A VALIDATED METHOD FOR RISPERIDONE IN HUMAN PLASMA USING UPLC/MS/MS

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INTRODUCTION

HPLC/MS/MS is the technique of choice for the quantification of drug substances in biological matrices during drug development and pharmacokinetic studies. The inherent sensitivity and selectivity of this technique allow the development of robust analysis methods with short chromatographic retention times to facilitate fast sample turnaround. However, there are potential challenges with LC/MS/MS. By reducing analysis times, the probability of the drug substance co-eluting with an interfering compound is increased. Ion suppression due to endogenous compounds in biological matrixes can lead to deterioration in the lower limit of quantification (LLOQ) and interferences from co-eluting drug metabolites can give falsely elevated responses for the drug substances. Improved sample preparation can reduce these effects, but it is often necessary to develop longer chromatographic methods to separate the drugs from interferences. This can result in reduced sample throughput.

In this project we have utilized Ultra Performance LCTM (UPLCTM) coupled to a tandem quadruple mass spectrometer to develop and validate a bioanalytical method for the determination of risperidone and it's major metabolite, 9–OH risperidone, in human plasma using clozapine as an internal standard. UPLC allows the use of shorter run times while maintaining or increasing the chromatographic resolution which reduces the probability of matrix interferences.

METHODS

HPLC Conditions

Waters Alliance® HT

Column:	Waters X-Terra® MS C18 2.1 x 50mm, 3.5µm			
Eluent A:	2mM ammonium acetate in water, pH 9.0			
Eluent B:	100% methanol			
Flow Rate:	0.3 mL/min			
Gradient:	Time (min)	%A	%B	Curve
	0	50	50	-
	0.5	50	50	6
	2.0	0	100	6
	3.5	50	50	11
Run Time:	5.5 minutes			
Column Temp:	40°C			
Injection Volume:	5µL			

UPLC Conditions

Waters ACQUITY™ UPLC™ System

Column:	Waters ACQUITY UPLC™ BEH C_{18}, 2.1 x 50mm, 1.7µm			
Eluent A:	2mM ammonium acetate in water, pH 9.0			
Eluent B:	100% methanol			
Flow Rate:	0.6 mL/min			
Gradient:	Time (min)	%A	%B	Curve
	0	50	50	-
	0.25	50	50	6
	0.75	0	100	6
	1.25	50	50	11
Run Time:	1.5 minutes			
Column Temp:	50°C			
Injection Volume:	5µl			

MS Conditions

Instrumentation:	Waters Quattro Premier™, ESI+				
Capillary Voltage:	1.0kV				
Desolvation Temperature:	350°C				
Desolvation Gas Flow:	800L/hour				
Cone Gas Flow:	50L/hour				
Collision Gas Pressure:	3.5e-3 mbar (argon)				
MRM Transitions:					
Compound	Precursor	Product	Dwell	CV	CE
Risperidone	411.3	191.3	30ms	40V	30eV
9-OH Risperidone	427.4	207.2	30ms	40V	30eV
Clozapine (IS)	327.1	270.3	30ms	35V	25eV
Inter-channel Delay:	10ms				
Inter-scan Delay:	10ms				

Sample Preparation

Sample: 100µL plasma spiked with Risperidone, 9-OH Risperidone and internal standard.

Protein Precipitation (PPT)

500µL methanol used to carry out protein crash
200µL of supernatant is transferred to a auto sampler vial and diluted with 200µL of water prior to injection

Solid Phase Extraction (SPE)

Water OASIS® MCX 30mg 96 well plate

Condition:	1mL methanol
Equilibrate:	1mL water
Load:	1mL of sample *(sample =100µL plasma + 1000µL water)*
Wash 1:	1mL 2% formic acid in water
Wash 2:	1mL 100% Methanol
Elute:	500µL (2 x 250µL) 5% NH_4OH in methanol

Dilute extract with 500µL of water prior to injection

The validation was carried out using the SPE method the PPT samples were only used for qualitative matrix effect experiments

RESULTS

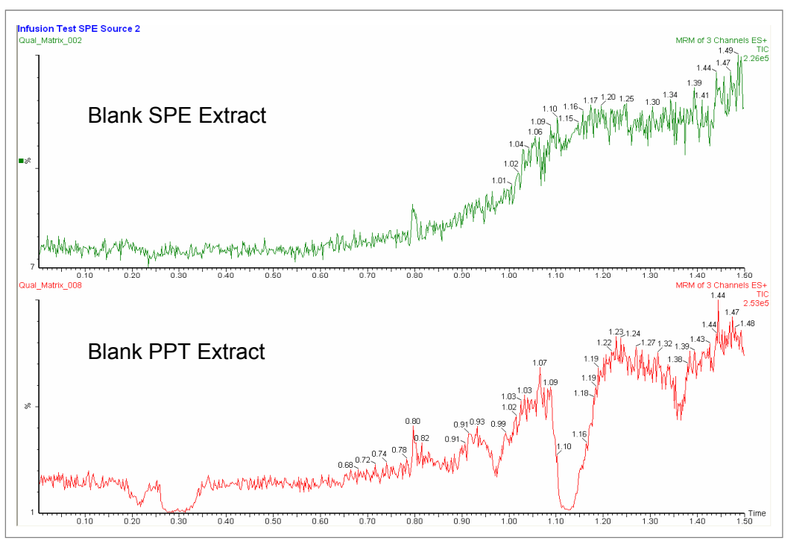

Figure 1. SPE vs. PPT qualitative matrix effect.

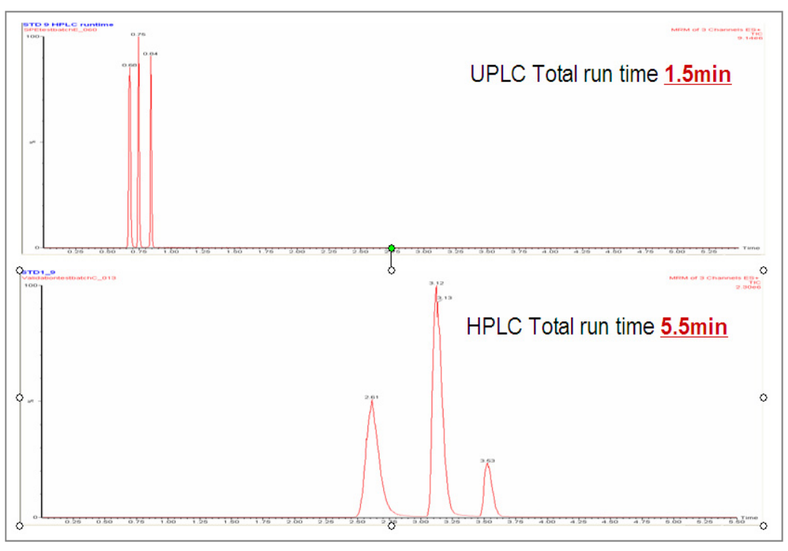

Figure 2. UPLC run time vs. HPLC run time.

Figure 3. Chromatographic resolution UPLC™ vs. HPLC

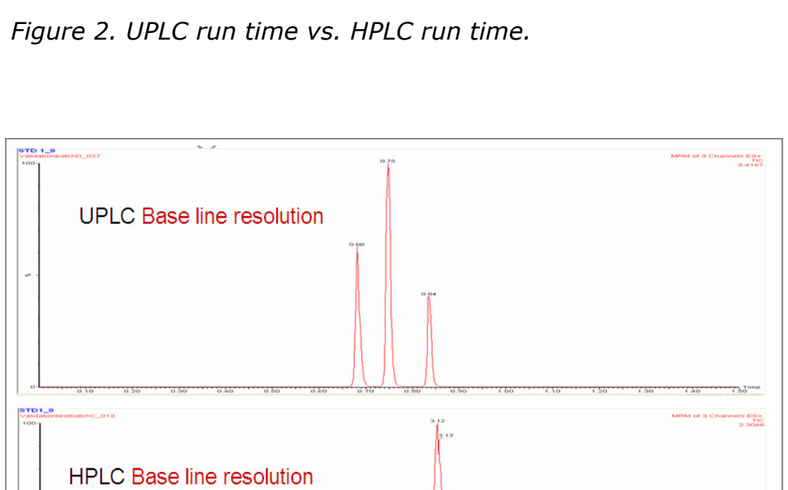

Figure 4. UPLC run time vs. HPLC run time.

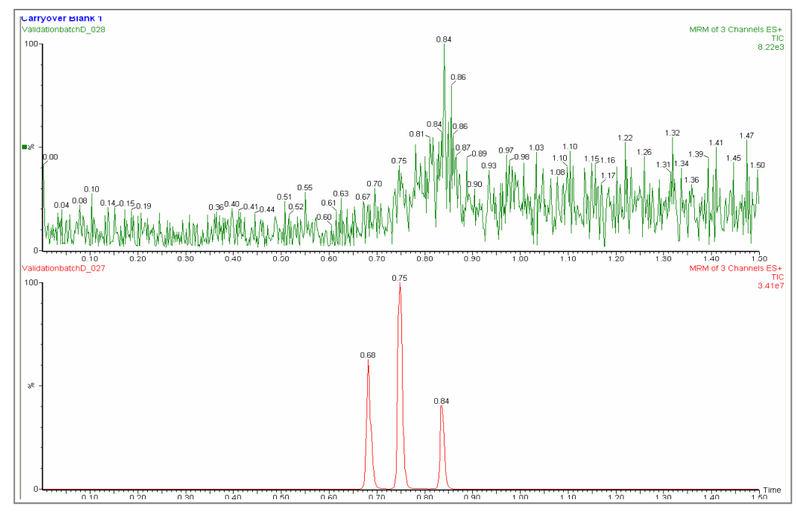

Figure 5. UPLC™ Carryover example , blank after high concentration standard.

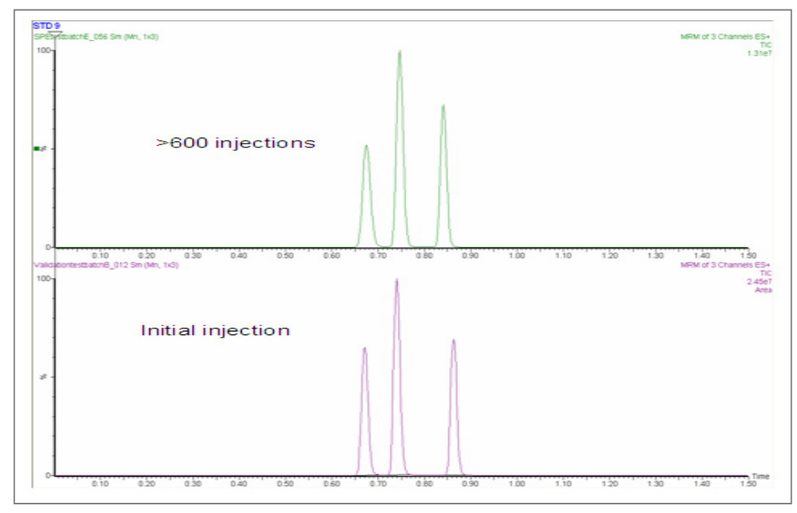

Figure 6. Stability of UPLC column after >600 injections.

DISCUSSION

A method for the determination of Risperidone and 9-OH Risperidone in human plasma has been successfully developed and validated. The accuracy and precision over the validation range of 0.1-200ng/mL was ±10% and ±5% respectively with correlation coefficients (r^2) of >0.998.

Figure 1 is an example of a SPE extract vs. a PPT extract, the qualitative matrix effect test shows that the use of SPE produces extracts that contain significantly less interferences that can lead to ion suppression when compared to PPT extracts. The matrix effects seen in the PPT sample are due to the very high concentrations of phospholipids in the plasma sample that are not removed by PPT. In this example we also separated the matrix effect from the peaks of interest chromatographically but in many cases this will not be possible and the required limits of quantification may not be reached. The use of the MCX (Mixed Mode Cation Exchange) SPE removes these interferences from the sample, therefore less time is required during LC method development.

The UPLC method that has been developed resulted in a 70% decrease in analysis time compared to HPLC (Fig 2.) without a loss of chromatographic resolution (Fig 3.) allowing a three fold increase in sample throughput, because of the very low system volume in the UPLC, long equilibration times are not required when gradients are being used. This increases the sample throughput allowing the efficient use of the MS/MS. This increase in sample throughput also applied to the method development process.

The very narow peak widths produced by the UPLC, typically 3 seconds wide at base, result in an increase in the signal to noise ratio. This will allow lower limits of quantification to be reached compared to HPLC, in this example (Fig 4.) a 3 fold increase in the signal to noise was achieved.

Figure 6. shows the robustness of the UPLC chromatography after more than 600 injections, to test the robustness of the column it was constantly stored under the UPLC conditions (pH9.0 @ 50°C), the system back pressure throughout the validation ranged from 9000-11000psi. These back pressures are produced by the use of the 1.7µm particle size in the UPLC column, a conventional HPLC system can not operate under these conditions.

CONCLUSION

- **A method for the analysis of Risperidone and 9-OH Risperidone has been fully validated**
- **Accuracy and Precision results exceed FDA guidelines for bioanalytical method validation**
- **SPE allows the generation of cleaner extracts that result in significantly less ion suppression than PPT**
- **Significant reductions in LC run time can be achieved using UPLC resulting higher sample throughput, while maintaining resolution**
- **UPLC allows lower limits of detection to be reached due to decrease in peak width and an increase in signal/noise ratio**

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