High-Throughput, Dual-Stream Micro-flow LC/MS/MS Bioanalysis

Brendon Kapinos[1], John Janiszewski[1], Wayne Lootsma[2], Steve Ainley[2] Mary Piotrowski[1], Nick Levitt[3]
[1]Pfizer Global R&D, Groton, CT, [2]Sound Analytics, Niantic, CT [3]TwoCenter Technologies, Cambridge, MA

Introduction

Pharmaceutical research is being challenged to decrease operating costs in a variety of ways; miniaturization and gains in efficiency during sample preparation are ultimately leading to lower sample volume available for analysis. Concurrently, more potent drugs necessitate lower limits of detection during analysis. Reducing reagent consumption and waste production are also key considerations driving costs and efficiency of LC/MS/MS analysis. Micro-flow LC (5-50µL/min) addresses these issues. Often however, these methods offer only modest sample throughput. We set out to investigate key parameters that limit throughput in the micro-flow range. Single stream LC methods were used to identify specific issues/limitations and potential resolution of throughput bottlenecks. We plan to scale-up the optimized system to dual-steam mode on the ADDA autosampler.

Materials and methods

- Agilent 1290 Infinity and Eksigent ExpressHT micro-flow pumps
- SCIEX 6500 QTRAP mass spectrometer with Analyst 1.6.2 software
- Apricot Designs Dual Arm (ADDA) equipped with high-pressure Rheodyne injection ports
- 30x0.5mm micro-flow columns and 65µ ESI electrodes from Eksigent
- Column heater from Analytical Sales and Services set to 55C
- 1/32”OD tubing from Analytical Sales and Services
- PicoFuze probes from New Objective, Woburn MA
- A method development cocktail in 10% methanol containing gabapentin, quinidine, propranolol, diltiazem, verapamil and diclofenac

System Optimization

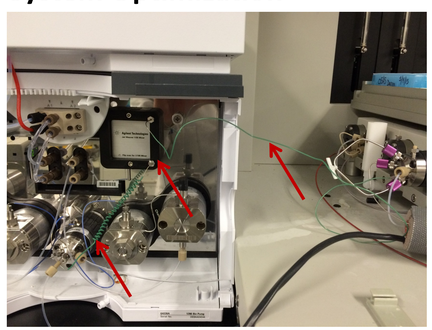

Fig 1a. When configured for HPLC/UHPLC, The Agilent 1290 Infinity has extensive dead volume (arrows) within transfer tubing and 35µL JetWeaver mixer. This makes high-performance micro-flow LC difficult. Efforts were focused on modifying setup and plumbing to accommodate high-performance micro-flow LC/MS/MS.

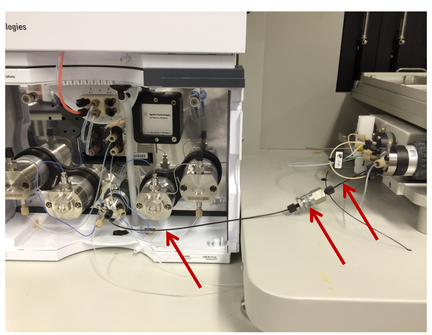

Fig 1b. To optimize the pump for micro-flow, low-volume PEEK tubing (0.1mm ID) and external 1µL mixer were installed using the shortest possible lengths to reach the injection port, reducing dead volume and delay time.

Assessment of System Performance

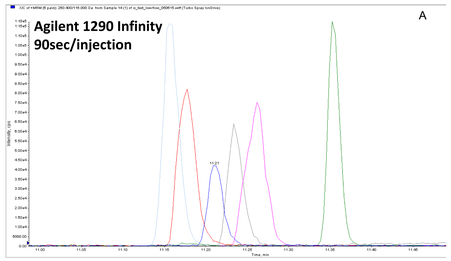

Fig 2a. 2µL injections of method development cocktail at 50µL/min (90sec/injection cycle time) using optimized Agilent 1290 Infinity UHPLC. Peak widths were ~4sec.

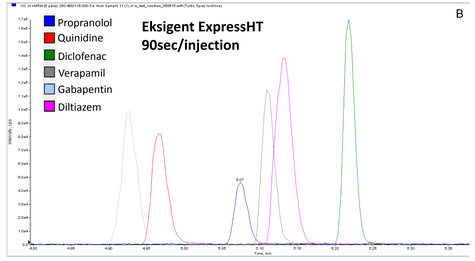

Fig 2b. 2µL injections of method development cocktail at 50µL/min (90sec/injection cycle time) using Eksigent micro-flow LC system. Peak widths were ~3sec.

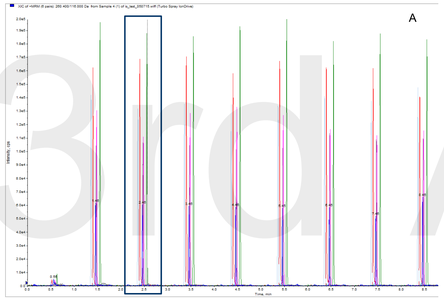

Fig 3a. Multi-injection chromatogram using Eksigent micro-flow LC system at 75µL/min, 60sec/injection.

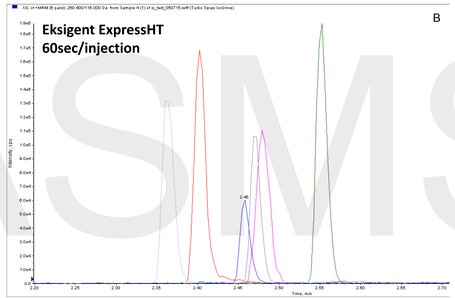

Fig 3b. Expanded view of a single injection from high-throughput (60sec/injection) multi-injected chromatogram.

Evaluation of PicoFuze technology

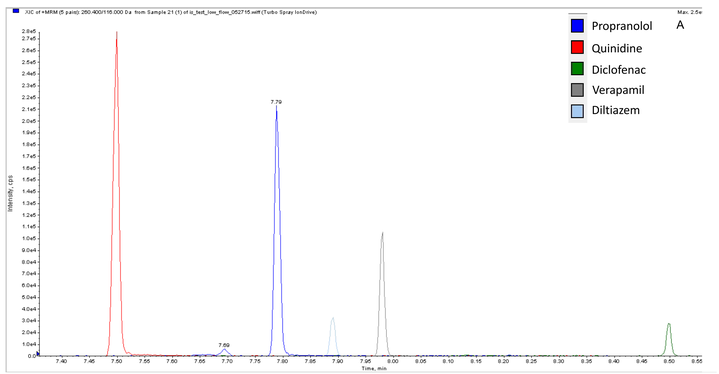

Fig 4a. Single injection of cocktail on PicoFuze probe using Eksigent ExpressHT pump, 3min/injection at 12µL/min. Gradient was linear from 0 to 90%B. **Peak widths were ~2sec.**

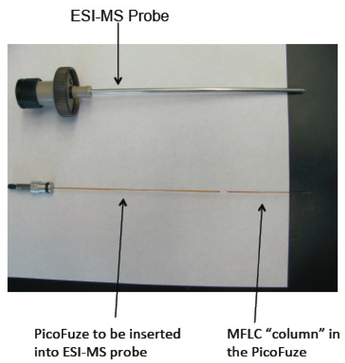

Fig 4b. A modified SCIEX probe houses the PicoFuze assembly. Dimensions were 50x0.2mm, tip size 20µ, and packing material was Reprosil-PUR C18, 3µ, 120A.

Results

The Eksigent ExpressHT pumping system performed significantly better than the Agilent 1290 pump at a flow rate of 50µL/min. There was better separation between analytes tested and peaks were sharper (Fig 2.). The enhanced performance was also evident for multiply injected files at 60 sec/inj throughput (Fig 3). LC flowrate was increased to 75 µL/min to achieve increased throughput. Key parameters used to optimize the system are summarized in Table 1. A PicoFuze LC probe was also tested (Fig 4.) The PicoFuze was installed in less than 5 minutes on an SCIEX IonDrive source, provided excellent separation of a variety of analytes and delivered good throughput at a low flow rate (12µL/minute). In this case the runtime was 3 min/injection (not high-throughput), but the separation and peak shape was superior.

Table 1.

Parameter	Initial Value	Optimized Value	Enhancement
Mixing volume	35µL	1µL	Delay volume, peak shape
Tubing ID	0.17mm	0.1mm	
Tubing volume	13.6µL	2.4µL	
Total delay volume	58.6µL	13.4µL	
Electrode ID	150µ	65µ	Peak shape

Conclusions

Reducing extra column volume can yield a significant enhancement in chromatographic performance, especially in the micro flow range.

Electrode inner-diameter is the main factor in performance enhancement in micro flow range.

The Eksigent ExpressHT micro-flow system delivers decreased gradient response time due to reduced dead volumes (3µL for Eksigent pump compared to 13.4µL for optimized Agilent 1290). In addition, enhanced mixing and laminar flow of this pump design significantly increase performance in the micro flow range.

With minor design modifications, the ADDA platform can be optimized for high-performance micro-flow LC (integrated with Sciex X500 instruments). By co-locating injection valve, pump and ion source, tubing runs become shorter, leading to improved peak shape, decreased dead volume and increased performance.

Acknowledgements

The authors would like to thank the following individuals for their contributions: Dan Warren and Joe Doktorski (SCIEX), Carla Marshall-Waggett and Helena Svobodova (New Objective)