Deploying an Ultra-Sensitive, High-Throughput, Integrated Microflow LC-MS/MS System to Support *in vitro* PK/PD Assessment

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Introduction

In drug discovery, project teams strive to rapidly interrogate pharmacokinetic and pharmacodynamic (PK/PD) profiles for promising candidates to optimize target exposure and inform clinical dose. *In vivo* studies are resource intensive and time-consuming; benchtop microfluidic systems can model PK/PD relationships *in vitro* at an early stage, affording teams a high degree of flexibility and control in their experimental designs, overall increasing agility. An ultra-sensitive, high-throughput microflow LC-MS/MS system was developed, and methods optimized specifically for enabling precise, high-throughput analysis of complex *in vitro* PK/PD samples. Based on a state-of-the-art triple quadrupole mass spectrometer featuring a microflow-compatible ion source, the system routinely provides rapid, robust analysis of resultant *in vitro* PK samples, and facilitates study designs with clinically-relevant dose and schedules.

Materials & Methods

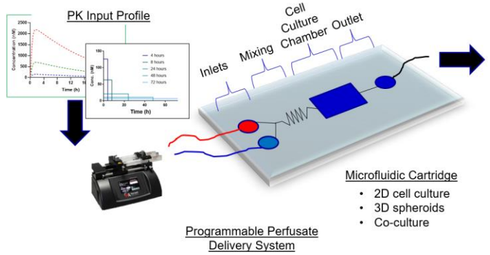

Fig1. Microfluidic Perfusion Platform
The PK Profile system uses programmable pumps to deliver user-defined dynamic drug concentration profiles to a downstream cell-containing microfluidic cartridge. Samples are prepared by protein precipitation, followed by reconstitution with LCMS-friendly diluent and further diluted 5-20x prior to analysis.

LS-1 sample delivery system
Fully integrated with LeadScape (LS) software, interfaces with SCIEX Analyst and SCIEX OS mass spectrometer software

ProLab Zirconium Microflow pump
4nL/min to 500uL/min flow range
15,000 psi maximum pressure
Dual flow controllers for precise LC gradient delivery

SCIEX 7500 QTRAP with OptiFlow Pro Ion Source
10-50uL/min electrode
Low Micro probe & E-Lens

Fig2. Ultra-sensitive microflow LC-MS/MS system & components

Sciex 7500 QTRAP was controlled by SciexOS software version 3.3.1 (SCIEX, Framingham MA) and paired with a Zirconium Prolab pump (Reinach, Switzerland).

LS-I sample-delivery system was controlled by LeadScape software (Sound Analytics, Niantic CT) and plumbed with 50µ ID Thermo NanoViper tubing. Microflow separation was performed with HSS T3 50x0.3mm columns (Waters, Milford, MA).

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Development of an integrated microflow LC-MS/MS (µfLC-MS/MS) platform

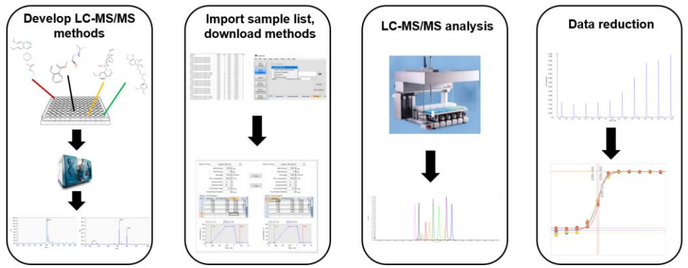

Fig3. Overview of automation supporting fully-integrated quantitative LC-MS/MS workflows
Accelerating delivery of physiologically-relevant *in vitro* PK/PD data enables project teams to make crisp decisions in real time. LeadScape software automates key aspects of the quantitative bioanalytical workflow-including integrated method development, sample batching and data review.

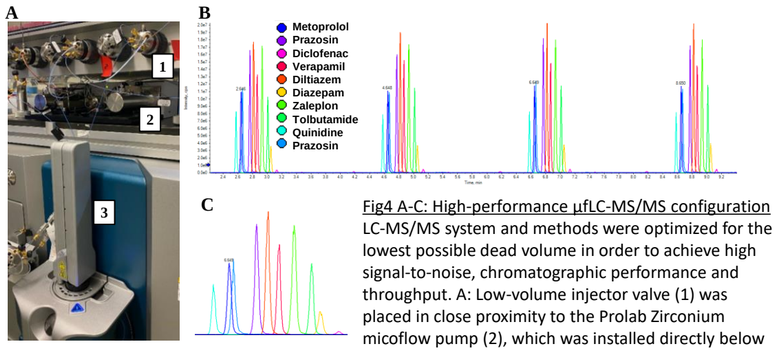

Fig4 A-C: High-performance µfLC-MS/MS configuration
LC-MS/MS system and methods were optimized for the lowest possible dead volume in order to achieve high signal-to-noise, chromatographic performance and throughput. A: Low-volume injector valve (1) was placed in close proximity to the Prolab Zirconium micoflow pump (2), which was installed directly below the LS-1 autosampler. The Optiflow Pro MS source housed a 50x0.3mm microflow column within an integrated column heater set to 40C. Low Micro probe and E-Lens were installed to ensure maximum stability at low flows (3). Samples were collected as multiply-injected chromatograms-analysis of a neat standard cocktail reveals good chromatographic performance at micro flow rates (B, C).

Table 1.

Parameter	Description
Mobile phase	A: 0.1% FA in water B: 0.1% FA in acetonitrile
Column	Waters HSS T3 5µ 50x0.3mm
Injection Volume	0.5uL
Cycle Time	120s
Flow rate	20µL/min
Flow Path	50µ ID NanoViper tubing

Table 1: Microflow-LC method parameters
50u ID NanoViper tubing was used for fluidic connections, resulting in a total system volume of 1uL. 0.3mm ID microflow columns were interfaced directly to the source electrode, eliminating post-column dead volume and resulting in very short peak widths.

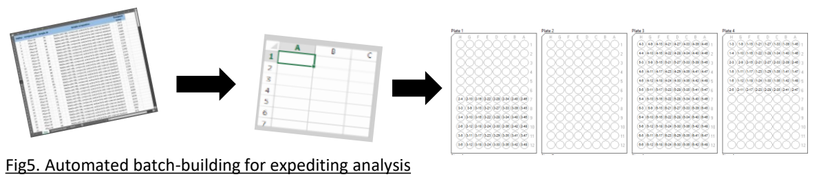

Fig5. Automated batch-building for expediting analysis
Software automation accommodates complex experimental designs and random-access sampling. Files are imported directly into LeadScape software, and ultra-sensitive and selective MS/MS methods are quickly accessed from a centralized DQ LC-MS/MS database, saving FTE time.

Results

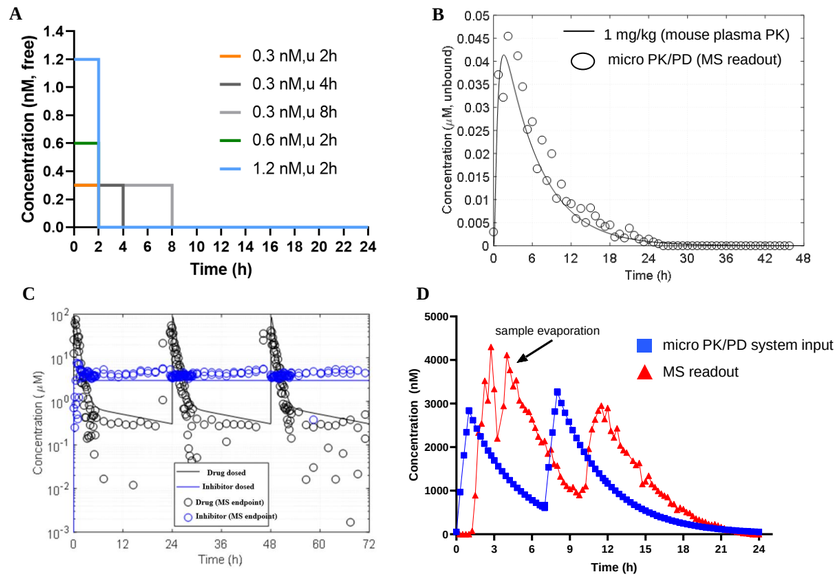

Fig6 A-D: *in vitro* PK/PD endpoints enabled with fully-integrated µfLC-MS/MS platform
To date the high-performance µfLC-MS/MS platform has delivered PK readouts across various *in vitro* PK/PD assessments. This includes target coverage studies, wherein dose and exposure are modulated to assess pharmacodynamic response (A), perfusion studies at relevant *in vivo* dose (B), and multi-day dosing designs with simultaneous measurement of drug and inhibitor (C). The microflow platform was also employed for *in vitro* PK/PD system troubleshooting and development, including assessment of actual drug delivery vs programmed profile. Drug profiles in the nM-pM range were routinely screened in complex matrices (cell culture media fortified with BSA).

[nM]	Area	Area Ratio	Used	Calculated Concentration [nM]	Accuracy
0.009144947	220	0.002	TRUE	0.010	99.61
~~0.027434842~~	~~543~~	~~0.005~~	~~FALSE~~	~~0.051~~	~~171.57~~
0.082304527	795	0.007	TRUE	0.080	99.9
0.24691358	2,431	0.023	TRUE	0.283	113.11
0.740740741	6,280	0.053	TRUE	0.683	92.34
2.222222222	17,910	0.162	TRUE	2.122	95.58
6.666666667	55,430	0.503	TRUE	6.633	99.45

Fig7. Linear dynamic range achieved with µfLC-MS/MS system
in vitro PK/PD capabilities were developed to support early-stage assessment of low [drug] target coverage and exposure. Microflow LC-MS/MS was leveraged to routinely deliver LLOQs in the low-pM range.

Conclusions

- ✓ ***In vitro* PK/PD platforms can provide teams with relevant pharmacokinetic/pharmacodynamic data at an early stage**
- ✓ **An ultra-sensitive, fully-integrated microflow LC-MS/MS system was developed to rapidly return data from complex *in vitro* PK/PD assessments**
- ✓ **A standardized approach was constructed to support highly-variable study designs, complex matrices, and low expected drug concentrations, enhancing clinical translation and value**