Exploring the Sensitivity Differences for Peptide Quantification in the Low Flow Rate Regime

*NanoLC™ 400 System for High Performance Nanoflow and Microflow LC*

Christie Hunter, Xiang Zhu, Remco van Soest

*SCIEX, USA*

A commonly held view of the protein / biomarker research pipeline is diagrammed in Figure 1. Typically, high end mass spectrometers are used to perform non-targeted discovery experiments where the goal is to quantitatively profile large numbers of proteins across small numbers of samples to find protein targets (blue). In the next phase, this subset of proteins is analyzed across a larger set of samples. The outcome of this verification step (green) is hopefully a small subset of very promising protein markers that are then taken forward to perform large scale validation across a much larger sample size (orange), in hopes to find a small panel that provides the desired read-out.

While much focus has been on the mass spectrometry innovations, the importance of the separation component for getting the highest quality data cannot be underestimated. The sensitivity, robustness and throughput of the LC strategy must also evolve as research progresses through the pipeline. The nanoflow regime is used extensively for high sensitivity discovery experiments but more recently life science researchers are exploring the use of the microflow regime for increased throughput and robustness for quantification. The sensitivity differences between nanoflow and microflow rates are explored here with the goal of establishing general sensitivity guidelines.

**Key Features of the NanoLC™ 400 System for Quantitative Proteomics**

- The NanoLC 400 system\(^1\) has the flexibility and reliability to support a broad range of workflows, from global discovery to targeted quantitation.
- Easy to change flow module cartridges allow the user to rapidly switch between flow rate ranges\(^2\).
- MFCPlus™ Technology provides flow stability for highest retention time reproducibility, with retention time variability below 0.35% RSD at 500 nL/min.
- Ultra-high pressure (10 000 psi) for high resolution separations.
- High precision autosampler enables excellent injection reproducibility with little or no sample waste.

**Figure 1. Protein / Biomarker Research Pipeline.** The NanoLC 400™ System has the flexibility and reliability to support a broad range of LC-MS workflows, from high sensitivity global discovery to high throughput targeted quantitation.
Methods

In this work, four different column diameters were assessed, all containing the ChromXP C18CL (120Å, 3 μm) reverse phase media and all 15 cm in length:

- 75 μm cHiPLC® column, 300 nL/min
- 200 μm cHiPLC® column, 1 μL/min
- 300 μm column, 4 μL/min
- 500 μm column, 10 μL/min

Experiments were done using MRM analysis on the QTRAP® 5500 system. Concentration curves were analyzed and the lower limits of quantitation (LLOQs) were measured for each peptide on each column to compare impact of flow rate and column diameter on sensitivity. LLOQ is defined as lowest point on concentration curve with <20% CV precision and accuracy between 80 and 120% and S/N >10.

Ten unlabeled tryptic peptides (6 Protein Mix, Michrom Bioresources) were used for this sensitivity assessment in order to establish some general sensitivity guidelines (Figure 2). The matrix used was a simple matrix so the sensitivity differences could be measured with minimal impact from interferences or ion suppression. The matrix was a Serum Albumin tryptic digest (Michrom Bioresources) at 10 fmol on column, held constant across the concentration curve. Standard concentration curves were analyzed using MultiQuant™ Software and LLOQs were computed for each peptide on each column to compare impact of flow rate and column diameter on sensitivity. The concentration range measured for each column diameter tested was 62.5 fmol down to 1.9 amoles of peptide standard.

Exploring Sensitivity Differences at Different Column Diameters

This experiment was straightforward to do with the NanoLC™ 400 system as switching between flow regimes takes minutes due to the easily exchangeable flow modules. The Nano flow module was used for the 75 and 200 μm ID columns, along with the NanoSpray® Source. For the 300 and 500 μm ID columns, the Low Microflow module was used, along with the Turbo V™ Source with the 25 μm ID hybrid electrode.

The LLOQ for each peptide was measured on each of the different columns using MRM analysis on a QTRAP® 5500 system. A set of 10 different tryptic peptides was used such that an average sensitivity difference could be determined. An example of the data acquired is shown in Figure 3, for the peptide VGDANPALQK from carbonic anhydrase. A concentration curve was run across a wide range of concentrations, the curve was assessed for linearity and the MRM signal for this peptide at the LLOQ of 3.8 amol on column is shown in the top right.
reproducibility and the LLOQ was determined to be 3.8 amol on column for the 300 µm ID column.

Table 1 shows the average increase in LLOQ (or the decrease in sensitivity) relative to running a 75 µm ID column at 300 nL/min for each column diameter. For the 200 µm ID column running at 1 µL/min, a ~2.5 fold decrease in sensitivity or increase in LLOQ was observed. Moving to the 300 µm ID column at 4 µL/min on the Turbo V™ Source, a 3x difference in sensitivity was seen. Finally, 4x differences in sensitivity was observed when compared to a 500 µm ID column at 10 µL/min as compared to the same experiment on a 75 µm ID column. Therefore, as flow rate increases, increased robustness and throughput can be obtained with just a small decrease in sensitivity. Obviously with the larger column diameters, more sample can often be loaded if available to offset this sensitivity difference.

As these averages are for only 10 different tryptic peptides, these relative differences should be used as a guidance for deciding on the right column diameter for the planned experiment.

Conclusions

The flexibility and reproducibility of the NanoLC™ 400 system makes it an excellent LC system for labs performing proteomics workflows, from high sensitivity protein expression analysis to high throughput peptide quantitation.

- User exchangeable flow modules enables both nanoflow experiments and microflow experiments to be used as the workflows demand
- Microflow experiments can be performed with higher throughput and robustness with a small decrease (~4x) loss in sensitivity
- The fast, high precision autosampler provided accurate, reproducible sample injections (<1% CV) even in µL-pickup mode with speeds compatible with high throughput microflow experiments.

References

2. Configuring the nanoLC™ 425 System for Both Nanoflow and Microflow LC-MS Workflows - Brief Instructions and Consumables List. SCIEX Technical Note RUO-MKT-02-7254-A.