200 µL/min
Wavelength : 254 nm
Temp: Ambient
Injection: 60 nL

FIGURE 7. Pierce Peptide Standard Mix (PPSM)
(Data courtesy of M. Bean, GSK)



1.9 µm Hypersil GOLD

Conclusions

In this poster we demonstrate the advantages of 1.9 µm Hypersil GOLD particles. We look at the the use of a proprietary manufacturing procedure in which small particles can be refined to give a very tight distribution. This in turn allows the chromatographer confidence that they are truly going to see the benefits of small particles.

We highlight the gains achieved with comparisons of particles sizes using applications of anabolic steroids and a peptide mix. Both applications show gains in resolution over traditional HPLC particle sizes while using fast, short run times.

While it has been shown that gains are made, further study and applications will help to prove this conclusively under a multitude of conditions and analytes. The novel proprietary manufacturing procedure can be expanded further to provide even smaller particles for use in HPLC and ultra high pressure LC.

References

(1)M. Woodruff; C. Blythe; H. Ritchie; S. Aspey; L. Pereira; Enhanced Sensitivity and Quantitation by Obtaining Symmetrical Peak Shapes for Basic Pharmaceuticals - Poster presented at HPLC 2004, Philadelphia.

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