HIGH THROUGHPUT PARALLEL LC/MS ANALYSIS OF MULTIPLE LIQUID STREAMS FOR DRUG DISCOVERY APPLICATIONS USING A MULTIPLEXED ELECTROSPRAY INTERFACE

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Introduction

Drug discovery groups are increasingly choosing to screen only chemical structures of controlled quality and purity. Since automated synthesis can now routinely generate arrays of thousands of chemical structures per week, the rate-determining step is likely to be compound QC.

Liquid chromatography in combination with atmospheric pressure ionisation (API) mass spectrometry (LC-MS) has become widely used for characterising synthetic arrays due to its specificity and sensitivity, and potential for high throughput. Compounds under investigation are introduced into the API interface of the mass spectrometer by either direct flow injection or fast, generic gradient liquid chromatography where inject to inject cycle times are often less than 5 minutes. On-line LC-MS data are preferable as they yield both molecular mass confirmation and purity information. There is little scope for significant increases in throughput with this serial analytical strategy. However, with the ability of orthogonal acceleration time-of-flight (oa-TOF) mass spectrometers to collect mass spectral data at high acquisition rates, there is the potential for rapidly sampling multiple liquid streams virtually simultaneously thus increasing sample throughput.

This poster describes the design and implementation of a multiplexed electrospray interface (MUX) that is capable of sampling eight individual liquid streams in rapid succession. The system has been integrated with the Z-Spray ion source of the Micromass LCT orthogonal acceleration time-of-flight mass spectrometer. Unlike previous work on parallel LC-MS systems, the sprays are indexed so that the system recognises which channel is being sampled at any one time and produces a discrete data file for each. Sample analysis using fast gradient LC analysis and the 8-channel MUX source is also described. The data shown herein relates to previous work performed using the 4-way MUX interface[1,2].

Experimental

A solvent gradient was delivered through 8 injection ports on a multi-injector autosampler. Each injector of the autosampler was connected to an LC column of the same diameter and packing material, allowing the solvent flow delivered from the pump to be split equally through each column. The eluent from each column was passed directly to a Valco T-piece arrangement where the solvent flow was reduced prior to each electrospray channel of the 8-channel MUX source. All data acquisition and hardware control was from MassLynx 3.4. **Figure 1** shows a schematic representation of the hardware arrangement for the experiment. The analysis conditions are described below.

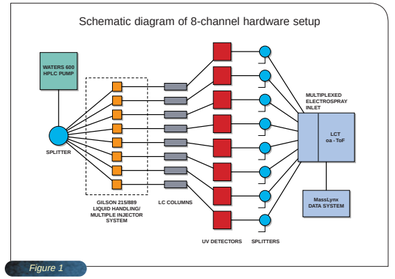

Figure 1

The following gradient LC conditions were used for the analysis:

LC:	Waters 600E
Autosampler:	Gilson Multiprobe 215/889
Column type:	Symmetry C18, 2.1 x 50mm, 3.5µ
LC flow rate:	8mL/min (nominally 1mL/min each column)
Inject volume:	20µL
Split ratio to MS:	10:1 (100 µL per electrospray channel)
Mobile Phases:	(A) = Water (0.1% formic acid)
	(B) = Acetonitrile (0.1% formic acid)
Gradient:	t = 0 mins, 100% A
	t = 3 mins, 70% B
	t = 3.1 mins, 100% B
	t = 3.5 mins, 100% A
	t = 3.6 mins, 100% A
	t = 5.0 mins, 100% A

The following MS parameters were used for data acquisition:

MS:	Micromass LCT with 8-way MUX interface
Ionisation mode:	ESI +ve
Capillary voltage:	3.5 kV
Cone voltage:	25V
Scan range:	100 - 1000 amu
Scan time:	0.1 sec
Inter-scan delay:	0.05 sec

Results and Discussion

1) 8-Channel MUX Design

The 8-channel MUX electrospray ion source interfaces directly to the conventional Z-Spray source of the LCT. The inner source housing contains an array of 8 pneumatically assisted electrospray probe tips that are directed at the sampling cone. The sampling cone is surrounded by a shield that obstructs the line-of-sight between the sprayers and the cone. The spray from each channel is admitted to the sampling cone region in rapid succession via a 5mm aperture in the shield that is driven between channels by a programmable stepper motor. The source is supplied with a heated stream of dry nitrogen that facilitates the desolvation of ions in the selected spray. To monitor the 8 separate electrosprays virtually simultaneously, the rotor is typically moved from position to position in 0.05 secs. Dwell times per channel for mass spectral acquisition are typically 0.1 secs, although both the inter-channel step and dwell times are user-definable from the control software. Thus, when the interface samples from each spray for 0.1 secs and step times between sprays are set to 0.05 secs, the complete duty cycle for monitoring all 8 channels is only 1.2 seconds.

During the acquisition periods, the data system holds 8 separate data files open simultaneously. The control software recognises which spray channel is being sampled at any one moment and writes data from that channel to its own specific data file. This allows easy access to the information acquired from each channel and means that the data files can be used with any of the standard processing available with MassLynx software, such as OpenLynx, MetaboLynx or quantification. **Figure 2** shows a photograph of the 8-channel MUX source whilst **Figure 3** shows a schematic representation of how the 8 channels are arranged internally.

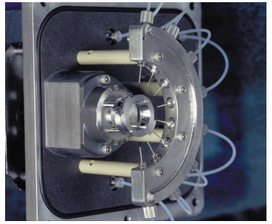

Figure 2

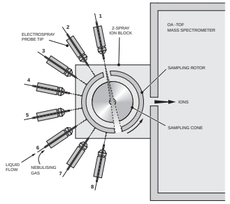

Figure 3

2) Analysis of a 3 Compound Mixture

Using the experimental conditions described above, two analyses using a 3 compound mixture containing diphenhydramine (M+H=256), oxybutynin chloride (M+H=358) and terfenadine (M+H=472) were performed. The first experiment involved injecting the 3 compound mixture onto each of the 8 columns simultaneously and monitoring each channel for elution of the analytes. **Figure 4** shows the chromatograms for each of the 8 channels acquired.

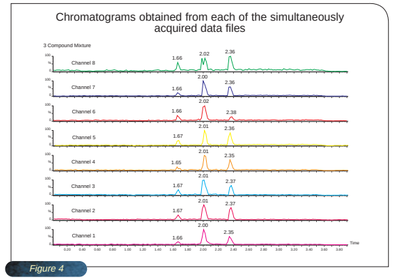

Figure 4

The elution order for the 3 compounds is diphenhydramine (t = 1.6 mins), oxybutynin chloride (t = 2 mins) and terfenadine (t = 2.3 mins). It can also be observed that the retention times for the 3 compounds are similar for all 8 channels.

The ability of the LCT to acquire mass spectra at very high rates (up to 10 spectra per second) allows the system to monitor all 8 channels in rapid succession and still produce high quality spectra. For the above experiment, where chromatographic peak widths were 3 seconds wide at half height, 6 mass spectra were typically obtained across each peak. **Figure 5** below shows the mass spectra for each of the 3 compounds taken from the chromatogram acquired on channel 1. In each case the $[M+H]^+$ for the compound is observed. The spectrum for diphenhydramine has a fragment ion at m/z 167. This was due to a single cone voltage being used for the acquisition which was higher than optimum for diphenhydramine resulting in in-source fragmentation. Reducing the cone voltage would eliminate this.

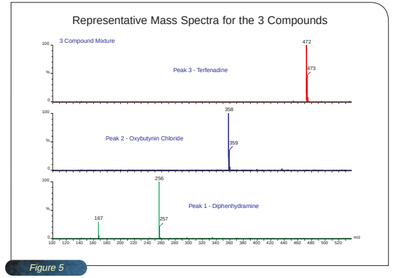

Figure 5

3) Inter-Channel Carryover

One potential concern in the design of a device such as the MUX interface is that a compound eluting from one channel should not produce an interference in the channel sampled immediately afterwards. This would result in the appearance of a 'ghost peak' in the adjacent channel. For this experiment, the same three compound mixture was analysed using the same conditions as for the previous experiment, but was only injected onto columns 1,3,5 & 7. Solvent blanks were injected onto columns 2,4,6 & 8. **Figure 6** shows the chromatograms acquired simultaneously for all 8 channels, whilst **Figure 7** shows the mass spectra from each of the channels taken at 2 mins (oxybutynin chloride eluting). As the results show, negligible inter-channel 'cross-talk' is observed, with the molecular ion for oxybutynin chloride not being observed in the adjacent channels of 2,4,6 & 8. These data were acquired at the fastest possible sampling rate on the device (0.1 sec sample, 0.05 sec step) and demonstrate the integrity of the individual sprays.

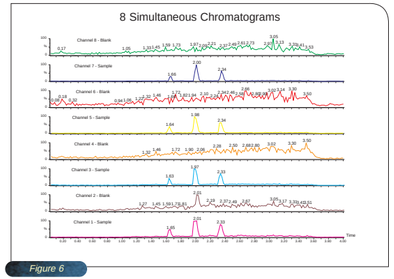

Figure 6

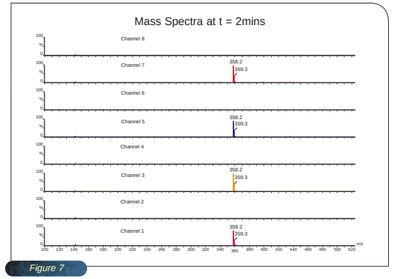

Figure 7

4) Analysis of a 96-Well Plate

Initial investigations have been carried out using the 8-way MUX source for the high throughput analysis of samples synthesised via combinatorial methodology. Impure samples provided in a 96 well microtitre plate were analysed using the same LC and MS conditions as described in the experimental section. Using these conditions, the whole plate was analysed in approximately 1 hour, facilitating an 8-fold increase in throughput.

Following sample analysis, the data files were processed as a batch using Micromass OpenLynx software, which automatically interrogates each acquired data file for the expected mass present from the associated microtitre plate position. The result of this processing is a reduced data set that summarises the success of synthesis across the microtitre plate. This reduced data set is visualised using a browser window shown in **Figure 8**.

Figure 8

Acknowledgements

Andrew Organ, Verner De Biasi and Neville Haskins at SmithKline Beecham, Harlow, UK for their contribution in the development of the MUX interface. Steve Taylor and Mandy Crowther at AstraZeneca, Alderley Park, UK for the provision of the 96 well microtitre plate

References

1) Sage, A *et al*, 'SCI Meeting on High Throughput Screening', Univ of Surrey, 7-9th June 1999.
2) Sage, Ashley B. and Giles, Kevin, Micromass Application Note No.241, June 1999.

Conclusion

This poster shows the design and implementation of an 8-way MUX electrospray interface that has been coupled to an orthogonal acceleration time-of-flight mass spectrometer, used for high throughput mass confirmation. The use of the 8-way MUX source has been applied to the parallel LC/MS analysis of both standard compounds and a combinatorial 96 well plate. Using fast gradient LC, the whole plate was characterised in approximately 1 hour. The data shown here also demonstrates that, with mass spectral data acquired from adjacent spray channels, inter-channel cross talk is negligible. With the ability of the Micromass LCT and MUX source to collect data points at fast acquisition rates, high quality spectra can easily be obtained.

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