**Exploring microLC/MS for Accelerating Peptide Quantitation Assays**

**ABSTRACT**

In this poster we will show how switching from nanoLC/MS to microLC/MS can improve throughput significantly for sensitive peptide quantitation.

**INTRODUCTION**

A common view of the protein/biomarker research pipeline is that one starts with high sensitivity global discovery at nanoflow rates and then proceeds to higher throughput targeted quantitation or discovery at microflow rates to control or reduce their ultimate utility. Many more samples must be analyzed with increased throughput and robustness, which means increased sensitivity and reproducibility. For high throughput analysis, sensitivity may decrease due to reduced ionization efficiency compared to nanospray ionization. There has been increased interest in working in the microflow regime (0.5 μL/min) to obtain a good balance between throughput, robustness and sensitivity. In this study, we will introduce microLC methods for high throughput, high sensitivity peptide quantitation.

**MATERIALS AND METHODS**

**HPLC**

An Eksigent microLC 200 or nanoLC 425 system in micro-mode was used. All nano-LC experiments were performed using the second gradient or loading pump that is part of the nanoLC-425 system. Mobile phase A was water with 0.1% formic acid and B was acetonitrile with 0.1% formic acid. An Eksigent HPLC column was used for the nanoLC experiments. See text and figures for columns, gradients, flow rates and injection volumes used.

**MS**

A 5600 system with Turbo V™ source or AB SCIEX QTRAP® 5500™ TripleTOF® 5600 system with Turbo V™ source or NanoSpray® II source was used. The electrode in the Turbo V™ source was changed to a microLC hybrid electrode with 25 μm ID (Eksigent). Data was processed with MultiQuant™ AB SCIEX software to perform quantitation and statistical analysis. Sample

Sample Preparation and Analysis

Samples were used as a beta-Galactosidase (BG) tryptic digest (AB SCIEX), a synthetic peptide standard with isotopically labeled peptides (CYP450 assay, AB SCIEX), a six protein mix digest (McDonald Bioresources), Bradykinin (Sigma) and CANP (CYP450 labeled tryptic peptides (AB SCIEX) was analyzed with 3 MRM transitions per peptide. The MRM chromatogram for the 10 peptides from the 75 μm ID column was averaged across the 10 peptides monitored. These results should provide a rough guide as to when to try to decrease which column flow rate will be best suited for the sample. See text and figures for columns, gradients, flow rates and injection volumes used.

**RESULTS**

**A comparison of the relative sensitivity between nanoLC/MS and microLC/MS was made using a selection of ten tryptic peptides from the Michrom six protein mix digest.** We compared both 75 μm and 200 μm ID chip nanoLC columns with 0.3 and 0.5 mm ID columns. Sensitivity only decreased by a factor 4 from a 75 μm ID chip column run at 350 nL/min to a 0.5 μm ID chip microLC column at 10 nL/min. This difference could be increased by almost a factor 2, because of less delay time and less post-column dispersion.

**Table 1. Comparison of Sensitivity Differences using different column IDs and flow rates.** The LLOQ difference for each peptide at each column diameter was computed relative to the LLOQ for the 75 μm ID column, then this difference was averaged across the 10 peptides monitored. These results should provide a rough guide as to when to try to decrease which column flow rate will be best suited for the sample.

**Figure 1. Comparison of the MRM Traces at the Lowest and Highest Flow Rate.** The MRM chromatogram for the 10 peptides monitored was obtained using a 15 min linear gradient, peptides are observed from t= 16 – 18 mins (top). At the highest flow rates, faster gradients can be used. Analyzing the same peptides on a 500 μm ID column shows a small decrease in sensitivity but an increase in throughput (7 minute linear gradient). However equivalent resolving power was obtained.

**High Throughput with Trap-Elute**

As a final example we developed a fast trap-elute method for the quantitation of Exanatide, a 39 amino acid peptide drug for the treatment of type 2 diabetes. A 0.5 x 5 mm column packed with 3 μm C18 phase (Dionex AB SCIEX) was analyzed with a 5 μL chip trap 5 μL C18 (Thermo Scientific). Flow rate for the separation was 40 μL/min with a gradient from 5-90%B in 1.5 min, while sample loading was performed at 50 μL/min for 1.5 min. 50 μL of sample was loaded and the trap was washed off in an equilibration step before the separation. Additional advantages is that any salts and other components not retained on the trap columns are washed away to waste, and can't contaminate the separation column and/or MS. The set-up is illustrated in Figure 4 below. Figure 5 illustrates the sensitivity gain that can be achieved by loading larger sample volumes of Bradykinin. IS spiked plasma samples were analyzed using an Eksigent 0.3 mm x 15 cm C18 3 μm column (Eksigent) operated at 4.2 μL/min and a 0.2 μm 10 mm C18 (Dr. Maech, Germany) loaded at 20 μL/min.

**REFERENCES**


**TRADEMARKS/LICENSES**

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