Evaluation of 150 µm ID Packed-tip Columns for Quantitative Peptide Analysis by LC-MS/MS

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Introduction

A predominant workflow for qualitative proteomics has been "GeLC-MS," a combination of 1- (or 2-D) gel electrophoresis with reverse-phase nanoflow liquid chromatography mass spectrometry (nLC-MS/MS). The limited protein quantity isolated from a single gel band coupled with column loading capacity maximums necessitate the use of 75 µm ID packed columns for optimal sensitivity. However, limitations on sample injection volume, gradient and flow characteristics, and excessive delay volume hinder throughput. Novel methods for fractionating complex biological samples with higher loading capacities and more efficient recovery, such as novel solution phase tube-gel fractionation and others, demand a column format which maximizes the extended dynamic range of these emerging techniques. Packed-tip columns with a larger ID (150 µm to 200 µm) facilitate higher sample loading capacity and enable higher flow rates for improved cycle time while maintaining the optimal sensitivity realized in the nanobore packed-tip column format. Using peptide standards, single protein digests and whole yeast digests improvements in cycle time and sample loading capacity using 150 µm ID packed-tip columns are demonstrated.

FIGURE 1 HOW MUCH PROTEIN CAN I LOAD?

There are three distinct measures of capacity for loading sample onto an RP-HPLC column

- Optimal capacity
  - Analytical separations
  - Consistent peak width
  - Excellent resolution
- Practical capacity
  - Preparative separations
  - Good peak shape
  - 10 – 50X optimal capacity
- Maximum capacity
  - Purification

Column Diameter (µm)	Flow Rate (µL/min.)	Sample Capacity (µg)
75	.25	.05
150	1.00	.20
300	5.00	1.00
500	10.00	2.00

Table showing optimal flow rates and sample capacity for capillary columns. The sample capacity in µg is the quantity of polypeptide which can be loaded onto a column without compromising the resolution, peak shape and peak width.

Adapted from David Carr *The Handbook of Analysis and Purification of Peptides and Proteins by Reversed-Phase HPLC*, 3rd ed., Hesperia, CA: Grace Vydac Technical Support Group, 2002.

Methods & Materials

Instrumentation

- 3-D ion-trap mass spectrometer (LCQ Deca, Thermo Fisher)
- Customized nanospray source (Digital PicoView, New Objective)
- nano LC-2D pump (Eksigent)
- Autosampler (Leap HTC Pal) equipped with 6-port micro-valve (VICI) containing 1.0 µl loop (for BSA standard) and 2.0 µl loop (for GelFree samples)

Columns

- # 1 - PicoFrit column (360µm OD x 75µm ID x 15µm tip) packed with 10 cm ProteoPep II (5µm, 300 Å, C18, New Objective)
- # 2 - PicoFrit column (360µm OD x 150µm ID x 15µm tip) packed with 10 cm ProteoPep II (5µm 300 Å, C18, New Objective)

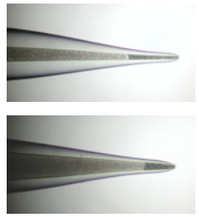
Reagents

- 500 µg Digested yeast lysate (Fluka) fractionated using an 8100 GELFrEE system (Protein Discovery)
- BSA Digest (MassPrep, Waters)
- 0.1% Formic Acid in Water (JT Baker)
- 0.1% Formic Acid in Acetonitrile (JT Baker)

Conditions

- Gradient: 30 minutes 2-50% B
  Mobile Phase A = 0.1% Formic Acid in Water
  Mobile Phase B = 0.1% Formic Acid in Acetonitrile
- Flow rate: 250 nl/min (75 µm ID PicoFrit) or 1000 nl/min (150 µm ID PicoFrit)
- On-column injection: variable concentrations

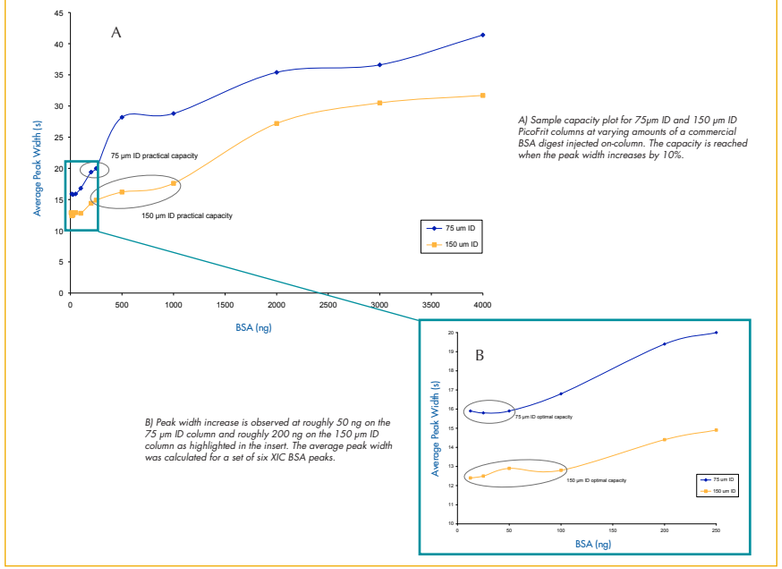
FIGURE 2



(top) 75 µm ID x 15 µm tip PicoFrit column

(bottom) 150 µm ID x 15 µm tip PicoFrit column

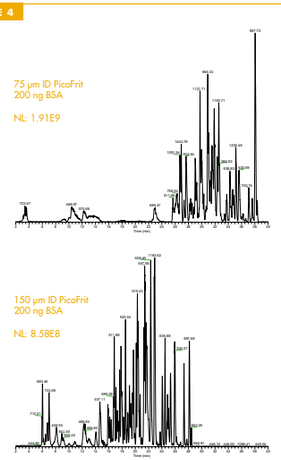
FIGURE 3 COLUMN CAPACITY: Practical/Optimal Capacity



A) Sample capacity plot for 75µm ID and 150 µm ID PicoFrit columns at varying amounts of a commercial BSA digest injected on-column. The capacity is reached when the peak width increases by 10%.

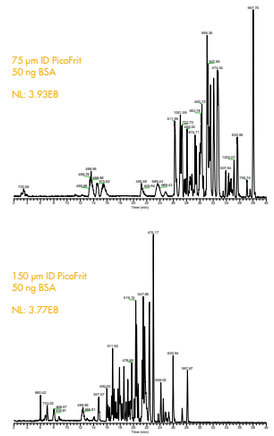
B) Peak width increase is observed at roughly 50 ng on the 75 µm ID column and roughly 200 ng on the 150 µm ID column as highlighted in the insert. The average peak width was calculated for a set of six XIC BSA peaks.

FIGURE 4



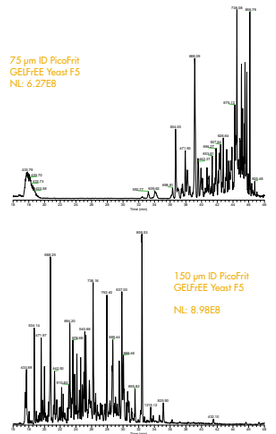
Chromatographic comparison of 75 µm ID vs. 150 µm ID PicoFrit column at 200 ng on-column injection of BSA. The increased flow rate used for the 150 µm ID column (1000 nl/min) demonstrates a definitive advantage in cycle time.

FIGURE 5



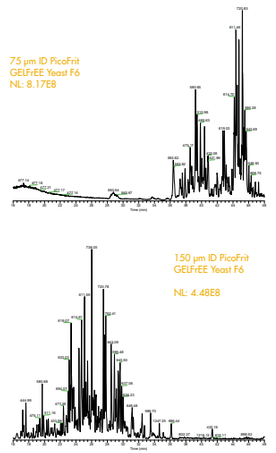
Chromatographic comparison of 75 µm ID vs. 150 µm ID PicoFrit column at 50 ng on-column injection of BSA

FIGURE 6



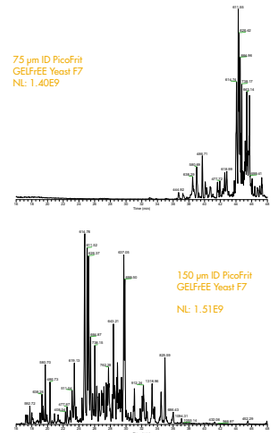
Chromatographic comparison of 75 µm ID vs. 150 µm ID PicoFrit column of GELFrEE Yeast F5

FIGURE 7



Chromatographic comparison of 75 µm ID vs. 150 µm ID PicoFrit column of GELFrEE Yeast F6

FIGURE 8



Chromatographic comparison of 75 µm ID vs. 150 µm ID PicoFrit column of GELFrEE Yeast F7

Conclusions

- The practical capacity of 150 µm ID PicoFrit column was determined to be 1000 ng for a BSA digest; 4X the practical capacity of a 75 µm ID PicoFrit column
- A 20% decrease in RT on a 150 µm ID PicoFrit column relative to a 75 µm ID PicoFrit column was observed, indicating improved cycle time for this format
- Using GelFree purified yeast lysate fractions:
  - Equivalent peak capacity on the 150 µm ID PicoFrit format relative to the 75 µm ID PicoFrit format was observed
  - Chromatographic quality was improved using the 150 µm ID PicoFrit
  - The 150 µm ID PicoFrit column demonstrated a 30% reduction in runtime

Future Work

- Evaluate 200 and 250 µm ID PicoFrit column formats
- Investigate 150 µm ID PicoFrit benefits in a quantitative workflow
- Evaluate the performance of 150 µm PicoFrit column format relative to other commercially available microbore columns

Acknowledgements
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