Increased Sample Loading Capacity For Peptide Analysis by LC-MS/MS Using 150 µm ID Packed-Tip Columns

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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 (LC-MS/MS). The limited protein quantity isolated from a single gel hand 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 delays volume lowering 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 nanoflow packed-column format. Using peptide standards, single protein digests and whole yeast digest improvements in cycle time and sample loading capacity using 150 µm ID packed columns are demonstrated.

Methods & Materials

- **Introduction**
- **Materials**
- **Methods**
- **Results & Discussion**

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

**Columns**
- 75 µm ID PicoFrit column (75 µm OD x 15 µm tip PicoFrit)
- 150 µm ID PicoFrit column (150 µm OD x 15 µm tip PicoFrit)

**Reagents**
- 500 µg Digested yeast lysate (Fluka) fractionated using an IL 3000 CE/GE system (Proteome Discovery)
- BSA Digest (MassPrep, Waters)
- 0.1% Formic Acid in Water (FT Baker)
- 0.1% Formic Acid in Acetonitrile (FT 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 nll/min (75 µm ID PicoFrit) or 1000 nll/min (150 µm ID PicoFrit)
- On-column injection: variable concentrations

**Chromatographic Comparison**

**Figure 1**

**Figure 2**

**Figure 3**

**Figure 4**

**Figure 5**

**Figure 6**

**Figure 7**

**Figure 8**

**Conclusion**

- The practical capacity of 150 µm ID PicoFrit column was demonstrated 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 GeLC 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 130 µm ID PicoFrit.
  - The 150 µm ID PicoFrit column demonstrated a 30% reduction in runtime.

**Future Work**

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