# Multiplexing Independent Streams to Increase LC/MS/MS Throughput

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## ABSTRACT

Our group routinely uses high-throughput LC/MS/MS methodologies for bioanalysis of *in vitro* ADME samples. The most common methodology being dual arm trap and elute. When this approach is applied to more complex samples requiring gradient analysis, we have often observed poor consistency data between two streams. This can be caused by small differences in performance from columns, even from the same lot. When analyzing a set of samples on two individual columns this minor difference in signal results in nonconformity of data. Here, we are creating a process using LeadScape software to run completely separate streams and separate acquisition files, thereby maintaining consistency of data while increasing throughput and ease of subsequent data analysis.

## Objective

Improved bioanalytical throughput by multiplexing separate streams/ LC systems to one mass spectrometer while acquiring to separate data files.

## METHODS

- A Sciex 5500 triple quadrupole mass spectrometer was coupled to an LC system consisting of two Agilent UHPLC pumps and a LeadSampler (LS-I) autosampler.
- LC mobile phases consisted of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. Kinetex C18 columns were used for separation.
- Data were acquired and processed with Analyst via LeadScape and Sound Review or MultiQuant software, respectively.
- Samples from protein binding studies were used for testing. The samples were injected by both the current, single arm workflow and the more efficient multiplexed independent streams workflow.
- Sample Run lists were created in Excel, containing information necessary to run and process data.
- This file contains file name, sample name, well/plate position, analyte name, sample type, nominal concentration and dilution factor. An additional field for the efficient workflow was added to indicate stream used for injections. This file is imported to LeadScape.

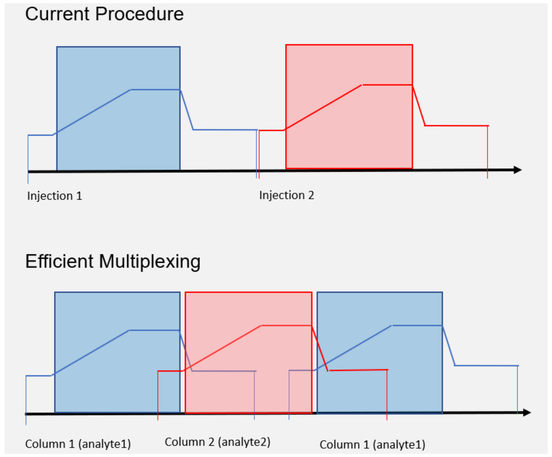


**Figure 1. LS-I Efficient multiplexing utilizes equilibration time to start next injection on a separate column.**

| SampleID | AnalyteID | VialPos | Plate | FileName | Stream |
|---|---|---|---|---|---|
| Blank | PF-001 | 1 | Plate 7 | PF-001-1 | 1 |
| 2 uM PF-001 MIX Human Liver Buffer @ eq | PF-001 | 1 | Plate 1 | PF-001-2 | 1 |
| 2 uM PF-001 MIX Human Liver Buffer @ eq | PF-001 | 2 | Plate 1 | PF-001-3 | 1 |
| 2 uM PF-001 MIX Human Liver Buffer @ eq | PF-001 | 3 | Plate 1 | PF-001-4 | 1 |
| 2 uM PF-001 MIX Human Liver Buffer @ eq | PF-001 | 4 | Plate 1 | PF-001-5 | 1 |
| Blank | PF-001 | 2 | Plate 7 | PF-001-6 | 1 |
| 2 uM PF-001 MIX Human Liver Matrix @ eq | PF-001 | 5 | Plate 1 | PF-001-7 | 1 |
| 2 uM PF-001 MIX Human Liver Matrix @ eq | PF-001 | 6 | Plate 1 | PF-001-8 | 1 |
| 2 uM PF-001 MIX Human Liver Matrix @ eq | PF-001 | 7 | Plate 1 | PF-001-9 | 1 |
| 2 uM PF-001 MIX Human Liver Matrix @ eq | PF-001 | 8 | Plate 1 | PF-001-10 | 1 |
| Blank | PF-001 | 3 | Plate 7 | PF-001-11 | 1 |
| 2 uM PF-001 MIX Human Liver Matrix - Stability | PF-001 | 9 | Plate 1 | PF-001-12 | 1 |
| 2 uM PF-001 MIX Human Liver Matrix - Stability | PF-001 | 10 | Plate 1 | PF-001-13 | 1 |
| 2 uM PF-001 MIX Human Liver Matrix @ T0 | PF-001 | 11 | Plate 1 | PF-001-14 | 1 |
| 2 uM PF-001 MIX Human Liver Matrix @ T0 | PF-001 | 12 | Plate 1 | PF-001-15 | 1 |
| Blank | PF-002 | 4 | Plate 7 | PF-002-1 | 2 |
| 2 uM PF-002 MIX Human Liver Buffer @ eq | PF-002 | 13 | Plate 1 | PF-002-2 | 2 |
| 2 uM PF-002 MIX Human Liver Buffer @ eq | PF-002 | 14 | Plate 1 | PF-002-3 | 2 |
| 2 uM PF-002 MIX Human Liver Buffer @ eq | PF-002 | 15 | Plate 1 | PF-002-4 | 2 |
| 2 uM PF-002 MIX Human Liver Buffer @ eq | PF-002 | 16 | Plate 1 | PF-002-5 | 2 |
| Blank | PF-002 | 5 | Plate 7 | PF-002-6 | 2 |
| 2 uM PF-002 MIX Human Liver Matrix @ eq | PF-002 | 17 | Plate 1 | PF-002-7 | 2 |
| 2 uM PF-002 MIX Human Liver Matrix @ eq | PF-002 | 18 | Plate 1 | PF-002-8 | 2 |
| 2 uM PF-002 MIX Human Liver Matrix @ eq | PF-002 | 19 | Plate 1 | PF-002-9 | 2 |
| 2 uM PF-002 MIX Human Liver Matrix @ eq | PF-002 | 20 | Plate 1 | PF-002-10 | 2 |
| Blank | PF-002 | 6 | Plate 7 | PF-002-11 | 2 |
| 2 uM PF-002 MIX Human Liver Matrix - Stability | PF-002 | 21 | Plate 1 | PF-002-12 | 2 |
| 2 uM PF-002 MIX Human Liver Matrix - Stability | PF-002 | 22 | Plate 1 | PF-002-13 | 2 |
| 2 uM PF-002 MIX Human Liver Matrix @ T0 | PF-002 | 23 | Plate 1 | PF-002-14 | 2 |

**Figure 2. Example of Import File.** An additional column containing stream is required to multiplex.

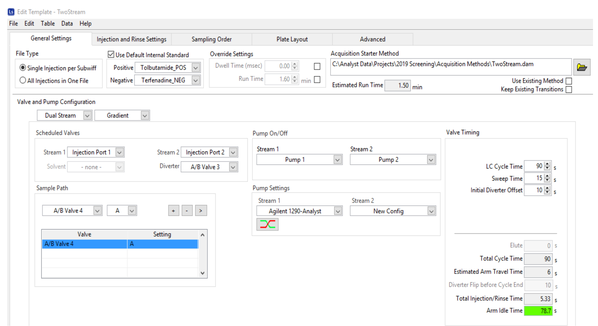


**Figure 3. LeadScape Template** contains File type, Valve and Pump configuration, Injection and Rinse settings, Sampling and Plate Layout, and Default mass spec acquisition method with source conditions.

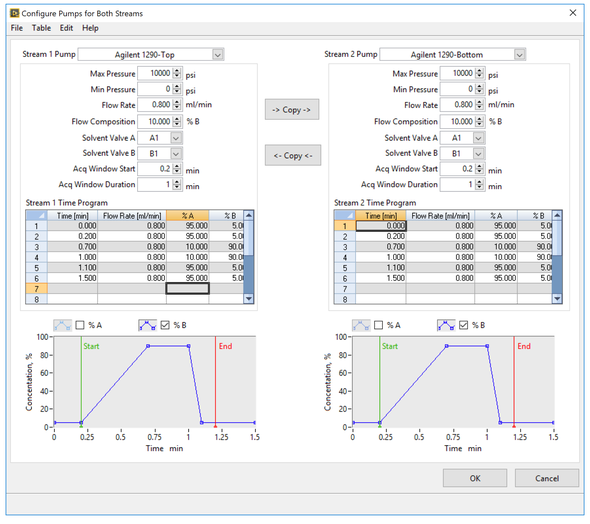


**Figure 4. LC Pumps Setup.** Acquisition start and duration for streams is entered in the Pump setup. Samples are run one injection per raw data file with data saved to wiff only during the specified acquisition window.

## RESULTS

Our group has previously shown dual arm analysis using trap and elute technology shortens the overall cycle time and increases throughput. In this approach, samples are run one after the other, alternating on separate short columns/cartridges while acquiring to the same data file. The samples are processed together independent of which column they were injected. Staggering injections saves potentially 50% cycle time depending on acquisition and equilibration needs. For simple in vitro samples this approach has been successful, however, it has been less successful when applied to gradient methodologies. Gradient methodologies are often required for more complex, multi component samples where greater separation is required. Gradient separations also require longer cycle times which would benefit more from faster throughput. Repeated attempts to use a dual arm approach have led to data inconsistency. The inconsistency stems mostly from a minor difference in signal coming from individual columns. We have observed individual columns of the same lot can show minor differences in signal intensity. Due to these small differences, samples run on different columns cannot be successfully processed together. Our recent efforts are focused on implementing a process via LeadScape software to multiplex two separate streams. In this new approach, one set of samples is run on one column and another set of samples is run on another column in a staggered manner. By isolating sets of samples to one particular column only, we avoid the problem of signal differences. One additional improvement allows the injections to be acquired as separate files and processed independently.

## CONCLUSIONS

- Routine sample analysis that requires gradient elution can benefit from using an efficient multiplexing approach.
- When applied to a 90 second gradient method, used to run protein binding samples, this approach saves ~33% cycle time.
- Acquisition/equilibration time necessary for an application will determine time savings.
- Acquiring data to separate files simplified the data processing step.