

OVERVIEW

- Carry out a full validation for the analysis of Risperidone and 9-OH Risperidone in human plasma
- Compare different sample preparation techniques for matrix effects
- Compare HPLC vs. UPLC™ for
 - Sensitivity
 - Speed
 - Resolution

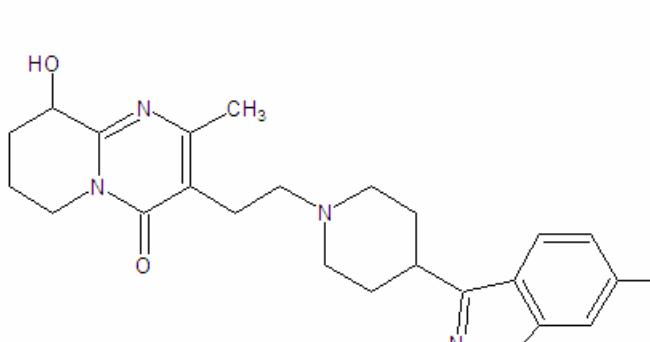
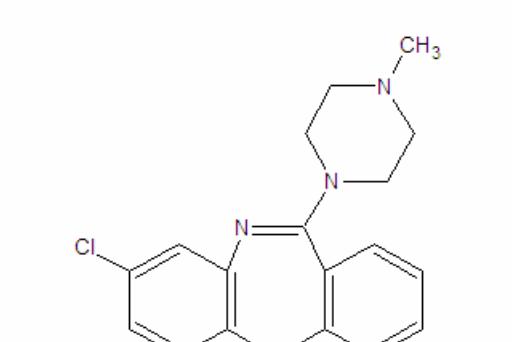
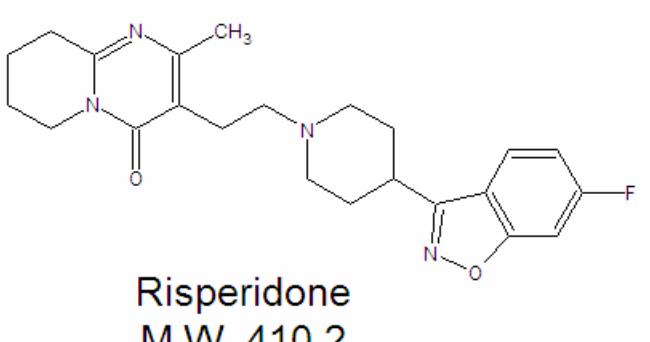
INTRODUCTION

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 robust analysis methods with short chromatographic retention time to be developed so that fast sample turnaround can be achieved. 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 matrices can lead to deterioration in the limit of quantification (LLOQ) or 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 results in reduced sample throughput.

In this project we have utilized Ultra Performance LC™ (UPLC™) coupled to a tandem quadrupole mass spectrometer to develop and validate a bioanalytical method for the determination of Risperidone and its major metabolite, 9-Hydroxyrisperidone, in human plasma using Clozapine as an internal standard. UPLC allows the use of shorter run times with maintaining or increasing the chromatographic resolution reducing the probability of matrix interferences.



METHODS

HPLC Conditions

Waters Alliance® 2795 HPLC System
Column: Waters Xterra® MS C₁₈, 2.1 x 50mm, 3.5μm
Mobile Phase A: 2mM ammonium acetate in water, pH 9.0
Mobile Phase 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

Total run time: 5.5min
Column Temperature: 40oC
Injection Volume: 5μL

UPLC Conditions

Waters ACQUITY™ UPLC™ System
Column: Waters ACQUITY UPLC™ BEH C₁₈, 2.1 x 50mm, 1.7μm
Mobile Phase A: 2mM ammonium acetate in water, pH 9.0
Mobile Phase B: 100% methanol

Flow rate: 0.6 mL/min

Gradient:

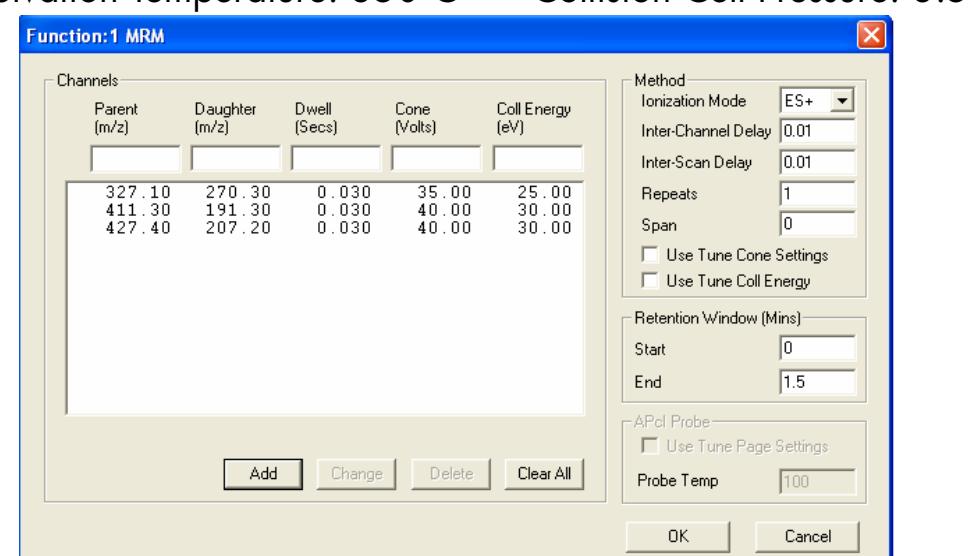
Time (min)	%A	%B	Curve
0	50	50	-
0.25	50	50	6
0.75	0	100	6
1.25	50	50	11

Total run time: 1.5min
Column Temperature: 50oC
Injection Volume: 5μL

MS Conditions

Waters Quattro Premier™ tandem quadrupole MS, ESI+

- Capillary Voltage: 1.0V
- Source Temperature: 120°C
- Desolvation Temperature: 350°C
- Desolvation Gas Flow: 800 L/Hr
- Cone Gas Flow: 50 L/Hr
- Collision Cell Pressure: 3.5e-3 mbar



SAMPLE PREPARATION

Sample: 100μL plasma (Na Heparin as anti coagulant) spiked with Risperidone , 9-OH Risperidone and internal standard

Linear range of 0.1-200ng/mL

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 with 1ml methanol
Equilibrate with 1ml water
Load 1ml of sample (100μL plasma sample + 1000μL water)
Wash 1 1ml 2% formic acid in water
Wash 2 1ml 100% Methanol
Elute 500μL (2 x 250μL) 5% NH₄OH in methanol
Dilute extract with 500μL of water prior to injection

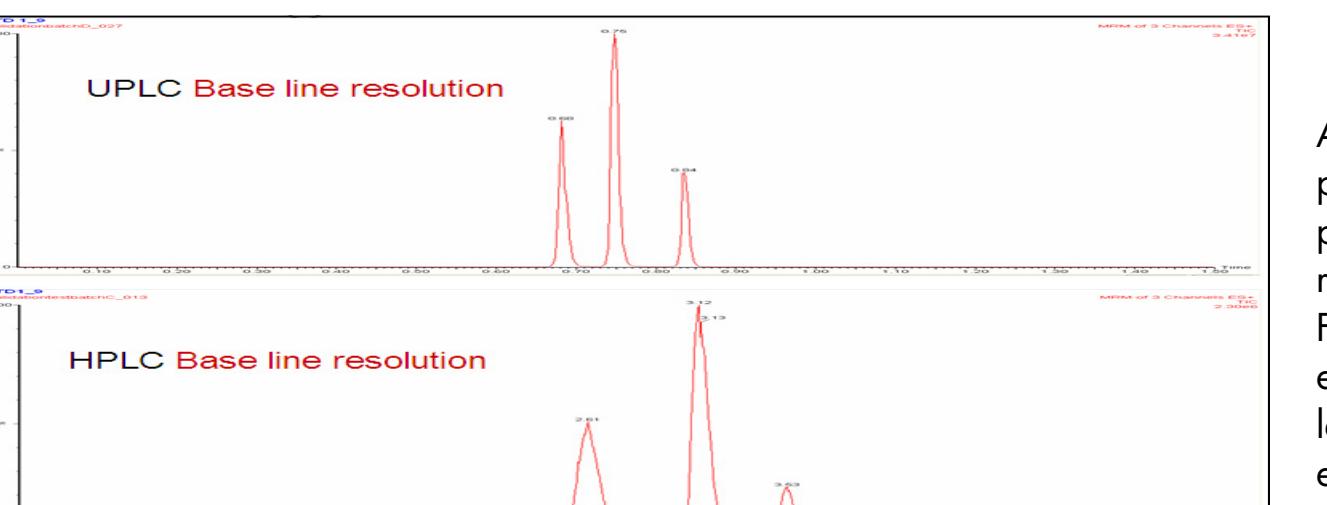


Figure 3. Chromatographic resolution UPLC vs. HPLC

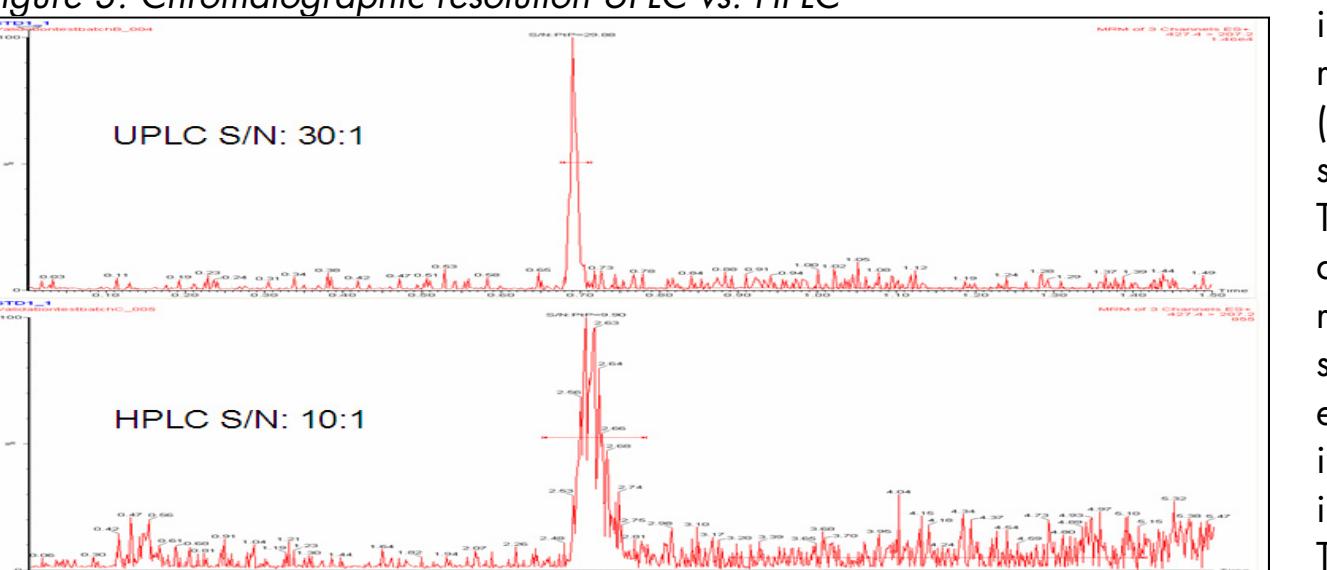


Figure 4. Increase in Signal/Noise UPLC vs. HPLC, 0.1ng/mL extract

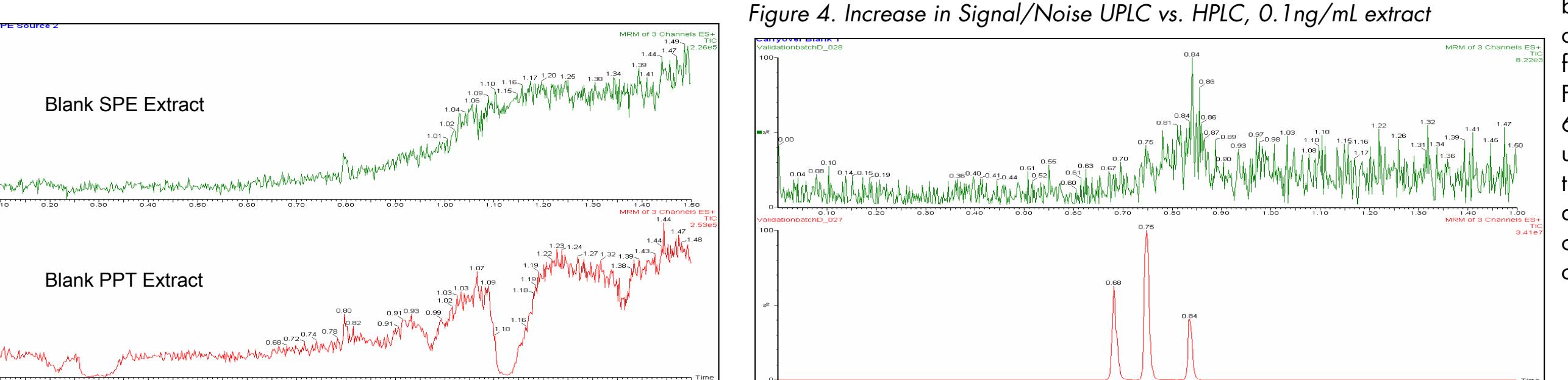


Figure 1. SPE vs. PPT qualitative matrix effect

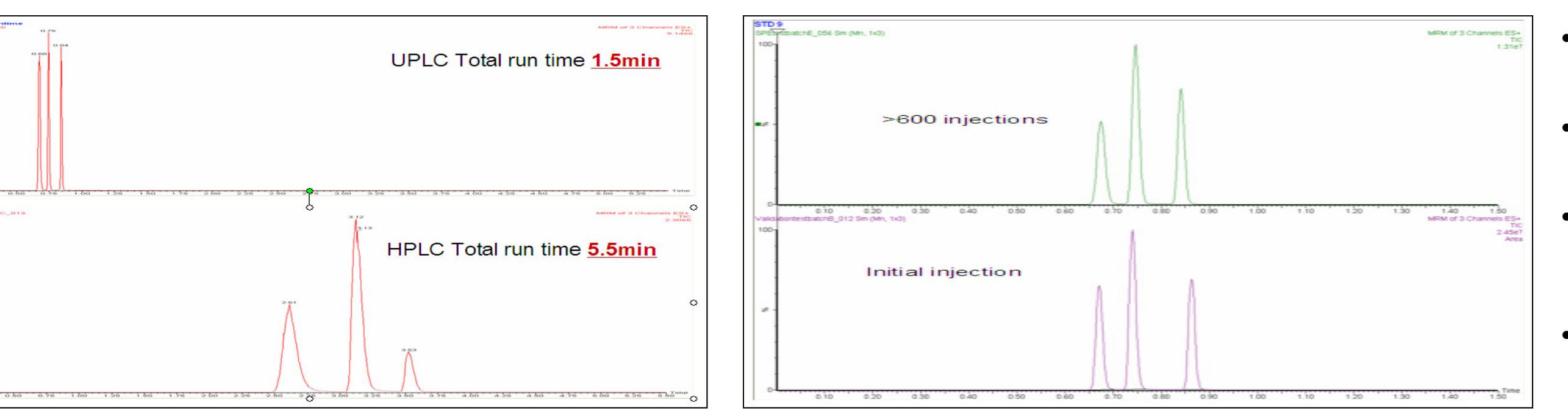


Figure 5. Stability of UPLC column after >600 injections

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 OASIS®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 in the same period of time, because of the very low system volume in the UPLC, long equilibration times are not required when gradients are being used, this again 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 small 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 the result of running the 1.7μm particles at their optimum linear velocity for optimum performance. This is only possible when using the UPLC system and cannot be achieved using traditional HPLC technology.

CONCLUSIONS

- 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