Improved Sensitivity for LC-MS Quantitation of Pharmaceutical Compounds in Human Plasma with MicroLC Using a New Microflow Source Design

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ABSTRACT

Microflow liquid chromatography has gained substantial popularity among the analytical community where ultrahigh sensitivity, high throughput, and operational cost reduction have been constant challenges. This study illustrates the capability of a SCIEX® QTRAP® 6500+ LC/MS/MS system with an OptiFlow™ Turbo V Ion Source with SteadySpray™ probe for the quantitation of pharmaceutical compounds using microflow LC. The comparison of LC-MS quantitation between microflow and analytical flow show an improvement in sensitivity for the small molecules studied.

INTRODUCTION

The drug discovery and development process requires robust and reliable pharmacokinetic data. With the increased potency of newer drugs sensitive and specific bioanalytical methods are essential. Because the volume of blood drawn from a small animal during DMPK studies is limited, there is pressure to extract more information from the same sample. Therefore, sensitivity is critical for success. In recent years, microflow liquid chromatography has gained substantial popularity among the analytical community because significant sensitivities gains have been observed for some compounds. Published methods using microflow have reported advantages over analytical flow chromatography, which include up to 14 times sensitivity gains, reduced source contamination and reduced solvent consumption, as well as lowered waste handling cost. Combining microflow LC with MS/MS detection enables more selective and sensitive assays because lower LLOQs are possible. In this poster, we describe sensitivity gains for several pharmaceutical molecules that were achieved using microflow LC combined with a new ESI source for the analysis.

MATERIALS AND METHODS

Software:
Analyst® 1.7 software with Hotfix 2 was used for data acquisition. MultiQuant™ 3.02 software was used or data analysis.

Sample Preparation:
Protein precipitation of rat plasma was performed using 2:1 acetonitrile:plasma. The mixture was vortexed for 15 seconds and then centrifuged at 15,000 rpm for 10 minutes. The supernatant was then diluted 5 fold with water. A selection of pharmaceutical molecules were spiked into the diluted crashed plasma. Isotopically labelled internal standards were used for the majority of the compounds.

HPLC Conditions:
Analytical flow: A Shimadzu Prominence LC system with a Phenomenex Kinetex C18, 50 x 2.1 mm column, 1.7µm was used. The column was kept at 35° C in the column oven. A gradient of water with 0.1% formic acid (eluent A) and acetonitrile with 0.1% formic acid (eluent B) at a flow rate of 500 µL/min was used. The injection volume was set to 2µL.

Microflow: An Eksigent ekspert nanoLC 425 system with a 1-10 µL/min gradient flow module and a Supelco Ascentis Express C18, 50 x 0.2 mm column, 2.7µm, was used. The column was kept at 35° C in the integrated column oven. A gradient of water with 0.1% formic acid (eluent A) and acetonitrile with 0.1% formic acid (eluent B) at a flow rate of 3µL/min was used. A 2µL full loop injection was utilized.

MS Conditions:
Eight different compounds were detected using positive ion MRM on a Sciex QTRAP 6500+ LC/MS/MS system. The mass spectrometer was equipped with 2 different sources. The MS source parameters (ISV, TEM, GS1 and GS2) were optimized for each source (Table 1). The CE, EP, DP and CXP values were kept constant for both the analytical and microflows.

Analytical flow: An Ion Drive Turbo V™ source with an Electrospray Ionization (ESI) probe was used. The protrusion the electrode and position the ESI probe was optimized carefully to give the best possible sensitivity for the compounds.

Microflow: A prototype OptiFlow Turbo V Ion Source with SteadySpray probe, a low microflow electrode and an integrated column heater were used (Figure 1). The electrode protrusion and position of the SteadySpray probe were not adjusted.

Table 1. The source conditions used for both the Ion Drive Turbo V (analytical flow) and the OptiFlow Turbo V (microflow).

Source Parameters	Analytical flow	Microflow
Curtain Gas	25	25
Collision Gas	High	High
IonSpray Voltage	4500 V	4500 V
Temperature	700°C	200°C
Gas 1	65	15
Gas 2	65	65

Figure 1. An OptiFlow Turbo V source with integrated column heater.

RESULTS

In this study, calibration curves were run back to back. The samples were first on the analytical flow configuration followed by the microflow configuration. The samples were run with a minimum of three replicate injections and at the lower end of the calibration curve five replicates injections were acquired.

The comparison of LC-MS quantitation between microflow and analytical flow show an improvement in sensitivity for the small molecules studied. The sensitivity gains between analytical and microflow results were compound dependent (Table 2 and Figure 2). These improvements in assay performance were achieved with microflow on an ESI source designed for ease of use and optimal sensitivity. The OptiFlow Turbo V source was designed for best spray conditions and no manual adjustment of the electrode protrusion and probe position was necessary to get optimal sensitivity.

Table 2. Average sensitivity gains (peak area and signal to noise) across several concentrations observed between microflow LC and analytical flow.

Compound	Ave. Peak Area Gain	Ave. Signal to Noise Gain
Naproxen	16	15
Haloperiodol	4.6	1.5
Alprazolam	5.5	22
α-Hydroxy-Alprazolam	9	8
Buprenorphine	6.5	3.3
Dextromethorphan	3.3	3.5
Imipramine	2.8	1.5
Propranolol	1.4	1.5

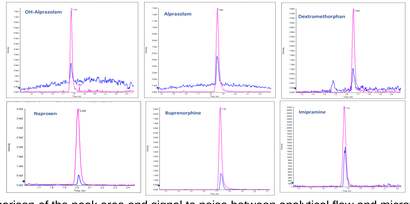


Figure 2. Comparison of the peak area and signal to noise between analytical flow and microflow assays. The two chromatograms are aligned to the analytical flow retention time. The analytical flow assay (blue trace) was performed at 500 µL/ min (2.1mm i.d. column). The microflow assay (pink trace) was performed at 3 µL/min (0.2mm i.d. column).

The sensitivity improvements achieved using the microflow LC method translated into an improved LLOQ for most compounds. Figure 3 compares the data collected for alprazolam near the LLOQ for the respective flow conditions. There is a dramatic improvement in the LLOQ for alprazolam using the OptiFlow Turbo V source. In this study, we also collected data for a metabolite of alprazolam (α-hydroxy-alprazolam). Similar results were also observed (Figure 4).

The calibration curves generated had good linearity for both analytical and microflows. Figures 5 and 6 show the calibration curves achieved for alprazolam and naproxen. The linearity, LLOQ and the precision and accuracy at the LLOQ for both flow rates is also provided.

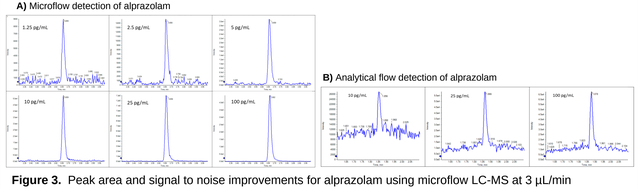


Figure 3. Peak area and signal to noise improvements for alprazolam using microflow LC-MS at 3 µL/min versus analytical flow LC-MS at 500 µL/min.

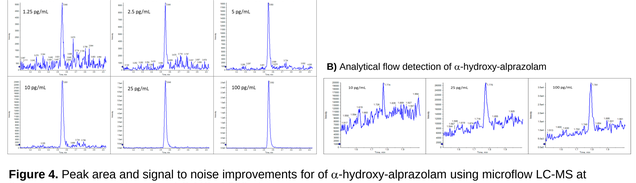
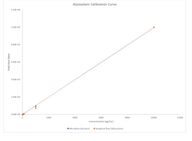
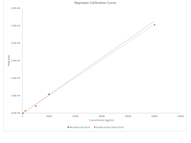


Figure 4. Peak area and signal to noise improvements for of α-hydroxy-alprazolam using microflow LC-MS at 3 µL/min versus analytical flow LC-MS at 500 µL/min.



Flow rate	r2 (no weighting)	LLOQ (pg/mL)	%CV at LLOQ (N=5)	Accuracy at LLOQ (N=5)
Microflow	0.9982	0.5	8.5	99.7%
Analytical flow	0.9946	5	15.4	106%

Figure 5. Comparison of the calibration curves for alprazolam acquired at analytical and microflows. The table summarizes some of the data calculated from these calibration curves.



Flow rate	r2 (1/x weighting)	LLOQ (pg/mL)	%CV at LLOQ (N=5)	Accuracy at LLOQ (N=5)
Microflow	0.9925	25	10.7	91.8%
Analytical flow	0.9763	100	2.3	79%

Figure 6. Comparison of the calibration curves for naproxen acquired at analytical and microflows. The table summarizes some of the data calculated from these calibration curves.

CONCLUSIONS

Sensitivity and LLOQ improvements were achieved for a selection of pharmaceutical compounds by using microflow LC combined with the OptiFlow Turbo V source with SteadySpray probes on the QTRAP 6500+ LC/MS/MS system. Gains in peak area and signal to noise were achieved using a microflow LC method coupled to an ESI source designed for ease of use and optimal sensitivity.

TRADEMARKS/LICENSING

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Document number: RUO-MKT-10-7866-A