Application of Acquity Premier Technology for Enhanced Chromatographic Performance of Pharmaceutical Compounds in RP-UPLCMS

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Introduction

The high-throughput screening of analyte molecules in ADME laboratories can be challenging when dealing with compounds that exhibit non-specific binding[1)]. The analysis of such compounds is characterised by poor chromatography, low recovery and reduction in sensitivity. This consequently results in poor assay sensitivity and reproducibility, prolonged data analysis and complicated method development. Recent advancements in chromatographic technology have been established to mitigate the effects of analyte/surface interactions[2)]. These UPLC platforms have an inert internal surface coating on the metallic components in the liquid chromatography flow path, column frits and column wall to reduce surface/analyte interactions[3]. This study demonstrates the benefits of using the Waters Premier LC system in carrying out ADME screening with improved chromatographic performance and increased sensitivity.

Methods

Analyses performed using the following instrument parameters:

LC-MS Conditions

LC System	Acquity Premier and Acquity UPLC I-Class
Columns	Acquity Premier HSS T3 2.1 X 50 mm, 1.8 µm; Acquity UPLC HSS T3 2.1 X 50 mm, 1.8 µm
Column temperature	40°C
Injection volume	4 µL
Flow rate	1 mL/min
Mobile phase A2	10mM Ammonium formate + 0.1% Formic acid
Mobile phase B2	HPLC gradient grade methanol
Mobile phase A for peptide work	10 mM Ammonium acetate in water
Mobile phase B for peptide work	HPLC gradient grade Acetonitrile
Ion source and polarity	ESI (+)
Desolvation temperature	650ºC
Dwell time	0.034 s

LC Gradient

Time / Min	% A	% B
0.00	99.9	0.1
0.03	99.9	0.1
0.60	5.0	95.0
0.65	5.0	95.0
0.80	99.9	0.1
0.90	99.9	0.1

MS/MS Parameters

Compound	Precursor (m/z)	Product (m/z)	Cone (V)	CE (V)
Bepridil	372.2	184.1	7.0	20.0
Hydralazine	161.0	88.9	7.0	20.0
Reserpine	609.3	195.1	49.0	50.0
Peptide-1	809.0	153.9	35.0	50.0
Peptide-2	672.0	200.0	42.0	40.0

Figure 1: Acquity Premier UPLC-MS/MS

Sample preparation

- Compounds dissolved in DMSO at 10 mM concentration prior to dilution to 50 nM with MeOH:H2O
- Standard curve prepared at 5 – 5000 nM with serial dilution
- Analysis performed in triplicate on both standard and premier systems
- Same mobile phase used throughout analysis

Results and Discussion

Linearity and QC Response: Reserpine

Standard curve prepared at 5-5000 nM concentration. Good linearity achieved in both premier and non premier systems with negligible differences as shown in Figure 3 below.

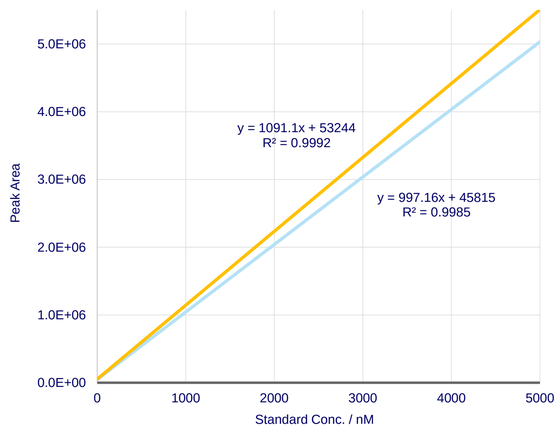

Figure 2: Reserpine standard curves for the standard configuration (Orange) and premier (blue) systems

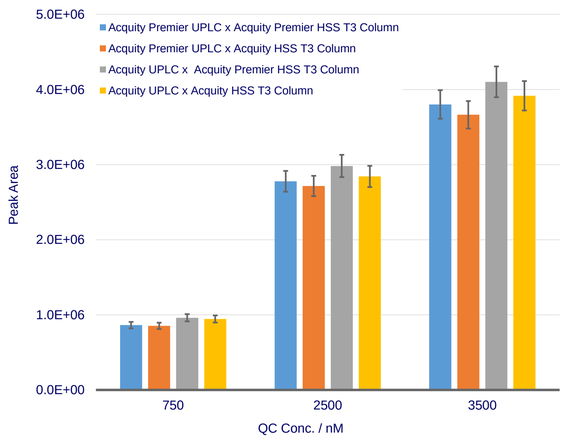

Figure 3: Reserpine QC response for 4 different system/column configurations

Carryover in matrix and solvent: Bepridil

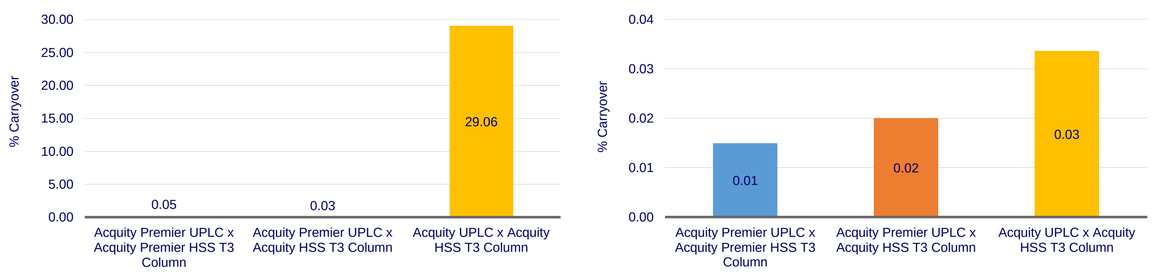

Figure 4: Bepridil carryover in matrix (crashed plasma, left) and solvent (1:1 MeOH:H_2O, right), between the Acquity and Acquity Premier UPLC systems with various column types

- Bepridil injected at 50 nM, carryover measured in following blank injection and expressed as a percentage of 50 nM response
- Samples in matrix exhibit much higher carryover than in solvent with Acquity standard UPLC column
- Matrix induced carryover significantly negated through use of Premier system
- Carryover attributed to injection ports and valves

Peak Shape at Low Concentration

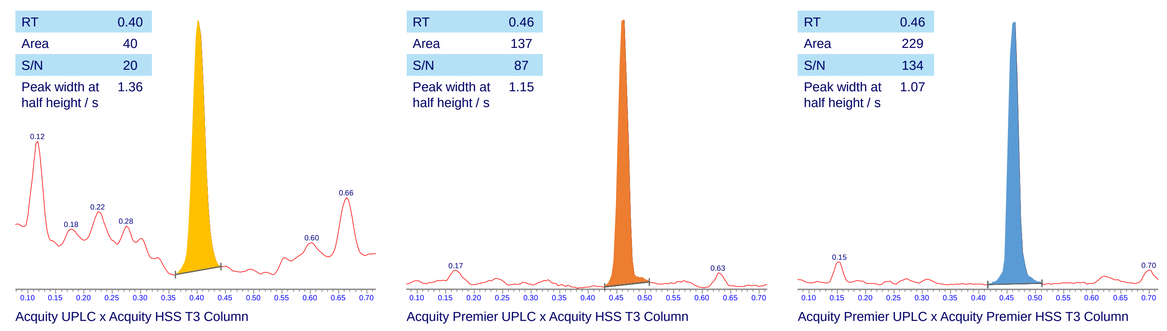

Figure 5: Peak shape of Hydralazine at 0.1 nM across 3 different system/column configurations

- Hydralazine injections at 0.1 nM conc, 2 uL injection volume
- Increased peak area from use of premier system, further increase with use of system and column – Higher S/N = Better limits of detection
- Decreased peak width/sharper peaks observed in premier system due to lower system volume, decreased band broadening and reduced interaction with internal surfaces
- Overall a significant improvement in chromatography through use of the premier system and column at low concentrations

Peak Shape Comparison: Peptide

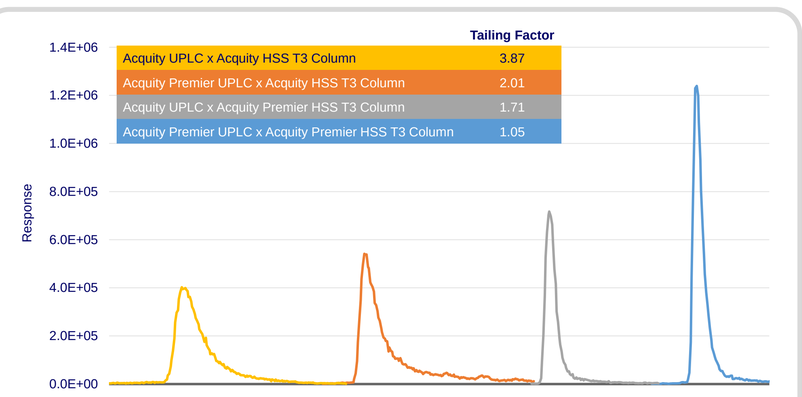

Figure 6: Peak shape & tailing factor of a peptide across 4 different system/column configurations

- Peak shape evaluation of a peptide injected at 50 nM on 4 system/column configurations
- Significant reduced tailing and sharper peaks achieved via use of premier column
- Further improvement with introduction of Premier system
- Column responsible for greatest reduction in tailing factor

Sensitivity of Problematic Compounds Across Systems

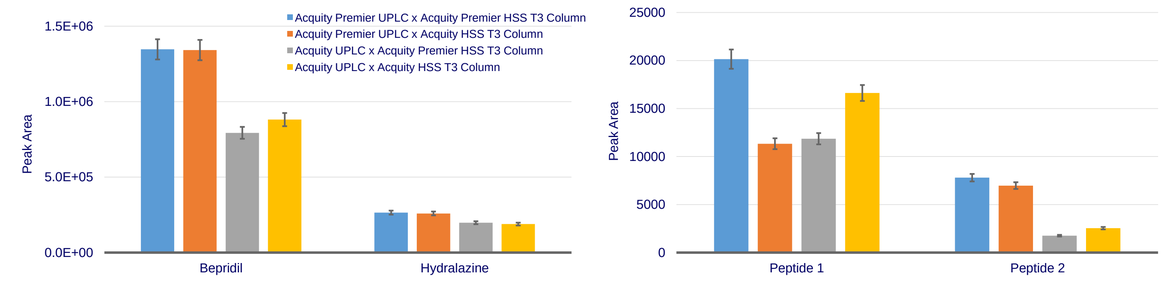

Figure 7: Peak areas of 4 problematic compounds across 4 different system/column configurations

- All compounds injected at 50 nM in triplicate
- Increase in signal intensity observed with fully inert premier system
- Samples run on premier column followed by standard column resulted in higher response observed in the non-premier system with standard column due to system passivation

Premier System Assay Application

Assessment of transporter/substrate binding. Final values are expressed as a ratio of compound (pmol) to cell material (mg), representing the amount of proprietary compound transported into the cell. Comparison to control group indicates importance of transporter proteins in the movement of the substrate.

1) Initial results suggest high uptake of test compound and therefore efficacious transporter/substrate binding
2) Carryover observed from high concentration standard curve injections resulting in overrepresentation of transportation
3) Premier system exposes non-specific binding
4) Assay repeats on Premier system indicate poor transporter/ substrate interaction

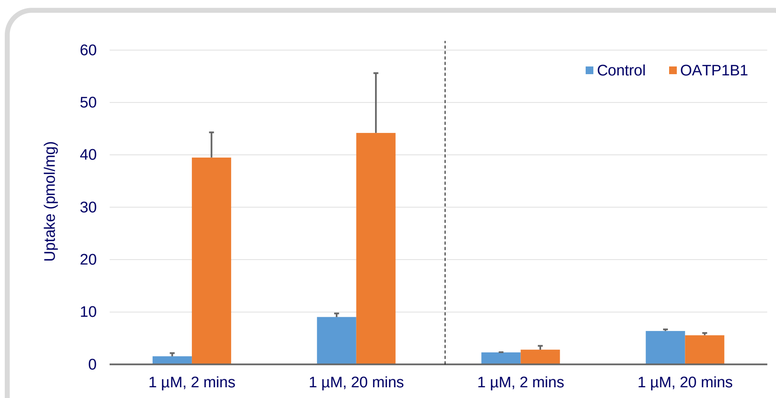

Figure 8: Transporter/substrate binding results from the standard (left) and Premier (right) systems

Conclusions

- The suitability of the Waters Acquity Premier was assessed for high-throughput analysis, differences in system configuration may cause barrier to application compared to current configurations
- Reproducible standard curves and QC responses as used for system checks and assay internal standards
- Lower internal system volume and inert internal surfaces in column and system provide improved peak shape, reduced tailing factors and Improved limits of detection
- Significantly reduced method development turnaround and rapid diagnosis of non-specific binding without passivation requirement
- Full premier system provides confidence of elimination of non-specific binding
- Future work will include oligonucleotide and phosphate containing compounds

References

1) Giddings, J.C. and Eyring, H. (1955). A Molecular Dynamic Theory of Chromatography. *The Journal of Physical Chemistry*, 59(5), pp.416–421.

2) Birdsall, R.E., Kellett, J., Yu, Y.Q. and Chen, W. (2019). Application of mobile phase additives to reduce metal-ion mediated adsorption of non-phosphorylated peptides in RPLC/MS-based assays. *Journal of Chromatography B*, [online] 1126-1127, p.121773.

3) Birdsall, R.E., Kellet, J., Ippolit, S., Ranbaduge, N. and Shion, H. (2020). Increasing Chromatographic Performance od Acidic Peptides in RPLC-MS-based Assays with ACQUITY PREMIER featuring MaxPeak HPS Technology, Increasing Chromatographic Performance of Acidic Peptides in RPLC-MS-based Assays with ACQUITY PREMIER featuring MaxPeak HPS Technology | Waters Accessed 21st April 2022