Design and Integration of a High-Performance Micro-flow LC-MS/MS system

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WORLDWIDE RESEARCH & DEVELOPMENT

Abstract

Biopharmaceutical R&D is challenged to reduce operating costs in spite of increasingly demanding portfolio needs. Sample volume, solvent consumption, waste generation, and cycle time are key considerations. Micro-flow (µF) LC-MS/MS reduces solvent consumption and waste for lower cost per analysis while delivering enhanced sensitivity. However, few µF-LC-MS/MS systems exist with optimal configurability, flexibility, and sample throughput. Parameters for high-performance micro-flow were identified, and drove design of a fully-integrated LC-MS/MS platform with very low system volume to support various LC-MS/MS analyses, including micro-flow. Instrument software automated batch building using conditions from a centralized DiscoveryQuant 3.0 database, and integrated micro-flow pump delivered precise gradient separations at 3-5µL/min.

Materials and Methods

- SCIEX 6500 TripleQuad mass spectrometer with Analyst 1.7 software
- TurboV IonDrive and OptiFlow MS/MS sources equipped with 25µ electrodes
- Prolab Zirconium Ultra micro-flow pump with flow control
- LeadSampler (LS-1) and LeadScape software
- 25 and 50µ ID, 1/32” OD tubing and fittings from Analytical Sales and Services
- 25 and 50µ ID, 1/16” OD NanoViper tubing from Thermo
- Luna Omega PS C18 50x0.3mm 3µ columns from Phenomenex
- Ascentis Express C18 50x0.2mm 2.7µ columns from Supelco
- Method development cocktail in 10% methanol containing propranolol, zaleplon, diazepam, verapamil, diltiazem, tolbutamide, terfenadine and diclofenac

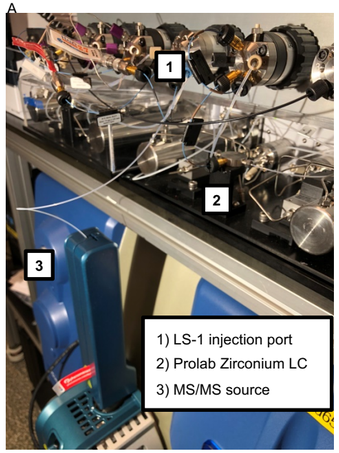
Fig 1. Micro-flow system configuration and components.

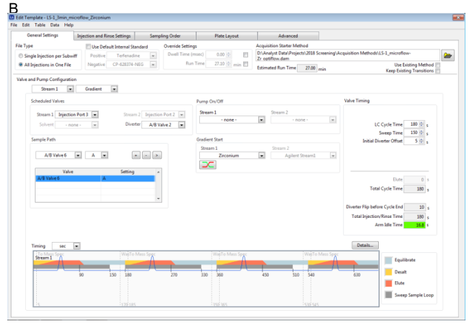
LS-1 autosampler
- High-speed, UHPLC-ready
- DiscoveryQuant database integration

Prolab Zirconium Ultra micro-flow pump
- 4nl/min to 500µL/min flow rate range
- 15,000psi maximum pressure
- 1350µL volume/channel

SCIEX 6500 TripleQuad
- Enhanced sensitivity and dynamic range

Micro-flow LC-MS/MS System Design and Integration





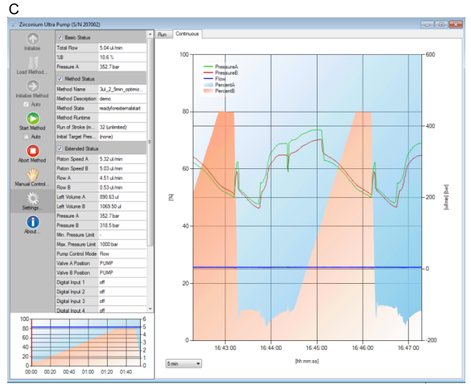


Fig 2a-c. Components of the micro-flow LC-MS/MS system were closely located for short flow path and low system volume (A). LeadScape software managed valve assignment and scheduling, and triggered pump program (B). Prolab Zirconium micro-flow pump software was employed for LC method building and provided real-time pressure and flow readings accessed from integrated sensors (C).

System Volume Optimization

Table 1. Comparison of flow path delay volumes across system configurations

	Pump → injector	Injector → column	Total delay volume (µL)
Configuration A	20µ x 150mm	20µ x 550mm	0.22
Configuration B	50µ x 150mm	50µ x 550mm	1.37

Table 1. Impact of delay volume on micro-flow performance was examined. The system was configured with 20 or 50µ ID NanoViper tubing for these assessments.

Fig 3a-b. Injections of standard cocktail with instrument Configuration A (top) and B (bottom).

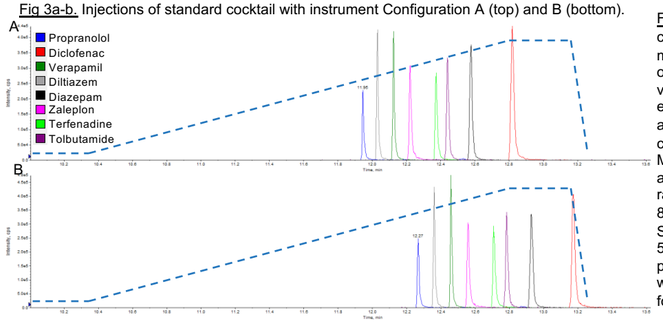


Fig 3a-b. An internal standard cocktail containing 8 small molecule analytes was injected on the system in each delay volume configuration. Analytes eluted ~20s earlier, on average, in Configuration A compared to Configuration B. Methods were identical for all assessments; 4µL/min flow rate, linear gradient from 4-85%B, 5-minute cycle time, Supelco Ascentis Express C18 50x0.2mm 2.7µ column, mobile phase A: 0.1% formic acid in water, mobile phase B: 0.1% formic acid in acetonitrile.

MS/MS Source Assessment

Table 2. LC gradient program

Time sec)	Flow (µL/min)	%B
0	4	4
20	4	4
170	4	85
190	4	85
195	4	4

Table 3. Method parameters

Parameter	Description
Mobile phase	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Column	Supelco Ascentis Express C18 2.7µ 50x0.2mm
Injection volume	0.3µL
Cycle time	5 minutes/injection
Flow path	20µ ID NanoViper tubing

Fig 4a-b. MS/MS sources were evaluated for low flow performance (1-5µL/min). Flow path and LC methods were identical (Table 2 and 3). SCIEX 6500 TripleQuad MS/MS was equipped with either TurboV IonDrive (A) or OptiFlow source (B) for analysis of a standard cocktail. Both sources were outfitted with 25µ electrodes, however 1/32” OD, 25µ ID x 100mm tubing with 1/16” to 1/32” adapter fitting and sleeve were installed on IonDrive source to interface the column with 1/32” electrode coupler.

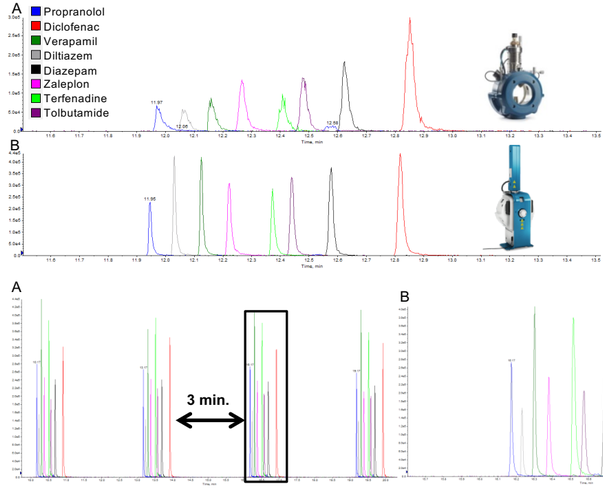


Fig 5a-b. Multiply-injected, micro-flow LC-MS/MS acquisitions of standards (n=4 injections) revealed good throughput and reproducibility using the OptiFlow micro-flow MS/MS source. Flow rate was 6µL/min at 3-minutes/injection cycle time (A). An expanded view of injection 3 shows sharp peak widths (3-5s) and near-baseline separation of 8 cassetted analytes (B). Method parameters were identical to those listed in Table 3. A linear LC gradient from 5-85% B over 2 minutes was programmed through Prolab Zirconium software and cycle time reduced to 3 minutes/injection.

Conclusions

- A micro-flow LC-MS/MS system was purposefully designed with integrated, high-performance components to minimize dead volume while maximizing throughput and flexibility.
- Delay volume was reduced by close placement of system components (valves, pump, and MS/MS source) and reduction in tubing ID.
- An innovative MS/MS source design (SCIEX OptiFlow) reduced post-column volume to ~60nL and consistently supported high-performance micro-flow analyses at 1-5µL/minute.
- Integration of LS-1/LeadScape, Prolab Zirconium pump, and SCIEX OptiFlow source allowed multi-injected acquisitions at very low flows while achieving good throughput (6µL/minute flow rate, 3 minutes/injection).