TechnicalNOTE

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FAST AND SENSITIVE QUANTITATIVE LC/MS/MS METHODS IN BIOANALYSIS: HPLC VS. UPLC™

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INTRODUCTION

HPLC/MS/MS is a widely utilized technique for quantitative bioanalysis due to its sensitivity and selectivity. There are three main challenges that face LC/MS/MS when developing new methods these are:

– Speed
– Sensitivity
– Resolution

Waters solution to the above challenges is the ACQUITY Ultra Performance LC™ System. This technical note demonstrates the benefits of UPLC compared to traditional HPLC for the analysis of Risperidone, 9-Hydroxyrisperidone, and the internal standard, Clozapine.

EXPERIMENTAL CONDITIONS

HPLC Conditions

HPLC System: Waters Alliance® HT System
Column: XTerra® MS C_{18}, 2.1 x 50 mm, 3.5 µm
Mobile Phase A: 2 mM $CH_3COO^-NH_4^+$ in H_2O, pH 9.0
Mobile Phase B: 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

Injection Volume: 5 µL
Sample Diluent: 0:50 v/v Methanol:Water
Column Temp: 50 °C
Total Run Time: 5.5 min

UPLC Conditions

UPLC System: Waters ACQUITY UPLC™ System
Column: ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Mobile Phase A: 2 mM $CH_3COO^-NH_4^+$ in H_2O, pH 9.0
Mobile Phase B: 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

Injection Volume: 5 µL
Sample Diluent: 50:50 v/v Methanol:Water
Column Temp: 50 °C
Total Run Time: 1.5 min

MS Conditions

MS System: Waters Micromass® Quattro Premier XE™
Ionization Mode: Positive Ion Electrospray (ESI^+)
Capillary Voltage: 3.00 V
Desolvation Temp: 380 °C
Desolvation Gas Flow: 800 L/hr
Cone Gas Flow: 50 L/hr
Collision Cell Pressure: $3.50e^{-3}$
MRM Transitions:
Dwell Time: 30 ms for all transitions
Inter-Scan Delay: 10 ms for all transitions

	Precursor Ion (m/z)	Product Ion (m/z)	Cone Voltage (V)	Collision Energy (eV)
Risperidone	411.3	191.3	35	25
9-OH Risperidone	427.4	207.2	35	25
Clozapine (IS)	327.1	370.3	40	30

RESULTS AND DISCUSSION

Speed and Resolution

The UPLC method that was developed resulted in a 70% decrease in analysis time compared to HPLC (Figure 1) with no significant change in chromatographic resolution (Figure 2), allowing a three-fold increase in sample throughput. The gradient and flow rates were optimized for both HPLC and UPLC and the reduction in analysis time results partly from the use of these conditions with the UPLC column, and partly to the very low system volume in the UPLC hardware. This low system volume also has a benefit in reducing the time required for equilibration when gradient elution is used, therefore further increasing sample throughput and allowing the efficient use of MS/MS.

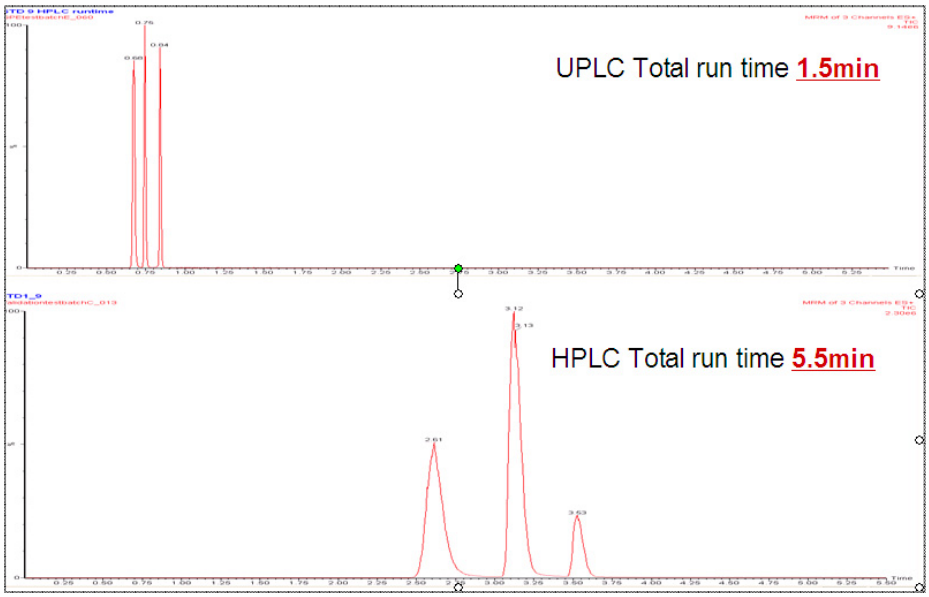

Figure 1. Risperidone, 9–OH Risperidone, and Clozapine (internal standard) run time, HPLC vs. UPLC.

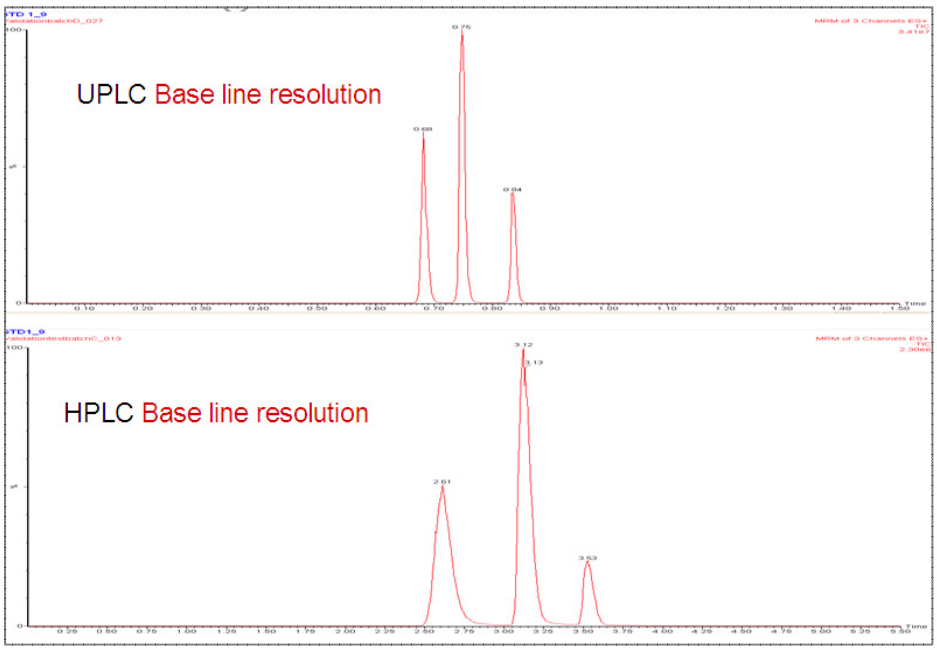

Figure 2. Risperidone, 9–OH Risperidone, and Clozapine resolution, HPLC vs. UPLC.

Sensitivity

The narrow peak widths produced by the UPLC, typically 2–3 seconds wide at base, result in increased peak intensity and improved signal-to-noise ratios. This allows lower limits of quantification (LLOQ) to be reached compared to HPLC. In this example (Figures 3 and 4), a 3–fold increase in the signal-to-noise was achieved for a 0.1 ng/mL LLOQ.

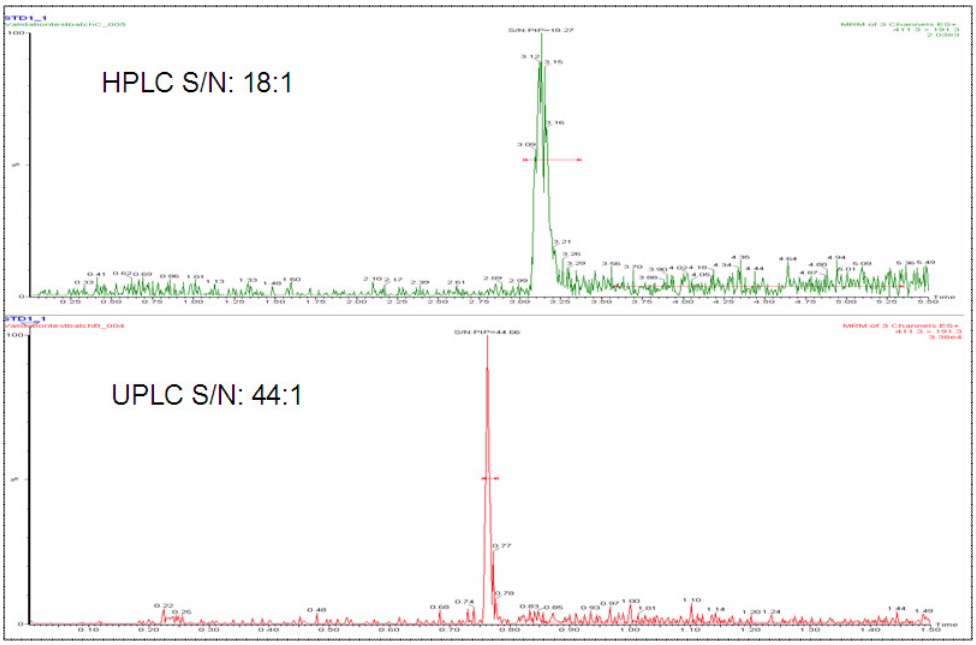

Figure 3. Risperidone sensitivity, HPLC vs. UPLC.

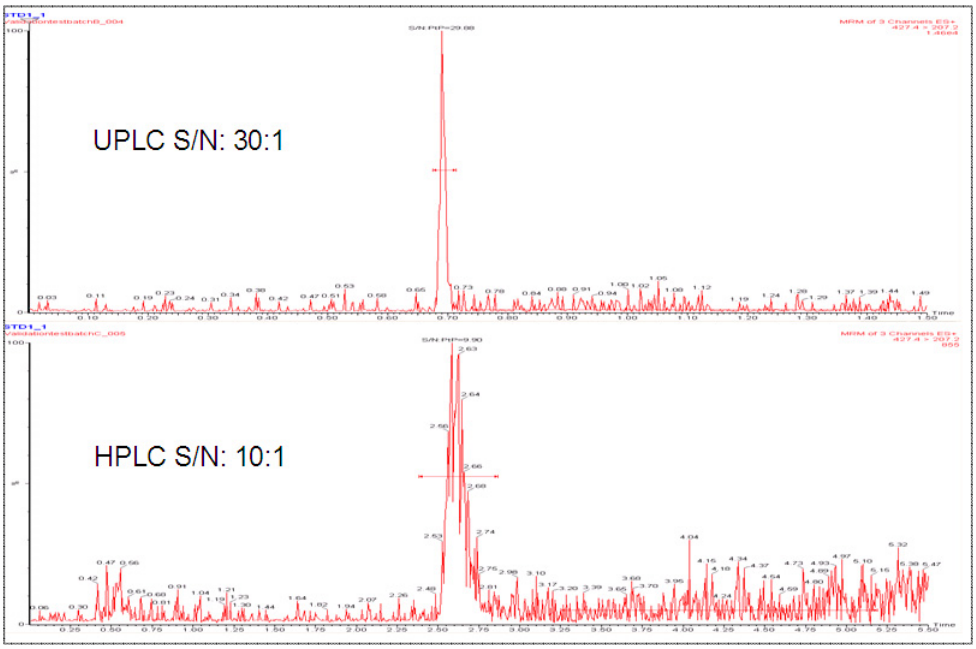

Figure 4. 9–OH Risperidone sensitivity, HPLC vs. UPLC.

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CONCLUSION

UPLC allows the development of fast and sensitive LC/MS/MS methods. When compared to conventional HPLC, significant reductions in analysis time and lower limits of quantification can be achieved, without the need to change the sample preparation method or the MS/MS system being used. In this example, a three-fold increase in both throughput and sensitivity was gained using the ACQUITY UPLC System.

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