WP 244

High-Throughput Dual-Arm, Dual-Stream LC-MS/MS Bioanalysis Leveraging the LS-II Autosampler

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Challenge

An increase in the throughput of bioanalysis support for *in vitro* assays is needed, while ensuring high data quality is maintained

Solution

The LS-II (Sound Analytics) platform offers dual-arm, dual-stream gradient liquid chromatography to increase throughput compared to single arm, single-stream Pal-XT

Impact

The LS-II delivered equivalent results to current Pal-XT based LC-MSMS in less than half the run-time (~2.4 throughput gain)

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- Automation Team

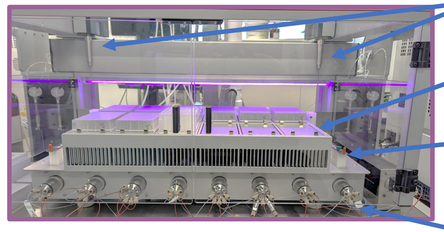
INTRODUCTION

- Our laboratory supports high-throughput LC-MS/MS bioanalysis across a variety of ADME in vitro assays.
- Each week, up to 768 analytes are tested in 11 high-throughput ADME assays, which require up to 1,536 total injections and offer a turnaround time of less than 24 hours.
- Instrument consistency and robustness are essential for delivering high-quality results quickly, while following optimized workflows for bioanalyzing hundreds of analytes.
- Here, we evaluated the LS-II autosampler from Sound Analytics as a replacement for the current Pal-XT based high-throughput LC-MS/MS workflows.

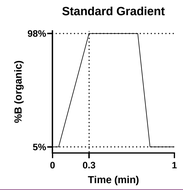
METHODS

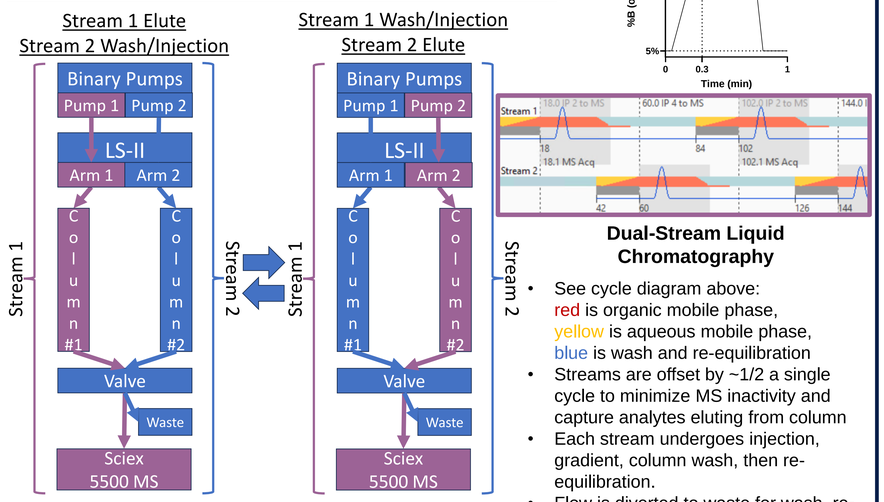
- LS-II is compatible with both Sciex software, Analyst and SciexOS, but requires LeadScape (Sound Analytics) for acquisition. LeadScape software was also used for the rapid review of acquired data. All LS-II data presented here was acquired using Analyst.
- For dual-stream bioanalysis, stability assays of mouse and human liver microsomes (LM) were analyzed concurrently using Multi Injected File (MIF) mode. MIF requires both streams to use the same MS method, after which the results are compiled into a single file.
- MIF works when analyzing assays consisting of multiple conditions, such as different matrices (i.e: mouse or human liver microsomes).
- In the presented data, human LM used stream 1 and mouse LM used stream 2.
- LM stability was performed using 6 timepoints of a 2-hour incubation and a blank

Instrument Configuration		
Autosampler	CTC Pal-XT	LS-II
LC Streams	One	Two
Binary Pump(s)	Agilent 1290 Infinity I	Agilent 1290 Infinity II + Sound Analytics SA1299
Software	Analyst + Discovery Quant	Analyst/SciexOS + LeadScape
Mass Spectrometer	Sciex 5500 Triple Quad	
MS Method	MRM	
MS Use	**50% of runtime**	**100% of runtime**



LC Method Details	
Stationary Phase	Waters X-Bridge C8 (2.1 x 30 mm, 5µm)
Flow Rate	800 µL/min
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in acetonitrile
MS Run Time	0.3 – 0.8 minutes



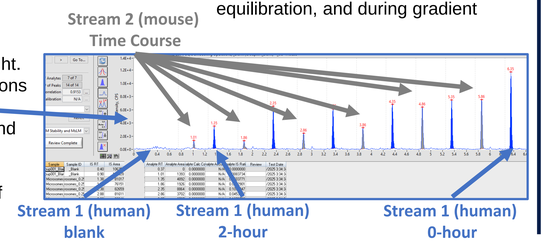


Dual-Stream Liquid Chromatography

- See cycle diagram above: red is organic mobile phase, yellow is aqueous mobile phase, blue is wash and re-equilibration
- Streams are offset by ~1/2 a single cycle to minimize MS inactivity and capture analytes eluting from column
- Each stream undergoes injection, gradient, column wash, then re-equilibration.
- Flow is diverted to waste for wash, re-equilibration, and during gradient

LeadScape Software

- Review of data in software shown on right.
- Chromatogram window shows all injections in .wiff file.
- Rapid assessment of chromatograms and validity of acquired signals.
- Can cycle through multiple .wiff files in seconds, allowing review of hundreds of injections in an hour



RESULTS

2.4-fold Increased Throughput in Liver Microsome Stability

Autosampler	Min/Inj	Time (h)
Pal-XT	1.42	21.2
LS-II	0.60	8.96

Figure 1. LS-II averaged 0.6 minutes per injection over 896 injections. LM stability consisted of 14 injections for every group of analytes (768 compounds cassetted into groups of 6), for a total of 1792 injections.

Instrument Response is Similar Between LS-II and Pal-XT methods

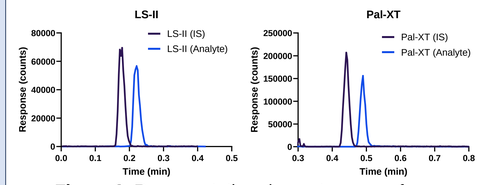


Figure 2. Representative chromatograms of same sample (analyte and internal standard) injected using LS-II and Pal-XT instruments. Response are similar regardless of autosampler.

Reduced Injection Variability in Each LS-II Arm compared to Pal-XT

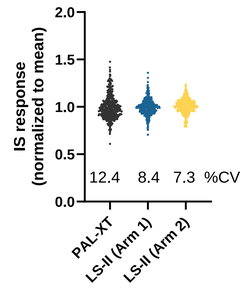


Figure 3. IS response (normalized to average response) showed less variability (%CV) in each LS-II arm than Pal-XT based single-stream over 448 injections

LM Stability Qualitative Validity is Equivalent Between LS-II and Pal-XT

Combined		Pal-XT	
86.5% Validity Agreement		Valid	NV
LS-II	Valid	577	54
	NV	49	88

Mouse		Pal-XT	
88.3% Validity Agreement		Valid	NV
LS-II	Valid	296	19
	NV	26	43

Human		Pal-XT	
84.9% Validity Agreement		Valid	NV
LS-II	Valid	281	35
	NV	23	45

Figure 4. A qualitative assessment of the LM Stability results shows that the LS-II BA method maintained assay quality without increasing non-valid (NV) results. NV represents samples failing to yield stability measurements. The combined validity agreement for mouse and human matrices was 86.5%, with Pal-XT exhibiting slightly more NVs (54) compared to LS-II (49).

Liver Microsome Cl_{int} Results Agree Between Dual-Stream LS-II and Single-Stream Pal-XT

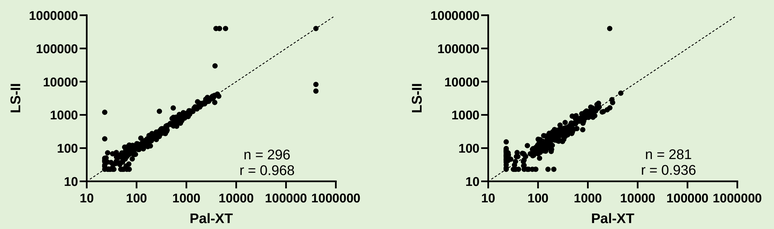


Figure 5. The quantitative endpoint for the Liver Microsome assay (Cl_{int}) is compared between both methods, LS-II and Pal-XT. LS-II reproduced similar Cl_{int} results, (spearman rho of 0.97 in mouse and 0.94 in human LM). These results compare favorably to the inter-day variability of the Pal-XT alone (spearman rho of 0.962 in mouse, n = 322; data not shown).

Conclusion

- Sound Analytics LS-II autosampler increased throughput 2.4-fold while still providing equivalent quality of data to lower throughput LC methods.
- LS-II is a suitable autosampler for workflows with room for dual-stream analysis and Sciex MS instruments.
- LeadScape software is a suitable replacement for Discovery Quant based workflows.
- Dual-stream chromatography has a learning curve due to the complexity. Thus, workflow must necessitate the need for increased throughput for implementation.

Future Direction

- Evaluate suitability of LS-II with different software such as SciexOS.
- Evaluate Single Injected File (SIF) acquisition mode. This mode allows for different MS methods on streams 1 and 2, allowing more flexibility in assays supported by LS-II.