APPLICATION OF ULTRA FAST LC-MS TO HIGH THROUGHPUT SCREENING ASSAYS IN ADME DISCOVERY

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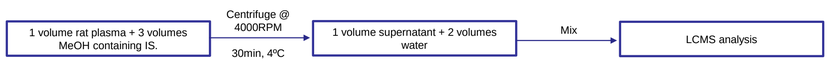
INTRODUCTION

A significant challenge for laboratories undertaking high throughput ADME screening is addressing the need for increased throughput. This is driven by the requirement to increase the number of drug candidates screened in a given time or reduce the turnaround time for a defined set of compounds to help speed up the decision making process in compound selection. Reducing assay sample LC-MS cycle time is one way of doing this and is effective when combined with increased sample preparation automation, automated data evaluation and/or elimination of the MS optimisation process by using HRAM MS. Here we compare the chromatographic quality of an ultra fast UHPLC method to our laboratory's current approach. The system was applied to a clearance assay for evaluation of its applicability.

METHODS

Sample Preparation

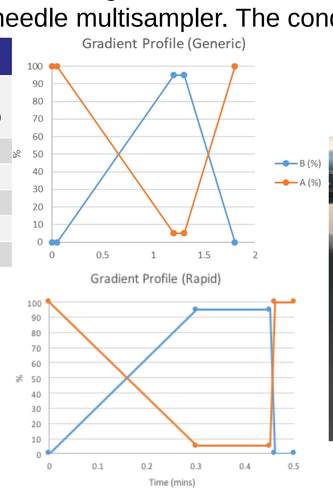
Samples were prepared in extracted rat plasma as follows:



Analytical Conditions

Samples were analysed using a Sciex Triple TOF 6600 high resolution accurate mass spectrometer and Agilent 1290 Infinity II UHPLC system with dual needle multisampler. The conditions were as follows:

Method	Rapid	Generic
Columns Used	Poroshell 120 EC-C18 (2.1µm) 5 x 2.1mm, VanGuard (2.5µm) 5 x 2.1mm	ACQUITY HSS T3 2.1 x 50mm (1.8µm)
Column Temp	RT (20ºC)	60ºC
Injection Vol.	1µL	8µL
Mobile Phase A	10mM ammonium formate + 0.1% formic acid (aq.)	
Mobile Phase B	HPLC gradient grade methanol	
Accumulation Time	0.04999s (96 points per peak)	



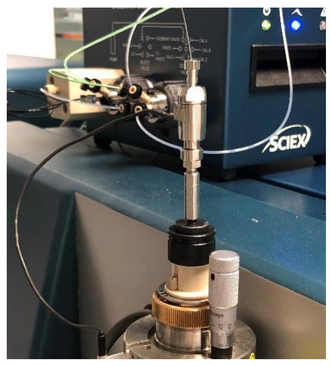


Fig. 1. The column-source connection

The MS acquisition conditions were:

Compound	m/z	Mass Range /Da
Nadolol	310.194	±0.02
Metoprolol	268.183	±0.02
Reserpine	609.273	±0.02
Mifepristone	430.267	±0.02
Rosuvastatin	482.546	±0.02

Ion Source	Turbo Spray (ESI+)
Source Temp	700ºC
Scan Mode	TOF MS

For the rapid system, the columns were attached directly to the source as in Fig.1 to minimise post column volume.

CHROMATOGRAPHY

The quality of the chromatography was assessed for multiple columns. A solution containing four model test compounds was injected to determine the chromatographic quality in terms of tailing factors and broadness. Representative chromatograms are outlined in Fig. 2:

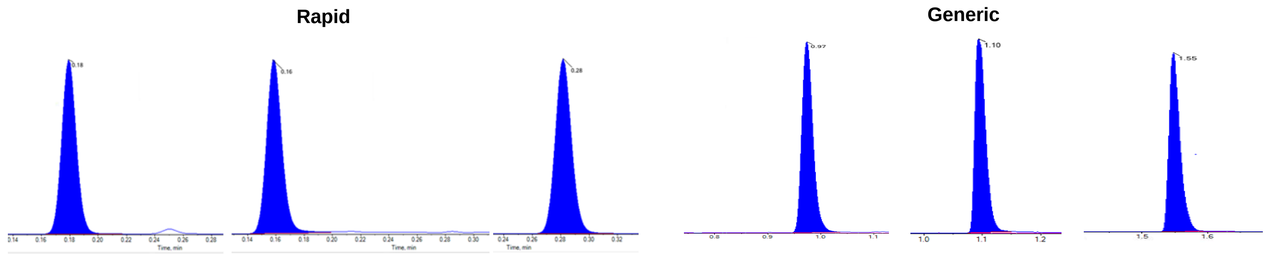


Fig. 2. Peak shapes for nadolol, metoprolol and mifepristone; respectively. Rapid and generic. Samples prepared at 500nM (5mM mifepristone) in 1:1 MeOH:H_2O. *Not to scale.*

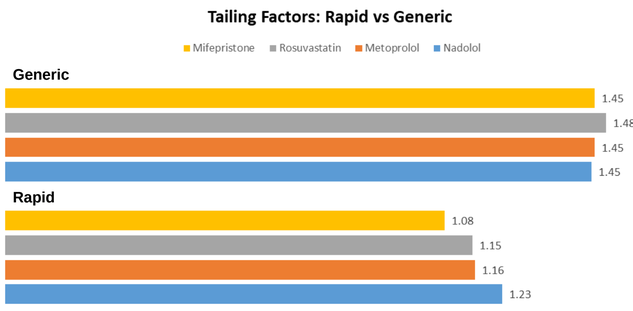


Fig. 3. Tailing factors

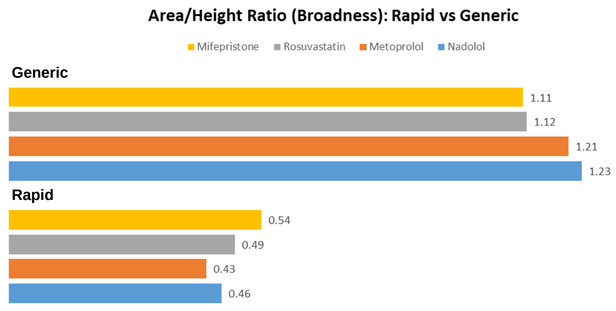


Fig. 4. Indication of peak broadness

Based on the two parameters outlined, both peak broadness and tailing factors were shown to be superior on the rapid system.

This data suggests that the ultra-fast chromatography provides comparable or better chromatographic data than our laboratory's generic method.

SENSITIVITY

The effects of a rapid flow rate of 2mL/min were also evaluated. Replicate plasma samples at 200nM (2mM mifepristone) were analysed at both 2mL/min (rapid) and 0.6mL/min (generic):

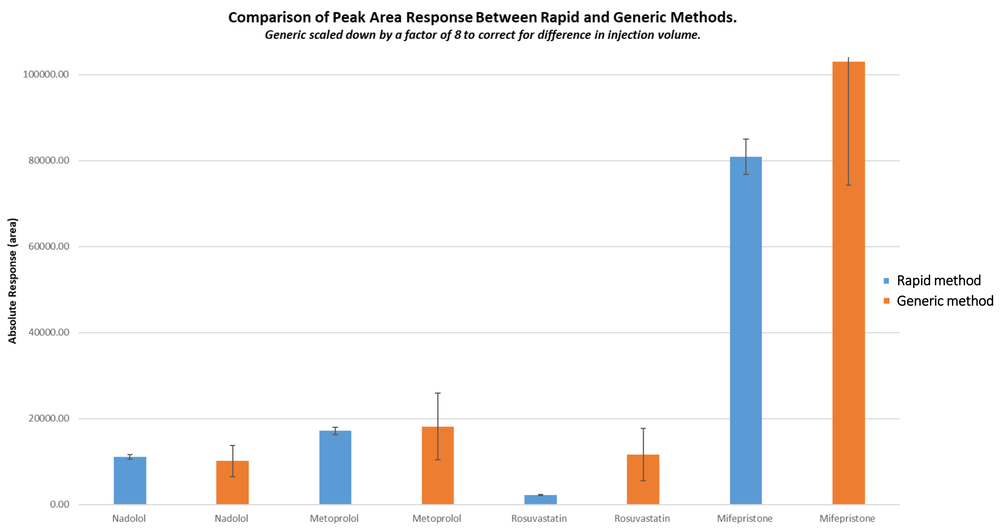


Fig. 5. Response comparison at rapid and generic flow rates

Fig. 5 indicates that the difference in sensitivity at different flow rates, for all compounds except rosuvastatin, was not statistically significant.

MATRIX EFFECTS

To rule out any ion enhancement or suppression at higher flow rates, the matrix effects were evaluated. Replicate 200nM (2mM mifepristone) samples were analysed by spiking extracted rat plasma and neat solvent to determine the matrix factor:

	Nadolol		Metoprolol		Mifepristone	
	Mean Peak Area	Matrix Factor (%)	Mean Peak Area	Matrix Factor (%)	Mean Peak Area	Matrix Factor (%)
Neat Solvent	9.99E+03	5.2	2.23E+04	6.0	8.75E+04	-12.5
Extracted Plasma	1.05E+04		2.37E+04		7.78E+04	

The calculated matrix factors indicate no significant (< ±20%) ion suppression or enhancement.

LINEARITY AND REPRODUCIBILITY

The linearity of the responses was evaluated at 2mL/min and 0.6mL/min to ensure calibration lines could be obtained over a comparable range. Fig. 6 indicates that the linearity between systems is comparable:

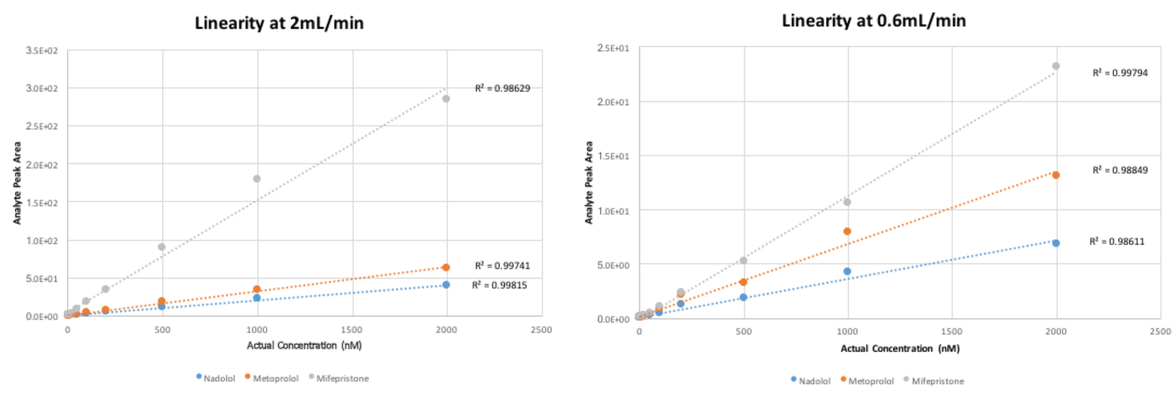


Fig. 6. Calibration lines produced on the ultra-fast method (LHS) and the generic method (RHS). *Mifepristone Std conc. 10 fold higher than shown on x-axis.*

Replicate QC injections for both systems demonstrated comparable reproducibility of the systems, within the acceptance threshold of 15%:

	Nadolol		Metoprolol		Mifepristone	
	Generic	Rapid	Generic	Rapid	Generic	Rapid
Mean Detected Concentration (nM)	208	228	224	216	2080	1670
CV (%)	10.7	8.9	11.9	9.4	6.8	8.9

ROBUSTNESS

Since the ultrafast columns are far shorter than the standard UHPLC columns, they may be more susceptible to degradation in performance during use. In order to rule out mid-assay column degradation, their robustness was assessed over replicate injections.

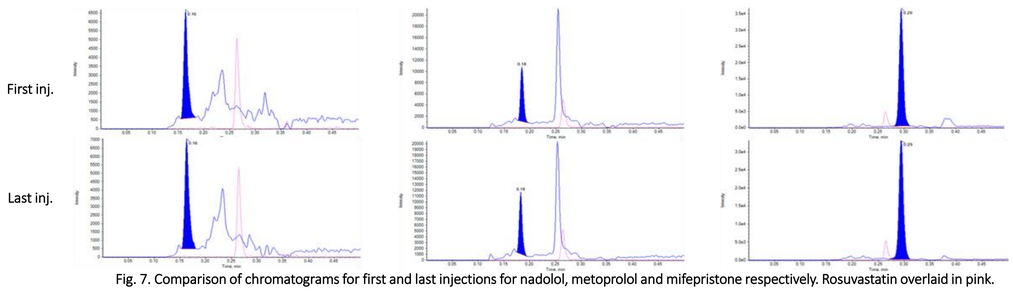


Fig. 7. Comparison of chromatograms for first and last injections for nadolol, metoprolol and mifepristone respectively. Rosuvastatin overlaid in pink.

Fig. 7 indicates no notable deterioration of the peak shapes over >800 replicate injections.

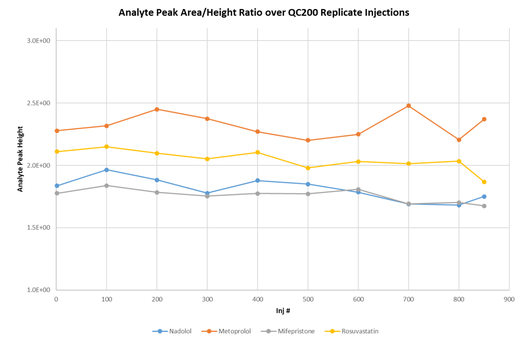


Fig. 8. Analyte peak area/height ratio over replicate 200nM injections (2mM mifepristone)

Fig. 8 indicates no increase in peak broadness over replicate QC200 injections, indicating no significant deterioration of the column.

APPLICATION TO HT-ANALYSIS COMPARATIVE DATA

The ultra-fast chromatographic system was applied to a hepatocyte stability assay using three benchmark compounds: prazosin, verapamil and umbelliferone. Clearance values (Cl_{int}) and half lives were determined using both the generic and ultra-fast methods.

Compound	Cl_{int} rapid (µL/min/10^6 cells)	Cl_{int} generic (µL/min/10^6 cells)	$t_{0.5}$ rapid (mins)	$t_{0.5}$ generic (mins)	% Difference Cl_{int}	% Difference $t_{0.5}$
Mouse						
Prazosin	46.2	48	15	14.4	4	4
Verapamil	460	535	1.51	1.29	14	17
Umbelliferone	UTD	UTD	UTD	UTD	UTD	UTD
Rat						
Prazosin	9.22	8.04	75.2	86.2	15	13
Verapamil	434	462	1.6	1.50	6	7
Umbelliferone	UTD	UTD	UTD	UTD	UTD	UTD
Dog						
Prazosin	0.82	1.2	849	576	32	47
Verapamil	112	133	6.17	5.21	16	18
Umbelliferone	433	477	1.6	5.79	9	72
Human						
Prazosin	8.36	9.18	82.9	75.5	9	10
Verapamil	92.2	120	7.52	1.45	23	418
Umbelliferone	147	197	4.72	3.52	25	34

Representative chromatograms are shown in Fig. 9:

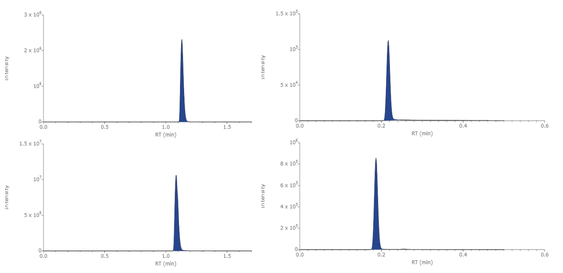


Fig. 9. Prazosin (top) and IS, metoprolol (bottom) at t=0. Generic method on LHS, rapid on RHS.

Fig. 9. shows the consistency of the chromatography between systems.

The data obtained was quite comparable between the two systems.

SUMMARY/ CONCLUSIONS

Ultra-fast chromatography with a 0.5min gradient at a flow rate of 2mL/min has been successfully demonstrated. Peak shapes obtained were consistent with those from our generic methods allowing for the successful quantification of model drug compounds in biological matrices.

The potential for application of the system to high throughput ADME-TOX assays has been demonstrated. The introduction of this system in our laboratory, in place of current analytical methods, would have a three fold improvement in LCMS cycle times providing a significant opportunity to increase throughput.