Development of High-Performance Micro-flow LC-MS/MS Methodology and Application to a High-Throughput Screening Workflow

Brendon Kapinos[1], John Janiszewski[1], Hui Zhang[1], Jianhua Liu[1], Brian Holder[1], Wayne Lootsma[2], Steve Ainley[2], Amanda Berg[3], Erik Hansen[3]
[1]Pfizer, Groton, CT; [2]Sound Analytics, Niantic, CT; [3]New Objective, Woburn, MA

WORLDWIDE RESEARCH & DEVELOPMENT

Abstract

Biopharmaceutical LC-MS/MS groups are challenged to enhance productivity and flexibility while simultaneously decreasing operating cost. Sample volume, solvent consumption, waste generation, and cycle time are key considerations for bioanalytical laboratories. Micro-flow LC-MS/MS can greatly reduce solvent consumption and waste, however available systems are dedicated and offer poor sample throughput. Parameters influencing micro-flow performance were identified, and drove design of a fully-integrated LC-MS/MS platform with very low system volume for maximum performance across an array of LC-MS/MS techniques including micro-flow LC. Instrument software performed automated batch building using MS/MS conditions from a centralized DiscoveryQuant3.0 database, and high-throughput micro-flow-MS/MS analysis of a cassetted global ADME screening assay. Solvent consumption was reduced by 150x over standard high-throughput analysis, while throughput was identical (15s/inj/cmpd).

Materials and Methods

- Eksigent ExpressHT Ultra micro-flow pump
- SCIEX 5500 TripleQuad mass spectrometer with Analyst 1.6.3 software
- LeadSampler (LS-1) and LeadScape software
- 25 and 50µ ID, 1/32" OD tubing and fittings from Analytical Sales and Services
- HALO C18 5µ 50x0.2mm PicoFuze probe from New Objective, Woburn MA
- Method development cocktail in 5% methanol containing buspirone, propranolol, clozapine, desipramine, diazepam, verapamil, diltiazem, tolbutamide and diclofenac
- Human liver microsome (HLM) stability samples were generated by following protocol: pooled human liver microsomes were incubated in the presence and absence of NADPH and 1µM control compound for one hour at 37°C. 20µL sample were taken at designated time and terminated with ACN. 10µL supernatant from each incubation per timepoint were pooled and dried under nitrogen. Samples were reconstituted in 5% methanol and vortexed prior to LC/MS/MS analysis.

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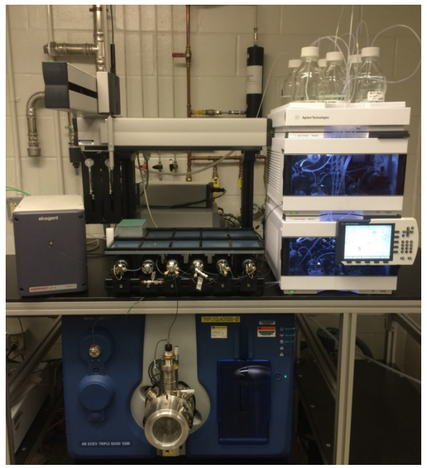

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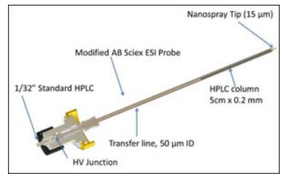

Fig 1a. System design focused on lowest possible dead volume, placing key flow path components in close proximity to SCIEX TurboV MS/MS source.

Fig 1b. PicoFuze incorporates a high-performance, micro-flow column within a SCIEX electrode, virtually eliminating post-column volume.

Optimizing Extra-Column Volume

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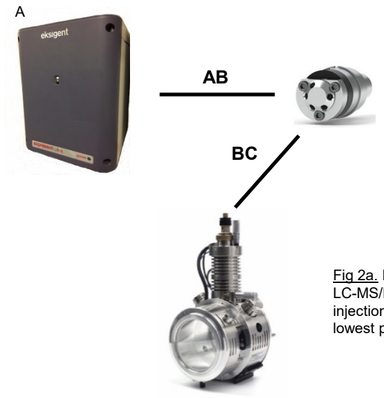

Table 1. Pre-column flow path parameters

Tubing Segment	Dimensions	Volume (µL)
Pump → injection valve (AB)	23cm x 25µ	0.113
Injection valve → PicoFuze (BC)	33cm x 50µ	0.648

Fig 2a. Reduced system volume drove high performance micro-flow LC-MS/MS. Dimensions of transfer tubing connecting pump, injection valve and MS/MS source (PicoFuze) were optimized for lowest possible delay volume.

Micro-flow LC-MS/MS Method

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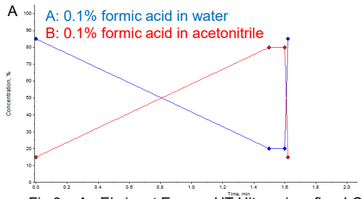

Table 2. Small molecule standards, selected physicochemical properties and MS/MS methods

	buspirone	propranolol	clozapine	desipramine	diazepam	verapamil	diltiazem	tolbutamide	diclofenac
									
LogD7.4	1.24	0.785	3.53	1.27	2.8	2.41	3.36	0.468	1.44
LogD2	-2.49	-0.2	-0.157	0.718	1.48	0.924	1.63	2.36	4.55
Mw_e	385.25	259.16	326.13	266.18	284.07	454.28	414.16	270.1	295.02
MRM	386.3→122.1	260.1→116.1	327.4→270	267.3→208.2	285.1→193.1	455.4→165.1	415.2→178.2	271.3→91.1	296.0→215.0

Fig 3a. An Eksigent ExpressHT Ultra micro-flow LC pump delivered a consistent linear gradient from 15-80%B at 13µL/min. Injection cycle time was 120s for all analyses.

HLM Stability Screening by Micro-flow LC-MS/MS

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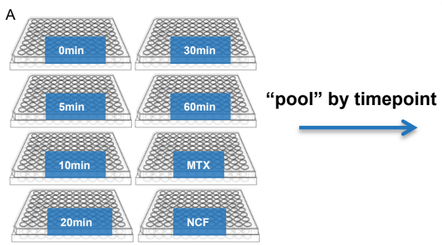

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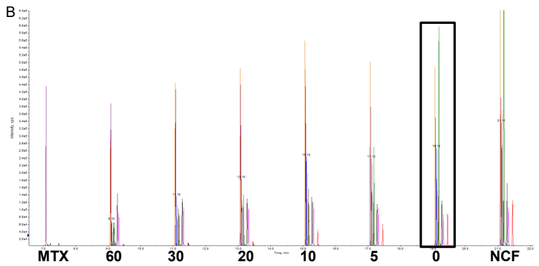

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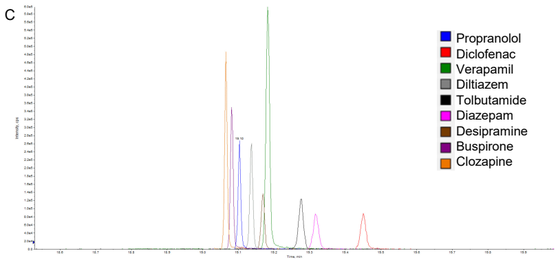

Fig 4a. Post-assay pooling scheme for HLM stability screen. Prior to micro-flow LC-MS/MS analysis, 10µL aliquots of quenched samples containing individual standards were pooled by timepoint, dried under nitrogen, and reconstituted in 5% MeOH. Injection volume was 0.2µL.

Fig 4b-c. Analysis of pooled HLM stability samples by micro-flow LC-MS/MS (13µL/min) was accomplished at 120s/injection and delivered baseline separation of 8 substrates + internal standard (buspirone).

Micro-flow LC-MS/MS performance

Table 3. Observed t ½ values of standards and respective CYP450 clearance pathway

Standard	Pathway	T ½ (min)
Desipramine	2D6	30-34
Diazepam	2C19, 3A4	>120
Diclofenac	2C9, 2C8	8-12
Diltiazem	3A4	26-30
Propranolol	2D6, 1A2, UGT	26-30
Verapamil	3A4, 2C9, 1A2	8-10
Clozapine	1A2	60
Tolbutamide	2C19	>120

Table 4. Comparison of sample throughput and solvent use across LC-MS/MS methods

	HT- "trap-and-elute" (2000µL/min)	Standard Gradient (800µL/min)	PicoFuze gradient w/ sample pooling (13µL/min)
Throughput (sec/inj/cmpd)	15s	120s	15s
Total runtime	12.8hrs	102.4hrs	12.8hrs
Total solvent usage	1,536 mL	4,915 mL	10 mL

Conclusions

Micro-flow LC-MS/MS can drastically reduce solvent consumption and required sample volume, decreasing operating costs and increasing return on investment. However, overall throughput and performance is often limited by extra-column volume, restricting application space for routine micro-flow LC-MS/MS. A fully-integrated LC-MS/MS platform (LeadSampler, LS-1) was designed outright for very low system volume. Key system parameters were identified and optimized for maximum performance across an array of LC-MS/MS techniques including micro-flow LC. The system was equipped with an MS/MS probe assembly with integrated, high-performance micro-flow LC column (PicoFuze), virtually eliminating post-column volume for good sample throughput (120s/injection) with baseline separation of 9 analytes at low flow (13µL/min). Excellent peak capacity and separations drove integration of post-assay sample pooling into this workflow. An HLM stability screen was pooled, and 8 substrates separated by micro-flow LC-MS/MS for increased efficiency and sample throughput. The combination of these methodologies delivered identical throughput (15s/injection/cmpd) with 150x-lower solvent consumption compared to existing HT-LC-MS/MS methods.