Dual channel microflow LC-MS: investigations of high sensitivity and high throughput

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ABSTRACT

A dual gradient microflow liquid chromatography (microLC) system has been coupled with a triple quadrupole mass spectrometer (MS) in a multiplexed arrangement which maintains the performance of the low flow system while allowing higher throughput separations. The system uses two separate gradient channels with their own injection valves and columns. Both injection valves are serviced by a single autosampler robot. The columns share a single column oven which is closely coupled to a stream selector valve mounted immediately at the electrospray source of the MS. Microflow electrodes are used to provide low dispersion electrospray. The system has been designed to provide the benefits of multiplexed LC-MS while maintaining the low dispersion coupling needed to realize the benefits of microflow chromatography. Analysis of both small molecule pesticides and peptides from tryptic digests of proteins is used to demonstrate the initial performance of the system. Nearly 2x increase in throughput using a 4 minute LC method is demonstrated. Data collection in both continuous collection mode for repeated injections (in one data file) as well as collection into a queued set of smaller data files is shown. Parallel collection using different samples and different flow rates is also demonstrated. The method can be extended to more disparate (and complementary) separation modes by using different chemistries and separation modes.

INTRODUCTION

Microflow LC-MS has proven to be a valuable tool for improving sensitivity while maintaining or improving throughput for many high-throughput applications, including those in drug discovery and development. The ability to augment standard microLC-MS workflows with functions for preconcentration (trap-elute) and multiplexing (parallel chromatography) can provide further improvements in both sensitivity and throughput. We present workflows that couple a dual gradient microflow LC with a high speed autosampler that preserve the critical performance of low-dispersion microflow separations while demonstrating the improved throughput of multiplexed workflows.

MATERIALS AND METHODS

Two microflow gradient pumping systems (SCIEX microLC 200) have been coupled in a custom hardware and software configuration that allows coordination of the pumping channel flows along with the required valve actuations. The pumping system is combined with a high throughput autosampler (CTC HTC-xt with DLW high speed washing; CTC Analytics) equipped with two, independent, small port injection valves and separation columns. In the current configuration, the columns share a single column oven which is mounted directly to the TurboV™ electrospray source of a triple quad mass spectrometer (SCIEX QTRAP® 4000). A nanovolume valve is also mounted directly to the MS source to allow low delay and low dispersion stream selection. The effluent from the stream selection valve is coupled with a microflow electrode (SCIEX) in the TurboV™ source.

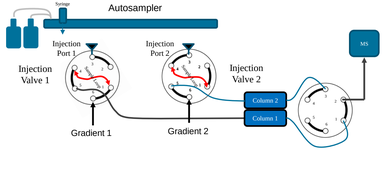


Figure 1. Plumbing diagram representing the experimental setup used for the parallel, multiplexed microflow chromatography. Two separate injection valves are filled by the CTC autosampler. A low volume stream selector is used to determine which column effluent is directed to the ESI source of the MS.

Separations using 0.5 mm ID x 50 mm length columns have been used for separation of both small molecule and peptide samples.
Chromatography conditions:

Mobile phase A: water with 0.1% formic acid
Mobile phase B: acetonitrile with 0.1% formic acid
Column: 0.5 x 50 mm HALO C18 2.7um particles (SCIEX)
Column temperature 35 C
Flow rate: 30 to 75 uL/min
Injection volume: 5uL

A mixture of seven triazine pesticides (Sigma) was used for small molecule analyses and a tryptic digest of β-galactosidase (SCIEX) for peptide analyses. Multiple reaction monitoring (MRM) transitions using the triple quadrupole function of the MS were used for detection.

MRM Transitions:

Compound	Q1 Mass (Da)	Q3 Mass (Da)
Ametryn 1	228.2	186.2
Ametryn 2	228.2	96.1
Atrazine 1	216	174
Atrazine 2	216	104.1
Prometon 1	226.2	142.3
Prometon 2	226.2	184.2
Prometryn 1	242.2	158.1
Prometryn 2	242.2	200.2
Propazine 1	230.2	146
Propazine 2	230.2	188.3
Simazine 1	202.1	132.1
Simazine 2	202.1	124.3
Terbutryn 1	242.2	186
Terbutryn 2	242.2	68.2

Table 1. MRM transitions for triazines. Two transitions per molecule.

Peptide Sequence	Q1 Mass (Da)	Q3 Mass (Da)
WVGYGQDSR	534.2	782.3
RDWENPGVTQLNR	528.9	855.4
APLDNDIGVSEATR	729.4	1176.6
DWENPGVTQLNR	714.9	884.5
DVSLLHKPTTQISDFHVATR	567.1	1045.5
VNWLGLGPQENYPDR	879.4	1075.5
LWSAEIPNLYR	681.4	1062.6
LSGQTIEVTSEYLFR	872	1143.6
TMITDSLAVVLQR	723.9	1101.6
IDPNAWVER	550.3	871.4
LPSEIFDLSAFLR	697.9	1184.6
GDFQFNISR	542.3	636.4
VDEDQPFPAVPK	671.4	755.5
WLPAMSER	495.4	690.3
YSQQQLMETSHR	503.4	760.3

Table 2. MRM transitions for tryptic peptides of β-galactosidase digest.

RESULTS

Microflow chromatography of both small molecule (triazine) and peptide (β-galactosidease protein digest) samples has been conducted using the dual channel system. Dual injectors with a post-column selector valve have been configured to allow multiplexed samples to be run. Initial experiments show an ability to gain nearly 2x in throughput over a single channel experiment, while maintaining good peak shapes and performance. Depending on the type of experiment, maximum throughput can be limited by multiple factors including autosampler speed, the speed of the individual assay, and for high sensitivity workflows, the flow rate and sample volume. An initial investigation of these factors and tradeoffs was conducted.

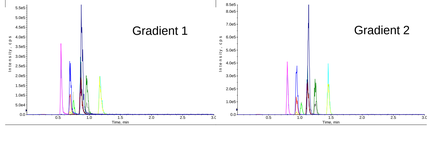


Figure 2. LC-MS-MS chromatograms of triazines collected individually on the two columns used for parallel analysis. This data was taken without the stream selection valve in line and represents a baseline for the chromatography. The two flow paths have slightly different plumbing lengths which contributes to slight differences in the elution times.

In the examples below, the separations were conducted using 4 minute methods composed of a 1 minute equilibration at the beginning, a 2 minute gradient, and a 1 minute wash at high organic at the end. The actual composition change during the 2 minute gradient was varied depending on the analytes (5-80%B for triazines, 5-40%B for the β-gal peptides). Multiple acquisition / data collection modes were employed for these experiments. In some experiments, a string of sequential chromatograms was collected in a single MS file, while in others the chromatograms were parsed to multiple MS files. With the exception of a small amount of system overhead (both from software and initialization) nearly the full 2x improvement in throughput was achieved.

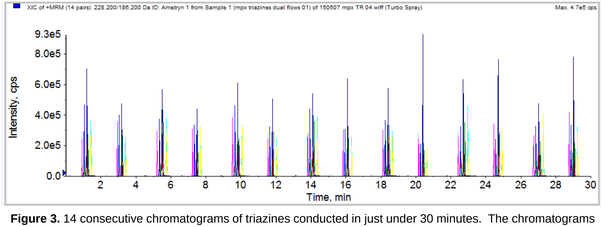


Figure 3. 14 consecutive chromatograms of triazines conducted in just under 30 minutes. The chromatograms were collected into a single MS data file. The chromatograms alternate between gradient channels and separation columns. At an average time of just over 2 minutes per chromatogram (using a 4 minute method), the throughput approaches the 2x improvement obtainable with a two channel multiplexed system.

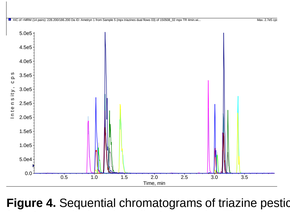
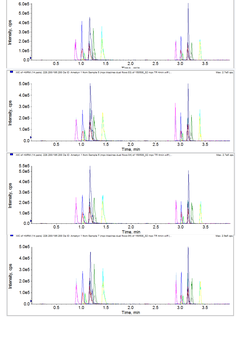


Figure 4. Sequential chromatograms of triazine pesticides from two multiplexed gradient channels. The chromatograms are synchronized between the two channels and collected in pairs into separate MS files.

Figure 5. To the right are shown eight successive separations collected as four pairs of two runs (one on each column) collected into four separate MS data files. Good retention time reproducibility is observed between each collection (data file).



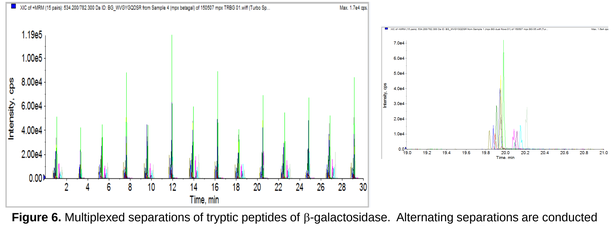


Figure 6. Multiplexed separations of tryptic peptides of β-galactosidase. Alternating separations are conducted on two different columns. In this example, the flow rate is varied between channels with Gradient 1 running at 40uL/min and Gradient 2 running at 75uL/min. To the right is a blow up of one of the individual chromatograms.

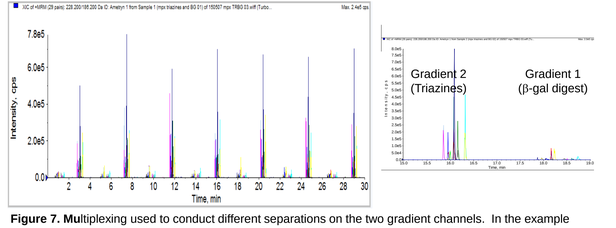


Figure 7. Multiplexing used to conduct different separations on the two gradient channels. In the example shown, β-galactosidase digest is separated on Gradient 1 using a shallow gradient (5-40%B in 2 min) at 40uL/min, while a mixture of triazines is separated on Gradient 2 using a steeper gradient (5-80%B in 2 min) at a higher flow rate (75uL/min). Note that the intensities of the two samples is considerably different. To the right is shown a blow of up one pair of sequential chromatograms.

CONCLUSIONS

A high throughput implementation of microLC-MS has been demonstrated using a dual channel LC system with two independent gradient pumping systems, sample injection valves, and separation columns. The effluent of the two columns is directed through a stream selection valve to the MS source. Through careful control of plumbing volumes and dispersion, including the mounting of both the column oven and the stream selection valve directly at the MS source, high performance microflow separations are maintained while nearly doubling the throughput of the original method. Such an approach promises to provide the benefits of microflow LC (including smaller sample volumes, higher sensitivity, lower mobile phase consumption, and reduced solvent load to the MS source) to high throughput, high performance workflows.

TRADEMARKS/LICENSING