

An Evaluation of the Suitability of 1.9µm Particle Packed Columns for Proteomics Applications

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Overview

In this poster the performance of 1.9 µm packing material is compared to the more traditional 5 µm packing material using protein digest samples of low and high complexity. The performance of the columns has been evaluated using peptide signal intensity, peak capacity and retention time variation.

Introduction

The use of sub 2 µm particles in LC and LC/MS applications has shown many advantages over traditional 5µm packing materials. To date, however, sub 2 µm particle packed columns have been employed predominately for small molecule metabolomics and biotransformation studies, although their application to large molecule proteomics research is increasing. Of particular interest for proteomics applications is the increased efficiency provided by sub 2 µm particles, which facilitates rapid separations with greater resolution, peak capacity and sensitivity. Due to the sample complexity associated with biomarker and proteomics applications, the need for increased chromatographic efficiency afforded by high resolution small particle LC is highly desirable.

When considering the often very small amounts of sample available, it is also very important to maximise the sensitivity of the LC-MS system. This can be achieved by incorporating in the nanobore column PicoFrit hardware, where a nanospray emitter is incorporated into the end of the column. This eliminates band broadening caused by dead volume between the column and the spraying tip.

Methods

- Columns:**
 - Hypersil GOLD™ 1.9 µm PicoFrit, 50mm x 75 µm x 15 µm tip;
 - Hypersil GOLD 5 µm PicoFrit, 50mm x 75 µm x 15 µm tip;
 - Hypersil GOLD 5 µm PicoFrit, 100cm x 75 µm x 15 µm tip (Thermo Fisher Scientific, Bellefonte, PA)
- Instrumentation:**
 - Rheos Allegra HPLC pump, fitted with splitter and flow sensor
 - CTC Pal autosampler
 - LTQ Orbitrap™ mass spectrometer
- Samples:**
 - Cytochrome C digest
 - BSA digest
 - Highly complex plant sample (*Arabidopsis thaliana* whole soluble fraction) digest
- Test method I (Cytochrome C digest – Figure 1, Figure 2; BSA digest – Figure 3):**
 - Mobile phase: A - H₂O; B - ACN
 - Gradient: 0-60% B in 20 minutes
 - Flow rate: 110 µl/min, split to give a flow rate of 200 nl/min on column
- Test method II (*Arabidopsis thaliana* digest – Figure 4, Figure 5):**
 - Mobile phase: A - H₂O; B - ACN
 - Gradient: 0-60% B in 150 minutes
 - Flow rate: 110 µl/min, split to give a flow rate of 200 nl/min on column

Results

i) Cytochrome C digest

Figure 1. Cytochrome C Identified Peptides and base peak chromatogram, 5 cm 1.9 µm PicoFrit column

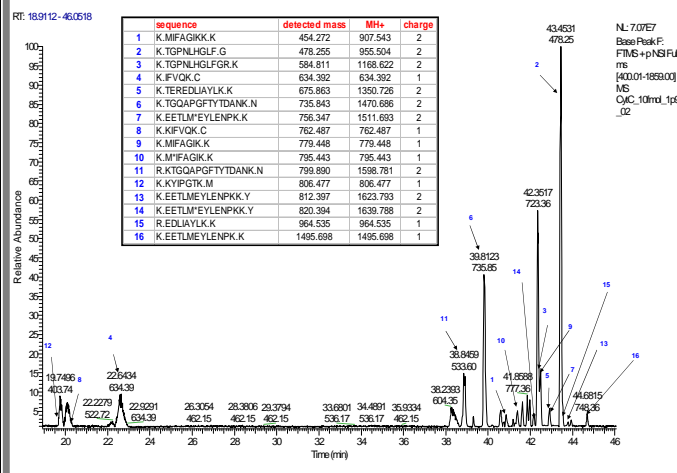


Figure 2. Extracted ion chromatograms for selected peptides using 1.9µm (upper) and 5µm (lower) columns

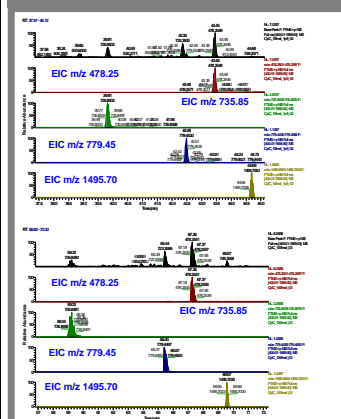


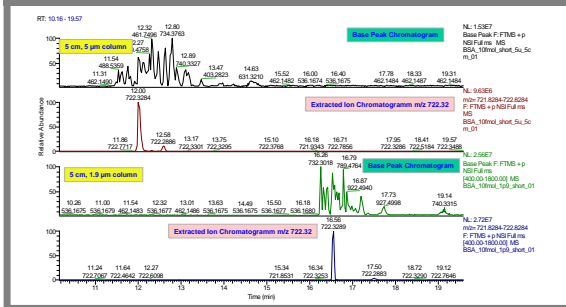
Figure 1 shows the base peak chromatogram for 10fmol digest of Cytochrome C obtained using the PicoFrit column packed with 1.9 µm particles. The sharp, narrow peaks result in excellent sensitivity and allow for the identification of 16 peptide fragments.

The Extracted ion chromatograms shown in Figure 2 provide a clearer indication of the peak shape. For the 1.9 µm column (upper set), the average peak width (measured at the base) is 0.23 minutes, compared to an average peak width of 0.50 minutes for the 5 µm particle packed column (lower set). This gives a much greater peak capacity – over the 70 minute gradient these peak widths result in a peak capacity of 305 for the 1.9 µm column and 141 for the 5 µm column.

The peak intensities are comparable – While on first appearance the peak intensities using the 5 µm seems greater, 100fmol was injected onto the 10cm column packed with 5 µm particles.

ii) BSA digest

Figure 3. Extracted ion chromatograms for selected peptides using 1.9µm (upper) and 5µm (lower) columns



The base peak and extracted ion chromatograms for 10fmol of BSA digest are shown in Figure 3 above. The column packed with 1.9 µm particles again gives excellent peak shape and sensitivity. The extracted ion chromatograms for m/z 722.32, show that the peak width is 0.1 minute, half of the peak width for the 5 µm particle packed column (lower trace). The observed ion signal for both the base peak and extracted ion chromatograms is 2-3 times higher for the 1.9 µm particle packed column.

iii) Arabidopsis thaliana digest

Figure 4. Base peak chromatograms for arabidopsis thaliana digest using 5 µm (upper) and 1.9 µm (lower) columns

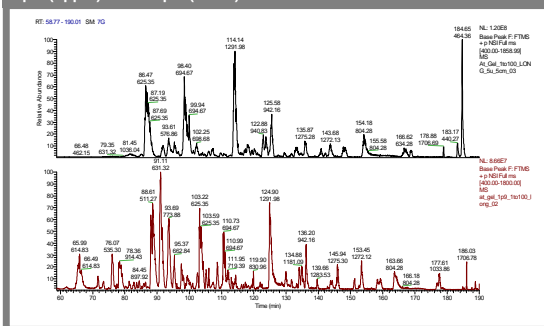


Figure 4 above compares the performance of the 1.9 µm and 5 µm particle packed columns over a long (150 minute) gradient run for a complex plant digest, *arabidopsis thaliana*. While peak intensities are similar for the two columns, the sharp, narrow peaks delivered by the 1.9 µm particle packed column can provide additional information over the 5 µm particle packed column.

Figure 5. Three repeat analysis of the complex plant digest, 35-180 minutes

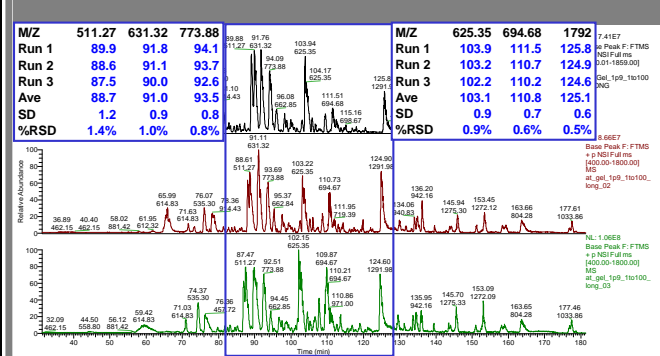


Figure 5 above shows the reproducibility of three repeat long gradient runs using the 1.9 µm particle packed column. For the most abundant ions observed in the base peak chromatogram (those eluting between 85 and 125 minutes), the reproducibility in retention times was observed to be typically 1.5 % RSD or better, as shown in the two insets.

Conclusions

In these preliminary investigations, the PicoFrit column packed with 1.9 µm Hypersil GOLD particles exhibited superb peak width and peak shape for protein digest samples of both low and high complexity. In comparison with the equivalent 5µm particle packed columns, the 1.9 µm particle packed column offer:

- Improvements in intensity and sensitivity
- Narrower peaks, allowing for increased peak capacity and the potential for the identification of a greater number of peptide fragments.

Additional Information

For additional information, please browse our chromatography resource centre, which can be accessed at www.thermo.com/columns

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