Significant gains in quantitative sensitivity using microflow chromatography

Comparing the SCIEX Triple Quad™ 7500 System – QTRAP® Ready to the QTRAP® 6500+ LC-MS/MS System

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Increasingly, microflow chromatography is being adopted in applications because of the "sweet spot" it occupies between increased sensitivity and solid robustness. Reducing the flow rate from analytical flow to microflow provides significant sensitivity gains while maintaining fast run times and robustness.[1,2] Increasing the flow rate from nanoflow rates to microflow does cause a reduction in sensitivity, but more sample is often available to account for this — and the ease of use and throughput improvements are significant.[3]

This led SCIEX to continue to evolve the Turbo V™ Ion Source into the OptiFlow® Pro Ion Source, a single source that would cover the full spectrum of flow rates for LC-MS analysis. The modular design of the source allows rapid switching with no downtime. The optimized probe and electrode design ensures that the high performance is achieved with minimal user tuning.[4] The ability to easily switch between flow regimes was also a critical part of the source design. Removing the barrier of

switching helps researchers choose the right flow rate for every project.

Here, the sensitivity of microflow on the SCIEX Triple Quad 7500 System was compared to microflow on the QTRAP 6500+ System, to characterize the sensitivity gains for this increasingly important flow regime. Peak area gains were observed for all the compounds tested, with an average area gain of around 8.5 fold (Figure 1).

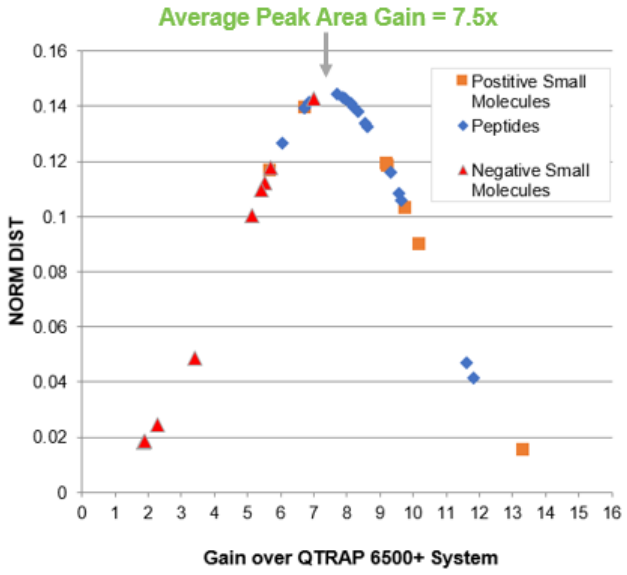

Figure 1. MRM peak area gains for microflow on the SCIEX Triple Quad 7500 System. Analysis of a small set of small molecules and peptide analytes on both the SCIEX 7500 System compared to the QTRAP 6500+ System showed significant gains in MRM peak areas with an average gain of ~7.5 fold.

Key features of the SCIEX 7500 System for microflow chromatography

- Sensitivity gains over the previous generation of instruments have been observed on the SCIEX Triple Quad 7500 System – QTRAP Ready[5]
- Similar sensitivity gains were also observed when performing microflow chromatography, with peak area gains of ~7.5 fold (Figure 1)
- OptiFlow Pro Ion Source provides high flexibility to meet diverse application needs with a single source solution
- Modular design allows fast switching between high and low flow regimes (Figure 2)
- Integrated E Lens™ Technology for microflow improves ESI droplet desolvation and provides more efficient ion collection
- High performance achievable across the flow rate range with very little need for tuning by user[4]

Methods

Sample preparation: Rat plasma was crashed with acetonitrile (2:1, acetonitrile/plasma). The sample was mixed and then centrifuged at 16,000 rpm for 6 minutes to pelletize the protein. The supernatant was collected and diluted 5 fold with water to use as the matrix.

A panel of small molecules was dissolved in methanol and mixed together to make a 10 µg/mL stock solution. This solution was diluted to create a concentration curve, which was then diluted 10x with crashed plasma to create the final solutions with matrix (covering the concentration range of 0.001 to 2500 pg/mL).

PepCalMix (a mixture of 20 different tryptic peptides, SCIEX) was diluted in 5% acetic acid, 2% acetonitrile in 0.1% formic acid to create a 10 fmol/µL stock solution. This was then diluted into simple matrix to create a standard concentration curve.

Chromatography: Separations were performed using a NanoLC™ 425 System (SCIEX) operating in low microflow range and controlled by contact closure.[6] The flow rate was 3 µL/min. The gradients used are outlined in Table 1.

Table 1. LC gradients used for the analyte sets tested.

Small molecule - positive ion mode		Peptides – positive ion mode		Small molecule - negative ion mode	
Time (min)	%B	Time (min)	%B	Time (min)	%B
0	*10*	*0*	*3*	*0*	*10*
1.75	*97*	*2*	*25*	*0.5*	*70*
3	*97*	*3.5*	*80*	*2*	*97*
3.1	*10*	*5.5*	*80*	*3*	*97*
4.5	*10*	*5.6*	*3*	*3.1*	*10*
		6	*3*	*4.5*	*10*

Mobile phase A - 0.1% formic acid in water
Mobile phase B - 0.1% formic acid in acetonitrile

Mass spectrometry: Small molecules and peptides were analyzed under very similar microflow LC conditions on both the SCIEX 7500 System and the QTRAP 6500+ System. Source conditions and some key instrument specific parameters were optimized between platforms.

Data processing: Data was processed using SCIEX OS Software 2.0 using both the Explorer and Analytics modules.

Figure 2. Switching from analytical flow to microflow. The modular nature of the source allows the flow regime to be switched rapidly, simply by changing the ESI probe and E Lens Technology to the microflow versions.

Small molecule quantification

A suite of small molecule compounds were used to benchmark the sensitivity gains between the SCIEX Triple Quad 7500 System – QTRAP Ready and the QTRAP 6500+ System in the

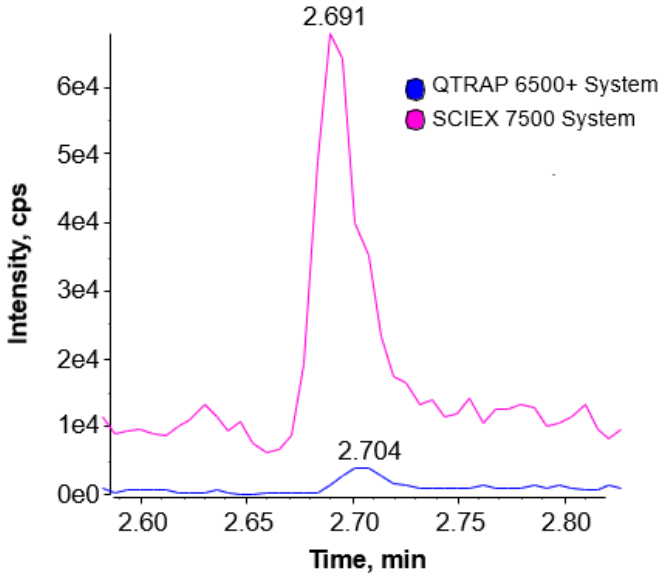

Figure 3. Peak area gains for tapentadol. (Blue) MRM signal for tapentadol at the LLOQ obtained on QTRAP 6500+ System. A peak area gain of about 9 fold was obtained for the same concentration on the SCIEX 7500 System (pink).

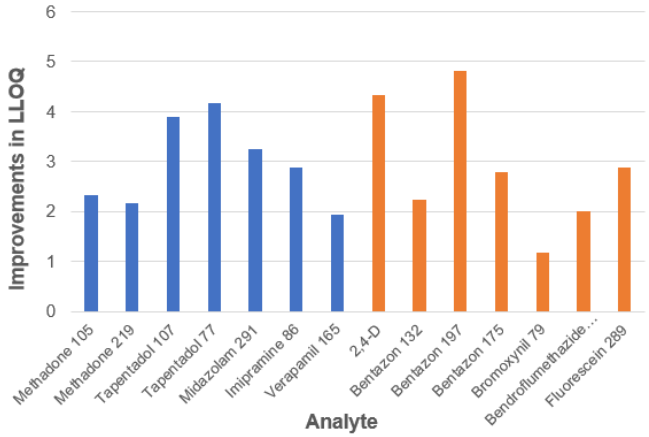

Figure 4. Improvements in lower limit of quantifications for small molecules. Lower LOQs were observed on the SCIEX 7500 System than on the QTRAP 6500+ System due to the higher sensitivity of the instrument. The LLOQs compared were the average of LLOQs determined from 3 systems of each type. An average improvement in LLOQ of 2.9 was observed.

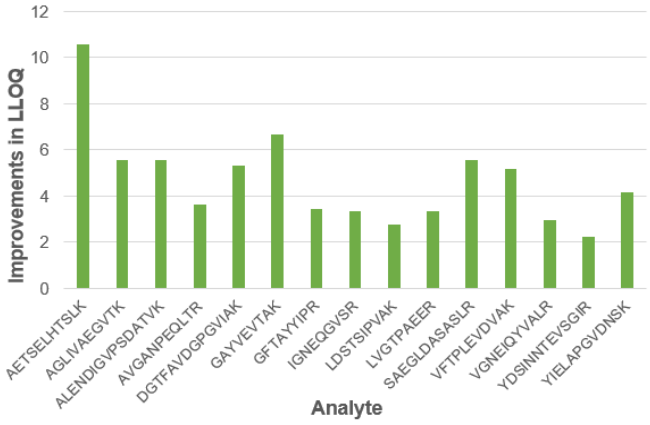

Figure 5. Improvements in lower limit of quantifications for peptides. Lower LOQs were observed on the SCIEX 7500 System than on the QTRAP 6500+ System due to the higher sensitivity of the instrument. LLOQs compared were the average of LLOQs determined from 3 systems in simple matrix. An average improvement in LLOQ of 4.6 was observed.

microflow chromatography regime. Three systems of each type were used to ensure a robust sensitivity comparison was obtained. Source and compound dependent parameters were optimized on each system to ensure best sensitivity on each instrument. Collision energies between the two platforms were optimized to the same values for each compound. Source settings did vary between platforms due to the differences between the OptiFlow Pro Ion Source and the IonDrive™ Turbo V Source. Calibration curves were run across a broad range of concentrations in protein-precipitated rat plasma, to compare the observed peak areas as well as determine the lower limits of quantification for the range of compounds.

Lower limits of quantification were compared for these small molecules in crashed rat plasma between the SCIEX 7500 System and the QTRAP 6500+ System (Figure 4), an average improvement in LLOQ of 2.9 was observed.

Peptide quantification

Similar experiments were performed on a set of tryptic peptides in simple matrix to determine typical sensitivity gains for peptides at microflow rates between the SCIEX Triple Quad 7500 System – QTRAP Ready and the QTRAP 6500+ System. Again concentration curves across a broad range of concentrations were run to compare both the peak areas (Figure 6) and the

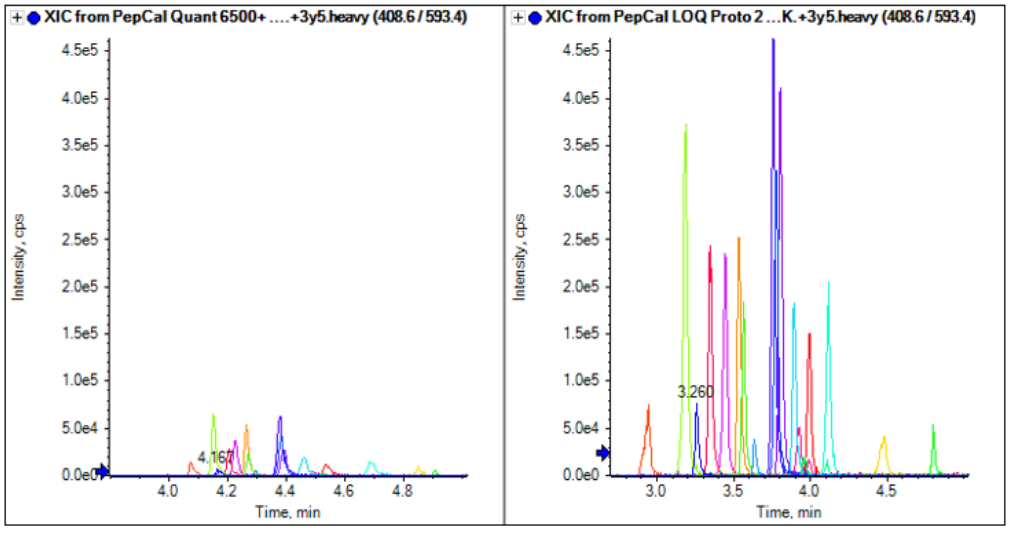

Figure 6. Peak area gains for peptides using microflow chromatography. (Left) MRM signal for PepCalMix at 100 amol on column obtained on a QTRAP 6500+ System. A peak area gain of about 8.7 fold was obtained for the same concentration on the SCIEX 7500 System (Right).

LLOQs (Figure 5). An average improvement for the SCIEX 7500 System over the QTRAP 6500+ System was a 4.6 fold lower LLOQ across the 15 peptides monitored.

Conclusions

The quantification workflows for peptides and small molecules were explored on the SCIEX Triple Quad 7500 System – QTRAP Ready, to characterize the sensitivity improvements over the previous generation of instruments. Calibration curves were run on three SCIEX 7500 Systems and three QTRAP 6500+ Systems, both with microflow LC, and the LLOQs and peak areas were compared. Average peak area gains of about 7.5 were observed across the set of compounds analyzed (Figure 1). LLOQ improvements of 2.9 for small molecules and 4.6 for peptides were observed for the SCIEX 7500 System. In all, this work demonstrates that the improvements in the SCIEX 7500 System also provide sensitivity gains in the microflow regime.

References

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