IMPACT ON DRUG IMPURITY PROFILING WITH SUB 3 µM HPLC COLUMN PACKINGS

Recently there has been great interest in the use of smaller column packing materials for HPLC separations. The smaller packing materials promise several benefits such as increased efficiency, increased resolution, and decreased run times. Over the last four years, several vendors have marketed sub 2 µm packing materials, and very recently some vendors have offered materials in the 2.2 – 2.5 µm range. In general, commercially available materials less than 2 µm in diameter have surface areas in the range of 170 – 200 m²/g. Last year, a series of 2.5 µm particles were introduced having surface area in the 400 m²/g range. Theoretically these higher surface area materials should provide a greater number of available stationary phase ligands to interact with the analytes in the column. Overall this increased surface area should increase the column loadability.

Chromatography Focus

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One key analytical area where column loadability is a major concern is drug impurity profiling assays, where a large amount of the main component must be loaded onto the column in order to increase the detectability of trace contaminants. Typically it is necessary to quantitate the impurities at the 0.05 - 0.1% level compared to the main peak area. In this paper, we examine the loadability of commercially available sub 3 µm stationary phases, and investigate the role that media surface area has on loadability and how this impacts impurity-profiling assays.

In recent years there have been significant investigations into the use of columns with smaller (< 3 µm) packing materials. The use of smaller packing materials promises several benefits including higher efficiencies per unit length and decreased resistance to mass transfer [1-3]. The lowered resistance to mass transfer allows for columns to be operated at significantly higher linear velocities (flow rates) and maintain equivalent performance.

The higher linear velocities in conjunction with shorter column lengths provide significant reductions in analysis times. There is, however, a seeming lack of consensus as to the optimum particle size, as there are commercially available columns packed with particles ranging from 1.5 – 2.5 µm. It has recently been demonstrated that the sub 2 µm columns fail to generate the efficiencies theoretically predicted for packing materials of this size, whereas 2.5 µm materials generate similar efficiencies as the commercially available sub 2 µm materials [4-12]. The sub 2 µm packing materials also have a significant increase in the resistance to flow through the column, making it necessary to operate these columns at much higher pressures in order to generate adequate mobile phase linear velocities through the columns. The drawback to higher operational pressures has been addressed to some extent by the commercialisation of HPLC equipment designed to operate at pressures of 600 – 1000 bar (8700 – 14500 p.s.i.). Because of these high operational pressures, the chromatographic media, as well as column packing structure and hardware must be designed to withstand the operational pressures and provide suitable column lifetime [13]. Most sub 2 µm materials have surface areas below 250 m²/g in order to have adequate mechanical stability for the particles under elevated pressure conditions. Luna® 2.5 µm C18 (2)-HST material has a surface area of 400 m²/g, which indicates that there should be significantly more carbon available for interaction with the analyte assuming equal bonding and packing density.

The lower surface area materials, however, are inherently more dense, therefore one must also consider the packing density of the material. Experimentally it has been determined that the packing density of Luna® 2.5 µm C18 (2)-HST is 0.58 g/mL, and the packing density of the various sub 2 µm media tested ranged from 0.51 g/mL to 0.76 g/mL. In order for the sub 2 µm medias

to have an equal number of moles of available carbon, the packing density would have to be twice as high, which was not demonstrated in the experimental tests. The greater quantity of ligands available for chromatographic interaction should lead to a larger column loading capacity. In many instances column loadability is not a significant concern, as the columns are loaded with masses well below the loading limit. The loadability does become a major concern when there is a significant dynamic concentration range of the analytes of interest. One particular application where the dynamic range is relevant is for impurity profiling and analysis of drug substances. In this particular application, a large amount of the drug substance is loaded onto the analytical column in order to adequately identify and quantify impurities in the drug substance. The quantification of the impurities is routinely reported in the range of 0.05 - 0.1% area of the parent compound peak.
In this poster, we investigate the effect of column load on peak width for a high surface area material (Luna® 2.5 µm C18 (2)-HST) as well as some commercially available sub 2 µm materials. We also show the peak spreading effects due to column overload on the ability to resolve impurity peaks, especially those that elute close to the main peak.

Table 1. Column Performance and Surface Area

Column	Stated Surface Area (m²/g)
Luna® 2.5 µm C18(2)-HST	400
Competitor A C18 1.8 µm	180
Competitor B C18 1.9 µm	220
Competitor C C18 1.7 µm	185

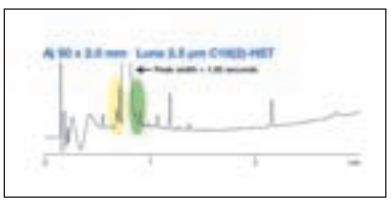

Figure 1. Separations of the impurities of Pindolol on Luna 2.5 µm C18 (2)-HST and several commercially available sub 2 µm columns at 50°C

Conditions
Mobile Phase: A: 0.1% TFA in Water
B: 0.1% TFA in Acetonitrile
Gradient: 5% B to 95% B in 2.9 min
Flow Rate: 1.1 mL/min
Injection Volume: 1 µL
Detection: UV @ 254 nm
Temperature: 50°C
Sample: Pindolol

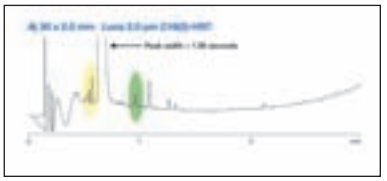

Figure 2. Separations of the impurities of Pindolol on Luna 2.5 µm C18 (2)-HST and a commercially available sub 2 µm column at 60 °C

Conditions
Mobile Phase: A: 0.1% TFA in Water
B: 0.1% TFA in Acetonitrile
Gradient: 5% B to 95% B in 2.9 min
Flow Rate: 1.1 mL/min
Injection Volume: 1 µL
Detection: UV @ 254 nm
Temperature: 60°C
Sample: Pindolol

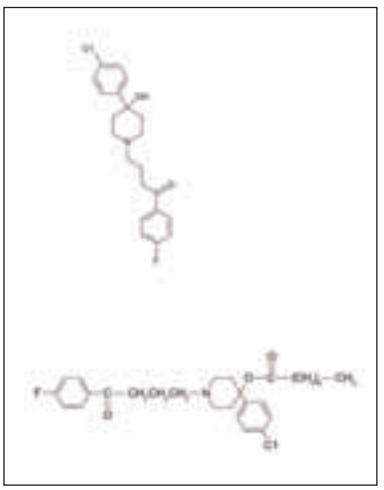

Figure 3. Structures of Haloperidol and Haloperidol Decanoate

Sample Information:
Haloperidol – 10 mg/mL
Haloperidol Decanoate – 5 mg/mL
Diluent – Methanol

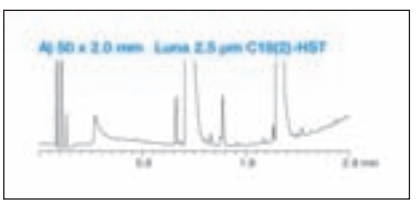

Figure 4. Impurity profile of Haloperidol and Haloperidol Decanoate on Luna 2.5 µm C18 (2)-HST and several commercially available sub 2 µm columns at 50°C

Conditions
Mobile Phase: A: 0.1% TFA in Water
B: 0.1% TFA in Acetonitrile
Gradient: 5% B to 95% B in 2.9 min
Flow Rate: 1.1 mL/min
Injection Volume: 1 µL
Detection: UV @ 254 nm
Temperature: 50°C

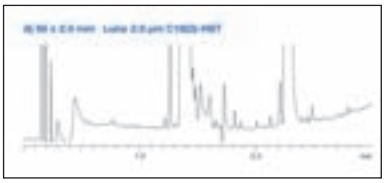

Figure 5. Impurity profile of Haloperidol and Haloperidol Decanoate on Luna 2.5 µm C18 (2)-HST and a commercially available sub 2 µm column at 60 °C

Conditions
Mobile Phase: A: 0.1% TFA in Water
B: 0.1% TFA in Acetonitrile
Gradient: 5% B to 95% B in 2.9 min
Flow Rate: 1.1 mL/min
Injection Volume: 1 µL
Detection: UV @ 254 nm
Temperature: 60°C

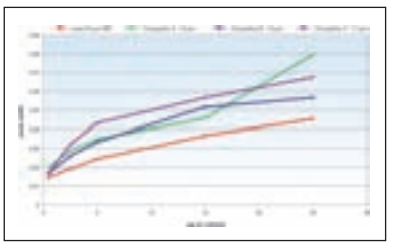

Figure 6. Peak width versus amount of sample on column for Haloperidol Decanoate on a 2.5 µm column and several commercially available sub 2 µm columns

Table 2: Linearity of the peak width vs. amount of sample on column for different concentration ranges for Luna 2.5 µm C18 (2)-HST and several commercially available sub 2 µm columns

Column	0.5 – 5.0 µg Linearity (R^2)	0.5 – 25.0 µg Linearity (R^2)
Luna 2.5 µm C18(2)-HST	0.99	0.98
Competitor A C18 1.8 µm	0.97	0.91
Competitor B C18 1.9 µm	0.98	0.90
Competitor C C18 1.7 µm	0.98	0.88

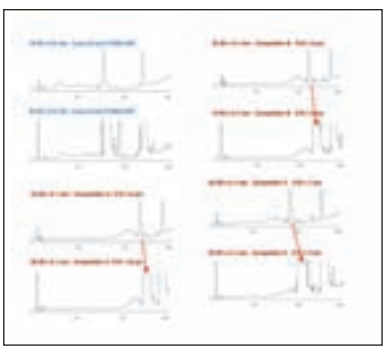

Figure 7. Impurity profile of Haloperidol and Haloperidol Decanoate on Luna 2.5 µm C18 (2)-HST and several commercially available sub 2 µm columns at 50°C at loads of 1.0 µg and 30 µg on column

In *Figure 1*, the separation of pindolol from its impurities is compared on a Luna® 2.5 µm C18 (2)-HST column as well as several commercially available sub 2 µm columns.

The width of the parent peak on the Luna® 2.5 µm C18 (2)-HST was 1.85 seconds, whereas the parent peaks on the sub 2 µm columns were all greater than 2.35 seconds (30% wider). The result of the major

compound peak broadening is that the trace impurities eluting close to the parent peak (illustrated with green and yellow circles) coelute with the broader parent peak. In *Figure 2*, a similar separation to the one presented in *Figure 1* is shown, however, in this case the separations were performed at 60°C, and Luna® 2.5 µm C18 (2)-HST was compared to a commercially available 1.7 µm material. As was the case in *Figure 1*, the 1.7 µm column produced a significantly broader parent peak, which caused the co-elution of the impurity peaks that elute close to the main peak. In *Figure 4*, a mixture of Haloperidol and Haloperidol decanoate (structures shown in *Figure 3*) were separated from their impurities using a Luna® 2.5 µm C18 (2)-HST and several commercially available sub 2 µm columns. In each separation a load of 10 µg was injected on column.
One interesting aspect of the separations in *Figure 4*, was that due to the smaller elution window between the two main peaks for the sub 2 µm columns, there is a significant decrease in the peak capacity for these columns. The peak capacity between the main peaks for the Luna column was 45, whereas it was 15 and 39 for the respective sub 2 µm columns (10% - 50% less). In *Figure 5*, a similar separation as presented in *Figure 4* is shown; in this case the separation was performed at 60°C. There are a significant number of additional impurity peaks present in *Figure 5a* vs the same separation performed in *Figure 4a* at a lower temperature.

> ***The higher linear velocities in conjunction with shorter column lengths provide significant reductions in analysis times.***

It is difficult to determine if the extra peaks present are a result of greater resolution provided from the elevated temperature separation, or rather if there is a significant degradation of the drug substances on column due to the elevated temperature. If one compares the separation in *Figure 5a* to *Figure 5b*, it is obvious that there is a significant increase in resolution of the impurity peaks that elute between the main peaks. This loss of resolution is partially due to the broader main peaks in the separation performed on the lower surface area 1.7 µm column due to overloading, and partially due to differences in selectivity between the two columns. The peak capacities between the main peaks for both columns were similar, 47 and 49, respectively. In *Figure 6*, the peak width for Haloperidol decanoate is plotted against the amount of the drug substance injected on column, for the columns tested in this study. As would be expected, the sub 2 µm columns with lower surface area show significantly broader peaks at higher column loading than the 2.5 µm material.

The linearity of the slopes of these curves were evaluated at loading ranges of 0.5 – 5 µg and also 0.5 – 25 µg. All the columns tested proved linear in the loading range up to 5 µg, however, only the Luna 2.5 µm had a linear correlation of peak width to loading over the entire range tested.

The non-linearity of the sub 2 µm columns at levels above 5 µg suggest that this is their loading limit, while the Luna 2.5 µm material has a loading limit five times higher than the sub 2 µm columns.

Another interesting consequence of high column loads on the sub 2 µm is that a 10% change in retention for the Haloperidol peak was observed (shown in *Figure 7*) as the load increased. The retention time of the Haloperidol peak increased on the sub 2 µm columns at higher loading, and it is difficult to accurately state the cause of this phenomenon.

The shifting retention time does cause quantitation problems since the retention time window must be significantly wider resulting in the mis-identification of peaks. One possible explanation for the shifting retention time is that the high concentration of Haloperidol decanoate acts as a mobile phase additive in the separation. We are currently investigating this phenomenon in attempts to determine the cause of this observation.

CONCLUSIONS

- Significant peak broadening was observed on lower surface area sub 2 µm materials at high concentrations of a drug substance in impurity profiling.
- The peak broadening observed reduced the resolution of impurities that elute near the main sample peak.
- There was a 5x increase in linear loading range when using Luna 2.5 µm C18(2)-HST in comparison to commercially available sub 2 µm materials.

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