Evaluation of a Novel Micro-Flow Electrospray Ion Source for MS/MS

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Introduction

Responding to the increasing sensitivity requirements for protein and peptide biomarker analysis as well as for mAb and therapeutic protein quantitation by LC-MS/MS or LC-HRMS in biological matrices, a newly developed electrospray micro-flow ion source for the Sciex 6500/6500+ tandem mass spectrometers was evaluated. Besides the sensitivity, attention was paid to robustness and ease of use compared to the original manufacturer source, as well as to the attainable duty cycle times which should ideally be less than 10 minutes. This is usually difficult to achieve with nano-flow electrospray sources or nano-HPLC systems.

The replacement of the electrode on the Turbo V Source to an electrode with an inner diameter of 25 µm reduces peak broadening, but will only gain in sensitivity by a factor of 3 to 4 [1] which is far below the theoretical expectation (factor 44, change from 2 mm ID to 0.3 mm ID columns). In addition to the source, the performance of the HPLC pump and the precision of the gradient is essential.

Ion Spray Source Design

At microliter flow rates (1-20 µL/min), proper ionization has to be supported by a nebulizing gas. The evaluated Prolab Microflow ion source uses an additional spray gas and a heated declustering channel for improved desolvation.

Methodology and Experimental

A therapeutic protein (approximately 30 kDa) was quantified in cynomolgus monkey plasma. The plasma sample was mixed with streptavidin-coated magnetic beads where a biotinylated anti-drug antibody was captured. After incubation of 1 h the beads were washed and subsequently the protein was digested with BrCN in 70 % formic acid for 1 h in the dark. A labelled peptide was added before the digestion step. The digestion mixture was injected directly onto a trapping column.

	Microflow setup	Regular flow setup
Mass Spectrometer	Sciex 6500+	Sciex 6500+
Ion Source	Prolab Microflow	Sciex Turbo V
Loading Pump	Agilent 1290	Agilent 1290
Analytical Pump	Prolab Zirconium Ultra	Agilent 1290
Trapping column	10 x 1 mm ID, C18 (Maisch)	10 x 2.1 mm ID, C18 (YMC)
Analytical column	150 x 0.3 mm ID, Triart C18 (YMC)	50 x 2.1 mm ID, XSelect CSH (Waters)
Mobile phase A	Water (0.5% formic acid)	Water (0.5% formic acid)
Mobile phase B	Acetonitril/2-propanol, 80/20 (0.5% FA)	Acetonitril/2-propanol, 80/20 (0.5% FA)
Trapping	2 minutes loading with 200 µL/min	2 minutes loading with 2 mL/min
Analytical run time	11 minutes	5 minutes
Analytical flow rate	4 µL/min	500 µL/min
Injection volume	10 µL	10 µL

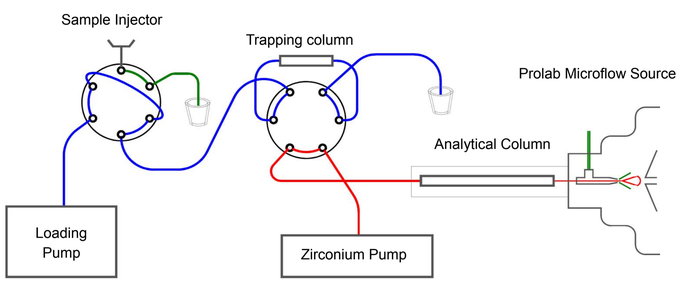

Figure 1: Schematic of the HPLC-MS setup

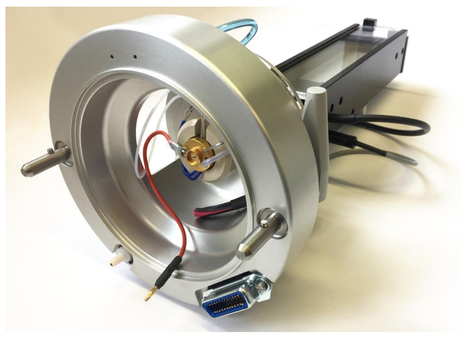

Figure 2: Prolab Microflow Ion Source with column oven

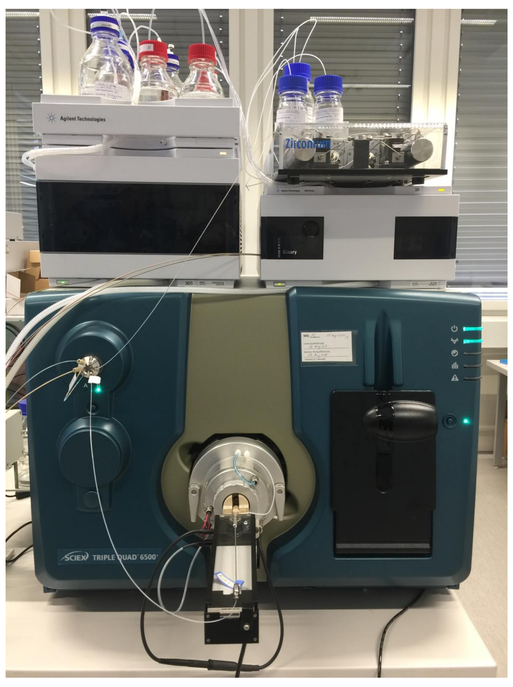

Figure 3: HPLC-MS/MS system with micro-flow setup

Discussion

A robust and highly sensitive assay was developed for the quantitation of a 30 kDa therapeutic protein in cynomolgus monkey plasma. The micro-flow method using Prolab's new Microflow ion source yielded an approximate 30-fold gain in sensitivity compared to the regular HPLC method where a 2.1 mm ID column was used.

With the micro-flow approach, a lower limit of quantitation in the range of 2.50 ng/mL could be achieved with the combination of immunocapturing and BrCN digestion of the protein.

A qualification run was performed, and good precision and accuracy of the method could be demonstrated.

Results

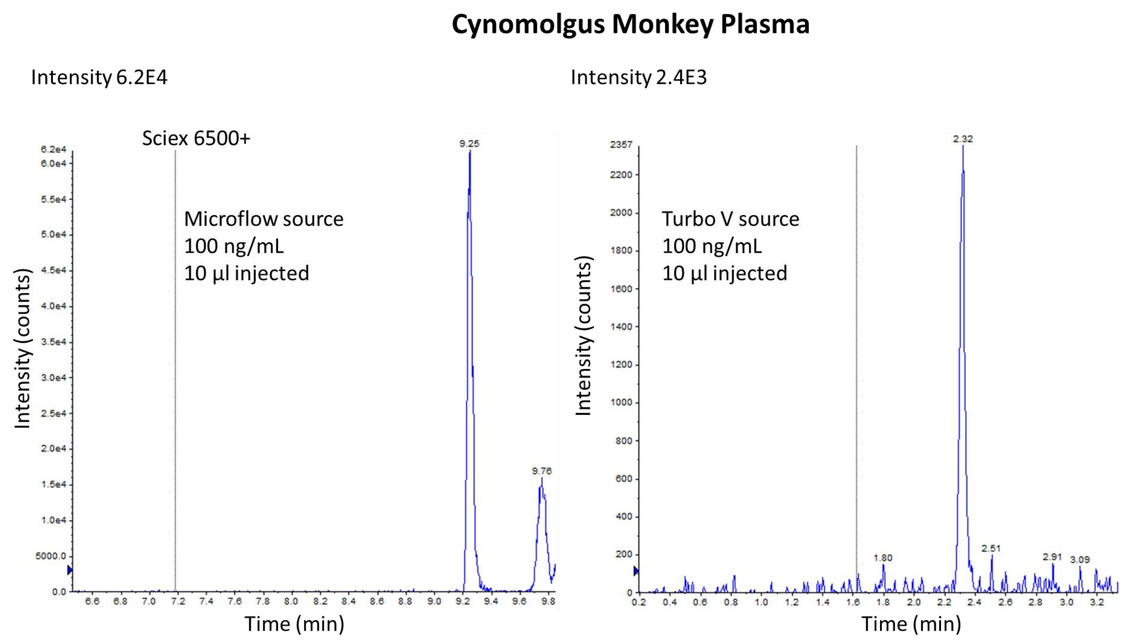

Figure 4: HPLC-MRM chromatogram of a 1 kDa peptide obtained after digestion with BrCN of a 30 kDa therapeutic protein.

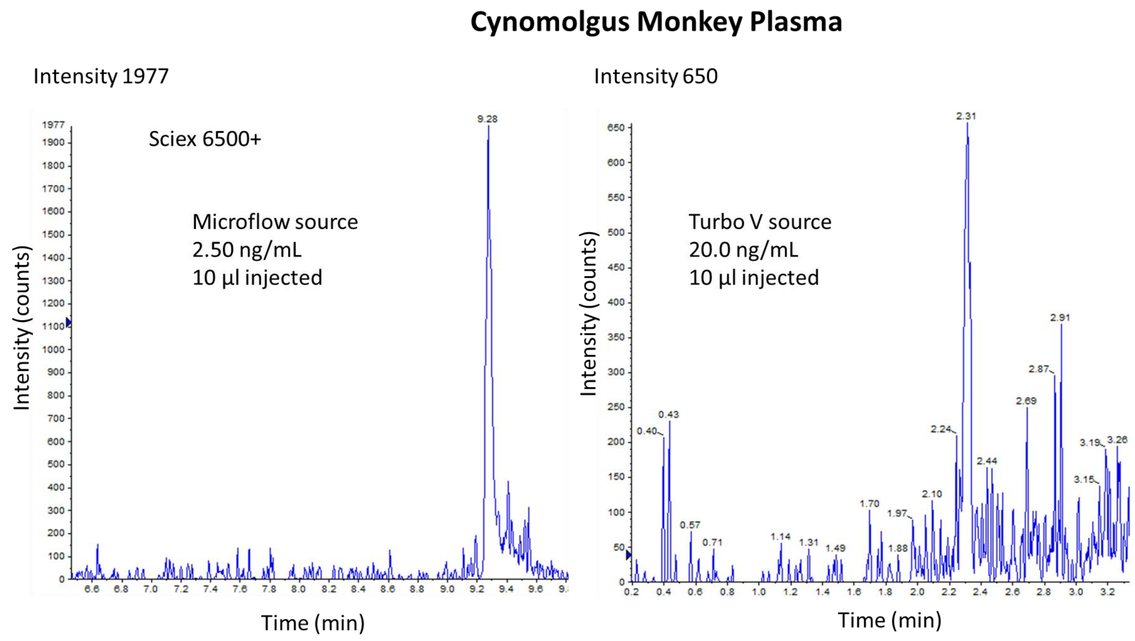

Figure 5: HPLC-MRM chromatogram of a 1 kDa peptide at the lower limits of quantification. An improvement of a factor 30 was achieved with the micro-flow setup (left, 0.3 mm ID column) compared to the regular flow setup (right, 2 mm ID column)

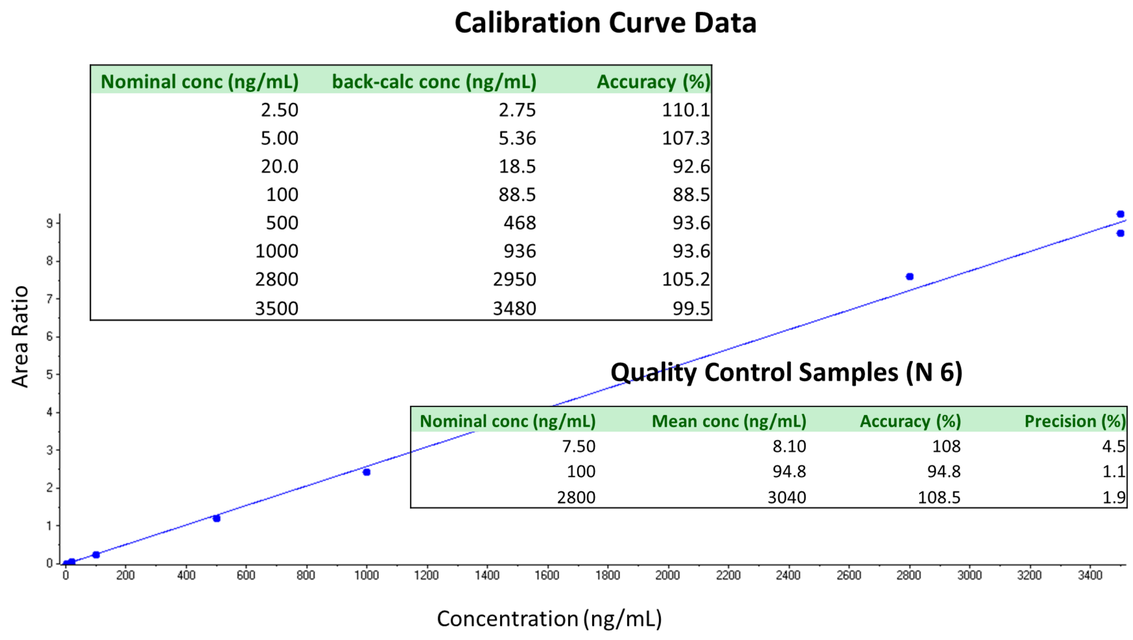

Figure 6: Method qualification data in cynomolgus monkey plasma within the concentration range of 2.50 ng/mL to 3500 ng/mL.

Conclusion

It was demonstrated that the Prolab Microflow ion source can be used for routine analysis. Combined with 2D chromatography using the Zirconium HPLC pump, excellent robustness and a very high sensitivity of 2.50 ng/mL for a therapeutic protein was achieved.

References

[1] Remco van Soest and Yihan Li SCIEX, Redwood City, CA, USA , Quantitation of Insulin Glargine in Human Plasma with a Combination of Immunocapture-Based Target Enrichment and Trap-and-Elute Microflow LC-MS/MS