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THE USE OF STAGGERED CHROMATOGRAPHY FOR HIGH THROUGHPUT BIOANALYSIS

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AIMS

High throughput bioanalysis plays a central role in the drug discovery and development process. The resulting data are used to determine a drug candidate's pharmacokinetic parameters that are crucial to defining its usefulness in combating disease. This poster describes the use of a tandem quadrupole mass spectrometer for the quantitation of a drug candidate using an integrated mass spectrometer data acquisition and processing software that has staggered injection functionality. The aim is to effectively multiply the throughput with staggered chromatography by a factor of 4 while maintaining good analytical accuracy and precision. We employed the use of staggered chromatography across four parallel HPLC columns interfaced to a single tandem mass spectrometer, cutting analysis time of a batch assay by 75%.

EXPERIMENTAL CONDITIONS

Sample Preparation

Rat plasma samples were processed using an automated 96-well solid phase extraction (SPE) method. C_{18} extraction disk plates were conditioned with methanol and water. Plasma samples (100 µL) were first mixed with 200 µL of IS working solution in ammonium acetate buffer and loaded to the extraction plate. The samples were washed with water and then an SPE wash solution. The samples were eluted with 200 µL of HPLC mobile phase B. 400 µL of mobile phase A were added into each well and mixed to produce 600 µL of processed sample from which 20 µL was injected.

LC Conditions

Mobile phase A:	2 mM Ammonium Acetate (0.2% acetic acid)
Mobile phase B:	2 mM Ammonium Acetate (0.2% acetic acid) Acetonitrile/Methanol
Flow rate:	300 µL/min
Column:	Betasil 5 µm, C_{18}, 100 x 2.1 mm
Injection volume:	5 µL

4x Waters 1525µ HPLC Pumps + Waters 2777 Sample Manager

Gradient Program

Time	A%	B%	Flow
0.00	70	30	0.30
0.10	70	30	0.30
1.00	10	90	0.30
2.20	10	90	0.30
2.30	70	30	0.30
2.50	70	30	0.30

MS Conditions

Instrument:	Waters® Micromass® Quattro Ultima Pt™
Polarity:	ESI Positive
Capillary (kV):	3.00
Cone (V):	60
Source Temperature (°C):	150
Desolvation Temperature (°C):	400
Desolvation Gas Flow (L/Hr):	883
Collision (eV):	20
Multiplier (V):	650
Collision Cell Pressure (mbar):	$4.98e^{-3}$

MRM Acquisition Parameters

Ionization mode: ES+

Transition (m/z)	Dwell (secs)	Cone Volt	Col. Energy (eV)
432.00 > 254.10	0.30	18.0	60.0
436.00 > 258.10	0.30	20.0	60.0

EXPERIMENTAL SETUP

To set the MassLynx™ data acquisition system to staggered chromatography mode the following options were selected (Figure 1).

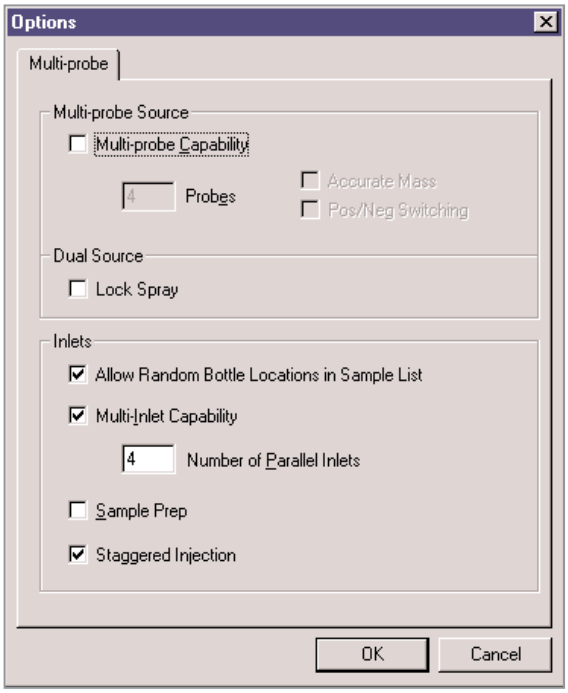

Figure 1. Setup of the Staggered Chromatography option.

The inlet control was configured to control four Waters 1525µ HPLC Pumps and a Waters 2777 Sample Manager. Using the inlet control dialogue box the LC program was entered (Figure 2). No changes were made to the LC gradient for staggered chromatography.

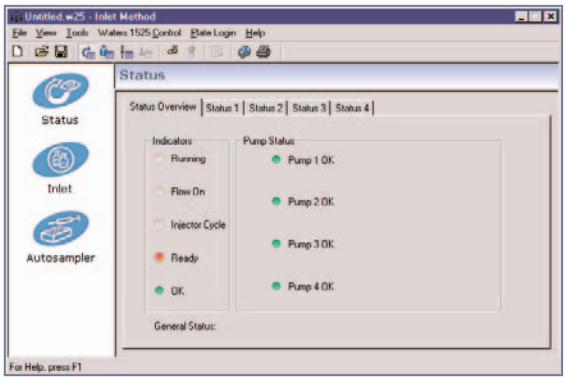

Figure 2. Inlet editor showing the four configured Waters 1525µ HPLC Pumps.

The CTC method editor sample manager was selected. The staggered injection MACRO was entered into the macro sequence. The method run time was used from the LC method (see Figure 3).

The parameter window "FRONT CUT TIME" in the CTC method editor is used to enter the time required from start of injection to start of MS data acquisition.

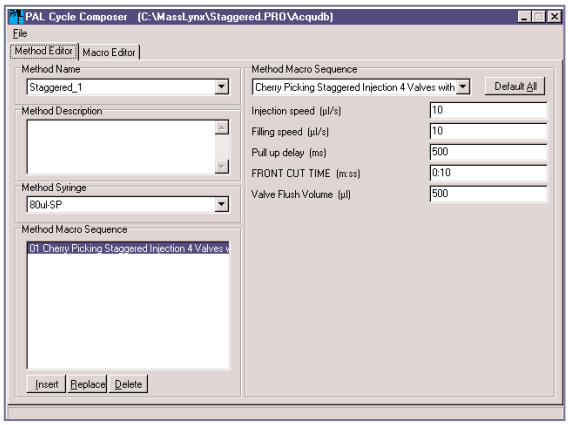

Figure 3. CTC Macro Editor for the Waters 2777 Sample Manager.

RESULTS

1. Figures 4 and 5 show the following product ion spectrum was obtained by direct infusion of a 10 pg/µL standard at 10 µL/min into the mobile phase flowing at 300 µL/min.

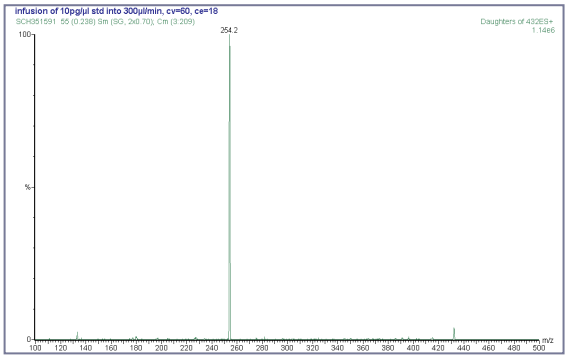

Figure 4. Product ion spectrum of compound SCH 351591.

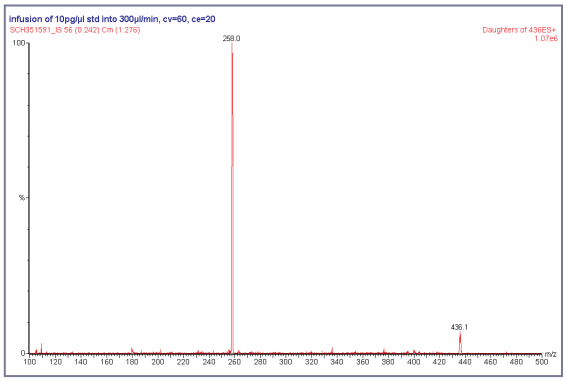

Figure 5. Product ion spectrum of Internal Standard.

2. Four-Channel Staggered Chromatography with biological samples: One Run on One Column Setup

The validation runs for Schering-Plough compound, 351591 (1-1000 ng/mL) were injected with the following setup:

Run 1 samples on Column 1, simultaneously while

Run 2 samples on Column 2, simultaneously while

Run 3 samples on Column 3, simultaneously while

Run 4 samples on Column 4.

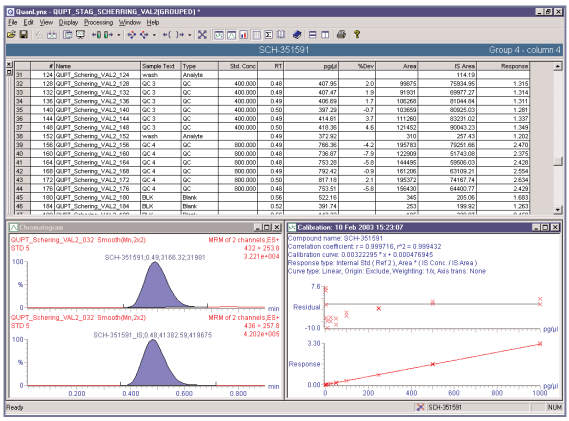

Figure 6. Quantitation report for extracted samples.

Figure 6 is an example of one of the four sets of analysis runs, showing very good linearity, accuracy and precision. The overall statistical data can be seen in Tables 1 and 2.

Calibration curve	R	R^2
1	09996	0.9993
2	0.9998	0.9996
3	0.9996	0.9991
4	0.9997	0.9994

Table 1. Statistical summary of the linearity obtained by using Staggered Chromatography over four columns.

		QC 1.00	QC 3.00	QC 400	QC 800
Run 1 Column 1	Mean	1.20	3.30	409	759
	Difference %	20.0	10.0	2.25	5.13
	RSD (%)	0.98	5.48	0.72	3.38
Run 2 Column 2	Mean	1.10	3.29	415	773
	Difference %	10.0	9.67	3.75	3.38
	RSD (%)	8.76	2.86	3.65	3.40
Run 3 Column 3	Mean	0.98	2.66	414	765
	Difference %	2.00	11.3	3.50	4.38
	RSD (%)	6.66	5.01	2.62	3.73
Run 4 Column 4	Mean	1.19	3.20	409	770
	Difference %	19.0	6.67	2.25	3.75
	RSD (%)	13.1	4.74	1.78	3.85
Between Runs	Mean	1.12	3.03	410	780
	Difference %	12.0	1.00	2.50	2.50
	RSD (%)	14.1	7.56	4.37	5.00

Table 2. Statistical summary of the quality control samples obtained using Staggered Chromatography over four separate columns.

3. Four-Channel Staggered Chromatography with biological samples: One Run on Four Column Setup

The validation runs for SCH 351591 (1-1000 ng/mL) were injected with the following setup:

Run 1 Samples on Column 1, Column 2, Column 3, and Column 4, followed by

Run 2 Samples on Column 1, Column 2, Column 3, and Column 4, then

Run 3 Samples on Column 1, Column 2, Column 3, and Column 4, finally

Run 4 Samples on Column 1, Column 2, Column 3, and Column 4.

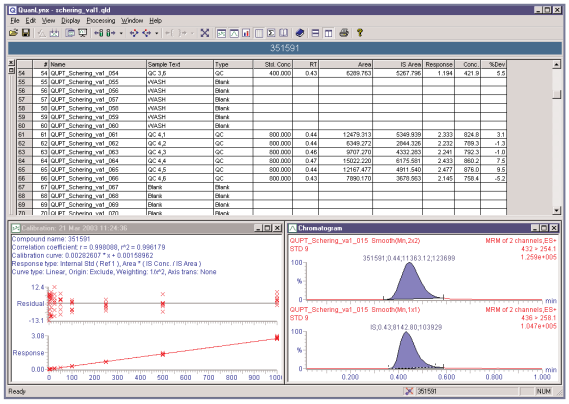

Figure 7. Quantitation results for 336 samples injected on all four columns.

The results of the quality control samples are summarized in Tables 3 and 4 with samples run across all four columns.

		QC 1	QC 3.0	QC 400	QC 800
Run 1	Mean	0.98	2.82	424	817
	Difference %	2.00	6.00	6.00	2.13
	RSD (%)	7.44	7.05	1.83	5.53
Run 2	Mean	1.06	3.14	415	786
	Difference %	6.00	4.67	3.75	1.75
	RSD (%)	5.70	2.27	4.60	3.22
Run 3	Mean	0.94	3.05	400	776
	Difference %	6.00	1.67	0	3.00
	RSD (%)	9.03	4.03	2.52	3.81
Run 4	Mean	1.03	3.14	399	744
	Difference %	3.00	4.67	0.25	7.00
	RSD (%)	9.16	3.38	4.04	2.10
Between Runs	Mean	1.00	3.03	410	780
	Difference %	0	1.00	2.50	2.50
	RSD (%)	11.7	7.56	4.37	5.00

Table 3. Overall precision and accuracy data for the validated assay using staggered chromatography for individual separated runs.

	QC 1.0	QC 3.0	QC 400.0	QC 800.0
Mean	1.60	3.06	403.29	765.94
Difference (%)	6.0	2.0	0.82	4.26
RDS (%)	10.34	7.49	4.37	5.25

Table 4. Accuracy and precision results for combined runs without separating out the individual runs.

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CONCLUSION

Staggered chromatography with four HPLC columns showed excellent linearity (r2 values greater than 0.999) over the range 1 to 1000 ng/mL for four batch runs injected in two different ways: one run on one column and one run across four columns.

Maximum throughput was achieved by performing sample analysis in multiples of four and completing the analysis in the same time as a single batch. A total of 336 samples were run, with a run time of 4 minutes per sample. This would take approximately 22.5 hours to run. With staggered chromatography the total run time for the same amount of samples was approximately 5.5 hours.

Furthermore, other commercially available products that can perform staggered chromatography often require a separate software package. With the Waters approach, the software is integrated into the mass spectrometer operating system, simplifying operation and reducing time and cost consuming validation efforts.

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