The Use of Sub 2µm 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 in HPLC has lead to interesting theories of how an expanded van Deemter curve can be utilized with smaller particles to increase efficiency and analysis speed. Columns packed with small particles can be operated over a wide flow rate range, with high efficiencies. Therefore, these high efficiencies enable considerable improvements in speed of analysis and resolution.

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. Combining this technology with the latest in bonding and end-capping advances, enables excellent peak shapes, speed and resolution. Short columns run at high flow rates can be used to obtain exceptional peak capacities with very short run times, allowing the analyst to increase productivity, without compromising the quality of qualitative or quantitative data.

Hypersil GOLD has already proved itself to be exceptional at producing good peak shapes for basic analytes[1], and therefore provide gains in sensitivity and resolution. This bonding technology has been incorporated into the 1.9 µm particles to ensure that when running high throughput applications, peak tailing does not compromise resolution.

Methods

Small particles provide very efficient separations. Figure 1 shows the efficiency gains that are obtained 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

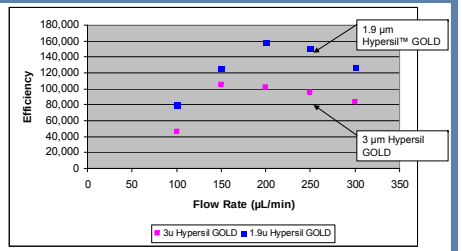


Table 1. Efficiency gain versus flow rate.

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

The flow rate for optimum efficiency is shifted to a higher value with the 1.9 µm particles. Table 1 shows that the gain in plates per meter when moving from 3 to 1.9 µm particle size is on average greater than 50%.

Analysis time and resolution are determined by the ratio of column length to particle size. Therefore, when reducing particle size, column length can be reduced proportionally to obtain faster analysis while keeping resolution.

Utilizing this new proprietary manufacturing procedure, 1.9 µm particles have been produced. This novel technology has two distinct advantages over existing methods: firstly, it allows particles of a much tighter distribution to be produced; secondly, 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 efficiency and resolution. Figure 2 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 2. Comparison of particle size distribution

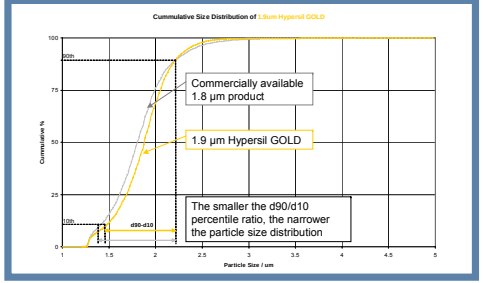
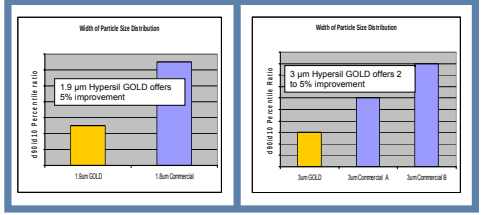


Figure 3 illustrates how the particle size distribution has been improved. Hypersil GOLD in 3 µm particle size improves monodispersity over other commercial 3 µm stationary phases by between 2 and 5%. The 1.9 µm Hypersil GOLD improves monodisperse character over an existing 1.8 µm phase by 5%.

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



Results

Figure 4 highlights an application of anabolic steroids differing only slightly in their chemical structure; maintaining both resolution and sensitivity while improving throughput of analysis is critical in such cases. Utilizing 1.9 µm particles allows all of these requirements to be achieved, with analysis under 1.5 minutes.

A selection of seven of the more common β-Blockers was separated using a 1.9 µm 20 x 2.1 mm Hypersil GOLD column (figure 5). This example demonstrates how the use of small particles enables very fast runs to be achieved due to the higher efficiencies provided. Sharp peak shapes are obtained on Hypersil GOLD columns for basic analytes such as β-blockers without the need for complex mobile phase systems, buffers, or additives. Instead simple generic gradients can be used, ensuring quick equilibration of the HPLC system, and therefore optimal overall turnaround time for HTS applications.

FIGURE 4. Increase in resolution as particle size is reduced for Anabolic Steroids

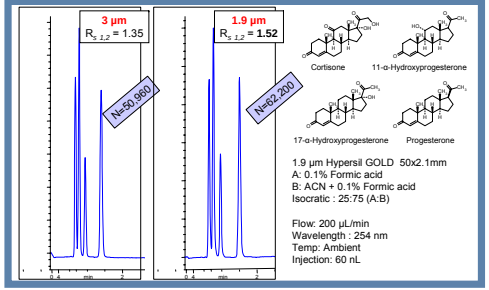
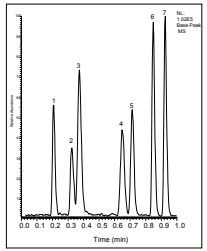


FIGURE 5. Separation of seven β–blockers in under 1 min.



Column: 1.9µm Hypersil Gold 20x2.1mm

Mobile phase: A - H_2O+0.1%formic acid

B - MeCN+0.1%formic acid

Gradient: 15 to 100% B in 1min

Flow rate: 0.5 ml/min

Temperature: 30°C

Detection: +ESI

System: Finnigan™ Surveyor™ and Finnigan Surveyor MSQ™

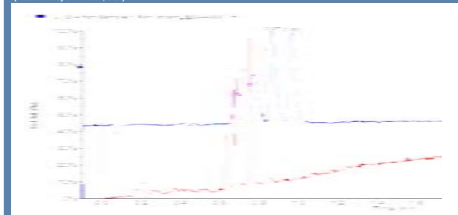
Analytes:

1. Atenolol
2. Nadolol
3. Pindolol
4. Timolol
5. Metoprolol
6. Oxprenolol
7. Propanolol

(Pressure at start of gradient – 186 bar)

FIGURE 6. Pierce Peptide Standard Mix (PPSM)

(Data courtesy of M. Bean, GSK)



Conditions for PPSM:

1.9 µm Hypersil GOLD 20 x 2.1 mm Javelin 5 µm AQUASIL 40 x 1 mm

A: 0.01% TFA

B: ACN + 0.01% TFA

Gradient: 3.5 – 97% B in 1.6 min., hold for 0.15 min.

Flow : 0.825 mL/min

It can be clearly seen that for the challenging peptide mix above, resolution is dramatically improved and analysis time is reduced with 1.9 µm Hypersil GOLD columns. The use of a small particle size, coupled with a stationary phase that provides excellent peak shapes, has contributed to this improvement.

Conclusions

In this poster the advantages of 1.9 µm Hypersil GOLD particles are shown. A proprietary manufacturing procedure in which small particles can be produced to give a very tight distribution is used. This tight particle size distribution ensures that the benefits of small particles can be explored. The novel proprietary manufacturing procedure used can be expanded further to provide even smaller particles for use in HPLC and ultra high pressure LC.

The gains achieved with 1.9 µm Hypersil GOLD particles in comparison with other sizes are illustrated for anabolic steroids and a peptide mix. Both applications show gains in resolution over traditional HPLC particle sizes while using fast, short run times.

Short 1.9 µm Hypersil GOLD columns can be used with high flow rates to obtain exceptional peak capacities with very short run times under 1 minute.

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.

Additional Information

For additional information, please browse our website: www.thermo.com/columns

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