Design and Optimization of an Integrated Trap-and-Elute Microflow LC-MS/MS Platform

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WORLDWIDE RESEARCH & DEVELOPMENT

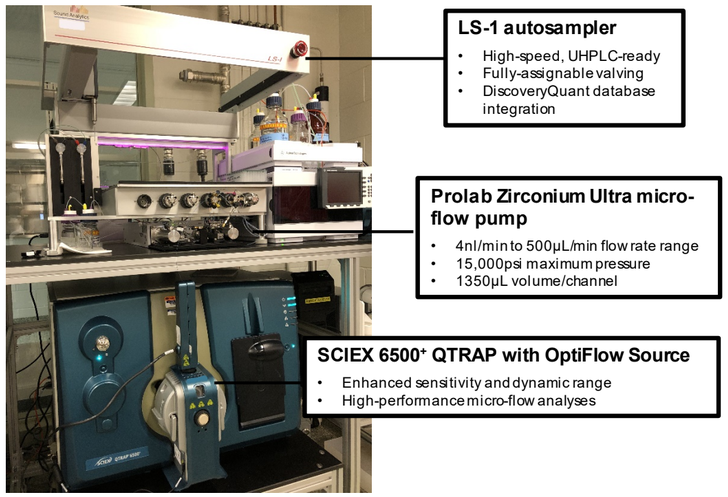
Abstract

Benefits of microflow chromatography (mflc) coupled to mass spectrometry are well-described, including increased sensitivity and drastically decreased solvent usage. Recent advances in available hardware and solutions have increased robustness and facilitated routine utilization. We seek to combine trap-and-elute methodology to mflc to expand application space and impact a production environment. For example, in proteomic analyses this combination workflow will enable larger injection volumes and remove interfering matrix components in samples, resulting in improved sensitivity and reproducibility. Enhancements to existing platform software enabled an integrated workflow based on an enterprise-level, high-throughput LC-MS/MS system. Specific platform features and parameters to support the technique are described in creating a highly-configurable, integrated trap-and-elute micro-flow LC-MS/MS system for enterprise proteomics and potentially many other applications.

Materials and Methods

- SCIEX 6500⁺ QTRAP mass spectrometer with Analyst 1.7 HF3 software
- OptiFlow MS/MS source equipped with 25µ electrode
- Prolab Zirconium Ultra micro-flow pump with flow control
- Agilent 1290 Infinity binary pump
- LeadSampler (LS-1) and LeadScape software
- 25 and 50µ ID, 1/16" OD NanoViper tubing from Thermo
- Luna Omega C18 20x0.3mm 5µ trap columns from Phenomenex
- Kinetex XB-C18 50x0.3mm 2.6µ micro-flow columns from Phenomenex
- Thermo Pierce BSA tryptic digest

Fig 1. 2D Micro-flow system and components.



Micro-flow Trap-and-Elute System Design and Integration

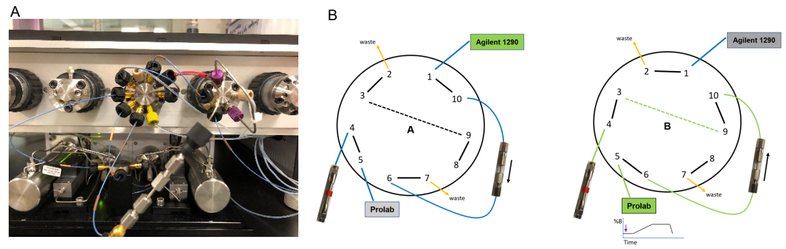


Fig 2a-c. LS-1 plumbing, highlighting close integration of injector, 10-port switching valve and micro-flow pump for lowest possible dead volume (A). 10-port valve schematic detailing plumbing and flow in each valve state, utilizing "backflushing" of trap column (B). LeadScape experiment template defines injection sequence, valve and pump assignments, and integrates fully-customizable, user-defined Timed Events that are automatically propagated for multi-injected data files (C).

Peptide LC-MS/MS method development

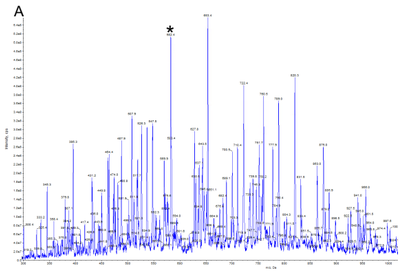


Table 1. Optimized BSA tryptic peptide MS/MS methods

Peptide Name	Q1 Mass	Q3 Mass	DP	CE
Peptide 1	820.49	720.46	20	25
Peptide 2	760.44	340.04	20	45
Peptide 3	653.39	251.18	20	35
Peptide 4	547.33	490.25	35	25
Peptide 5	777.86	662.85	95	35
Peptide 6	615.38	720.43	65	15
Peptide 7	507.89	397.22	35	25
AEFVEVTK	461.75	722.41	60	29
YLYEIAR	464.35	651.36	50	25
LVNELTEFAK	582.38	951.30	65	25

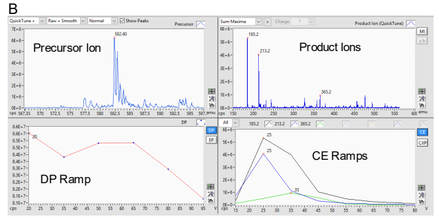
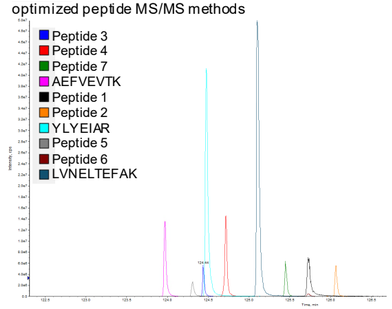


Fig 3a-b. BSA tryptic peptide method development
A BSA tryptic digest was selected for evaluation of the 2-D LC system. FIA revealed abundant peptides that were selected for further method development (A). LeadScape Optimization performed automated MRM method-building on selected peptide masses for optimal performance (B).

Figure 4. Injection of BSA tryptic digest in 10% methanol using optimized peptide MS/MS methods



Assessment of 2D-LC performance

Table 2. LC gradient program

Time (sec)	Flow (µL/min)	%B
0	11	3
35	11	3
390	11	29
410	11	29
440	11	90
460	11	90
470	11	3

Table 3. Method parameters

Parameter	Description
Mobile phase	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Trap Column	Phenomenex Luna Omega C18 5µ 20x0.3mm
MFLC column	Phenomenex Kinetex XB-C18 2.6µ 50x0.3mm
Injection vol.	10µL
Cycle time	10min/injection
Flow path	50µ ID NanoViper tubing

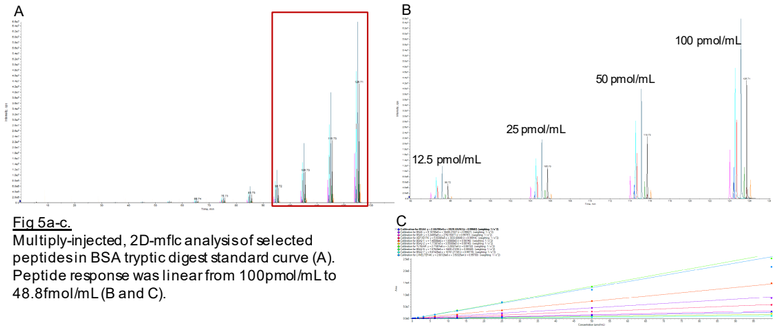


Fig 5a-c.
Multiply-injected, 2D-mflc analysis of selected peptides in BSA tryptic digest standard curve (A). Peptide response was linear from 100pmol/mL to 48.8fmol/mL (B and C).

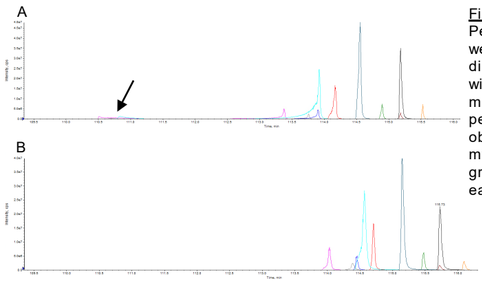


Fig 6a-b.
Performance of direct-inject and 2D methods were evaluated in a complex matrix. BSA tryptic digest was prepared in 10% methanol fortified with protein-precipitated human liver microsomes (HLM). Excessive tailing of polar peptides and loss of retention (arrow) was observed with the direct-inject method (A). 2D-mflc analysis with Luna Omega trap column greatly improved peak shape and retention of early-eluting peptides (B).

Conclusions

- An integrated, 2D micro-flow LC-MS/MS system was designed for minimal dead volume, maximum throughput and flexibility.
- LeadScape software integrated pumps and valves, enabling fully-customizable, user-defined sequences and complex 2D-LC methods.
- A 2D-mflc workflow used large injection volumes (10µL) for analysis of complex matrices, peak shape and retention of early-eluting peptides was enhanced compared to direct-inject methods.
- Further evaluation of trap and analytical column pairing can improve peak shape and resolution in challenging 2D-LC applications.