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アプリケーションノート

A Validated UPLC-MS/MS Method for Risperidone and 9-Hydroxyrisperidone in Human Plasma

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Abstract

This application note details the full validation of a UPLC-MS/MS method for the determination of risperidone and its active metabolite 9-hydroxyrisperidone concentrations in human plasma, using clozapine as an internal standard.

Introduction

Risperidone is from a class of compounds called atypical antipsychotics. Its main use is in the treatment of schizophrenia by blocking serotonin 5-HT2 and dopamine D2 receptors. Risperidone is rapidly absorbed by the body after administration and is metabolized to 9-hydroxyrisperidone. The monitoring authorities require the safety and efficacy of drugs and their active metabolites to be assessed. This application note details the full validation of a UPLC-MS/MS method for the determination of risperidone and its active metabolite 9-hydroxyrisperidone concentrations in human plasma, using clozapine as an internal standard (Figure 1). The full validation was carried out in accordance with FDA Guidance for Industry for Bioanalytical Method Validation and included the assessment of:

- Sensitivity
- Linearity
- Carryover
- Intra- and inter-batch accuracy and precision
- Selectivity
- Stability
- Recovery
- Ion suppression

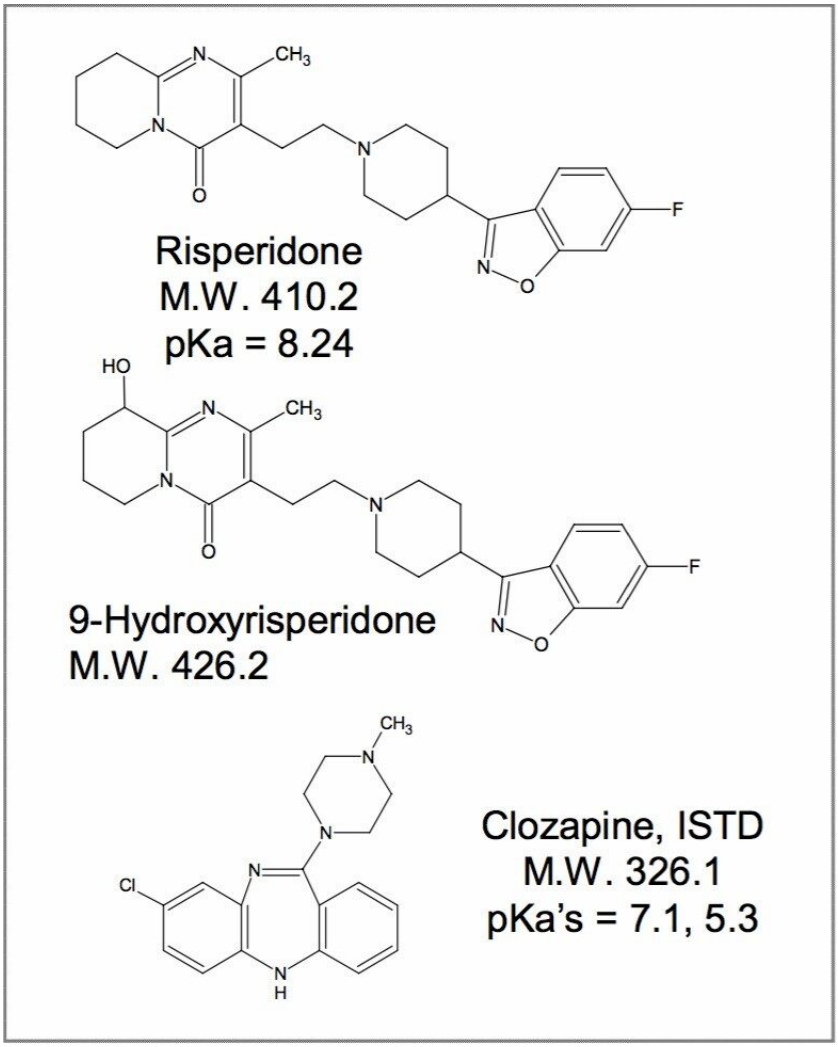

Figure 1. Structures of risperidone, 9-OH risperidone, and clozapine (internal standard).

Experimental

UPLC Conditions

LC system:	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
Injection volume:	5 µL
Sample diluent:	50:50 v/v methanol:water
Column temp:	50 °C

Waters ACQUITY UPLC System with the Waters Micromass Quattro Premier XE Mass Spectrometer.

Gradient

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

MS Conditions

MS system:	Quattro Premier XE Tandem Quadrupole Mass Spectrometer
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:	3.50 e^{-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

Standard Solutions

Stock solutions (1 mg/mL) of risperidone, 9-hydroxyrisperidone and clozapine were prepared by dissolving the appropriate amount of compound in methanol. Working solutions containing both risperidone and 9-hydroxyrisperidone were then prepared by diluting the appropriate volume of stock solution with 50:50 v/v methanol:water to give the following concentrations:

Calibration working solutions:

0.2, 0.4, 1.0, 2.0,10, 40, 200, 320, and 400 ng/mL.

Quality Control (QC) working solutions:

0.002, 0.006, 1.4, 3.0, and 4.0 µg/mL.

An appropriate volume of clozapine was diluted with 50:50 v/v methanol: water to give a internal standard working solution of 0.5 µg/mL.

All solutions were stored at 4 °C for 1 month.

Sample Preparation

A calibration curve for the determination of the risperidone and 9-hydroxyrisperidone in human plasma was prepared fresh on the day of analysis at the following concentrations:

0.1, 0.2, 0.5, 1.0, 5.0, 20, 100, 160, and 200 ng/mL.

Additionally, bulk plasma QC samples were prepared at the following concentrations:

0.1, 0.3, 70, 150, and 200 ng/mL.

Bulk QC samples were stored at –20 °C until required.

Extraction Procedure

Samples were prepared by taking 100 µL of spiked plasma and adding 50 µL of internal standard working solution (0.5 µg/mL). A 1 mL aliquot of water was then added to dilute the sample.

SPE using the Waters Oasis MCX µElution Plate, 30 µm particle, 96-well, Part Number 186001830BA <https://www.waters.com/nextgen/us/en/shop/sample-preparation--filtration/186001830ba-oasis-mcx-96-well--elution-plate-2-mg-sorbent-per-well-30--m-1-p.html>

- Condition wells with 1 mL methanol then equilibrate with 1 mL water
- Load 1.0 mL of prepared sample
- Wash with 1.0 mL 2% formic acid in water
- Wash with 1.0 mL methanol
- Elute with 500 µL 5% NH_4OH in methanol
- Dilute with 500 µL water prior to injection

Calibration Curves

Calibration curves were generated using the Waters QuanLynx Application Manager for MassLynx Software by plotting the peak area ratio of Risperidone or 9-hydroxyrisperidone to the internal standard for each calibration concentration (Figures 2 and 3). The linear regression was constructed from 0.1–200 ng/mL (excluding the origin) and a weighting of $1/x^2$ was applied. For all validation batches, the calibration curve was analyzed in duplicate. All of the calibration curves generated had an $r^2 > 0.996$ and all calibration points were within ±15% of their theoretical concentration.

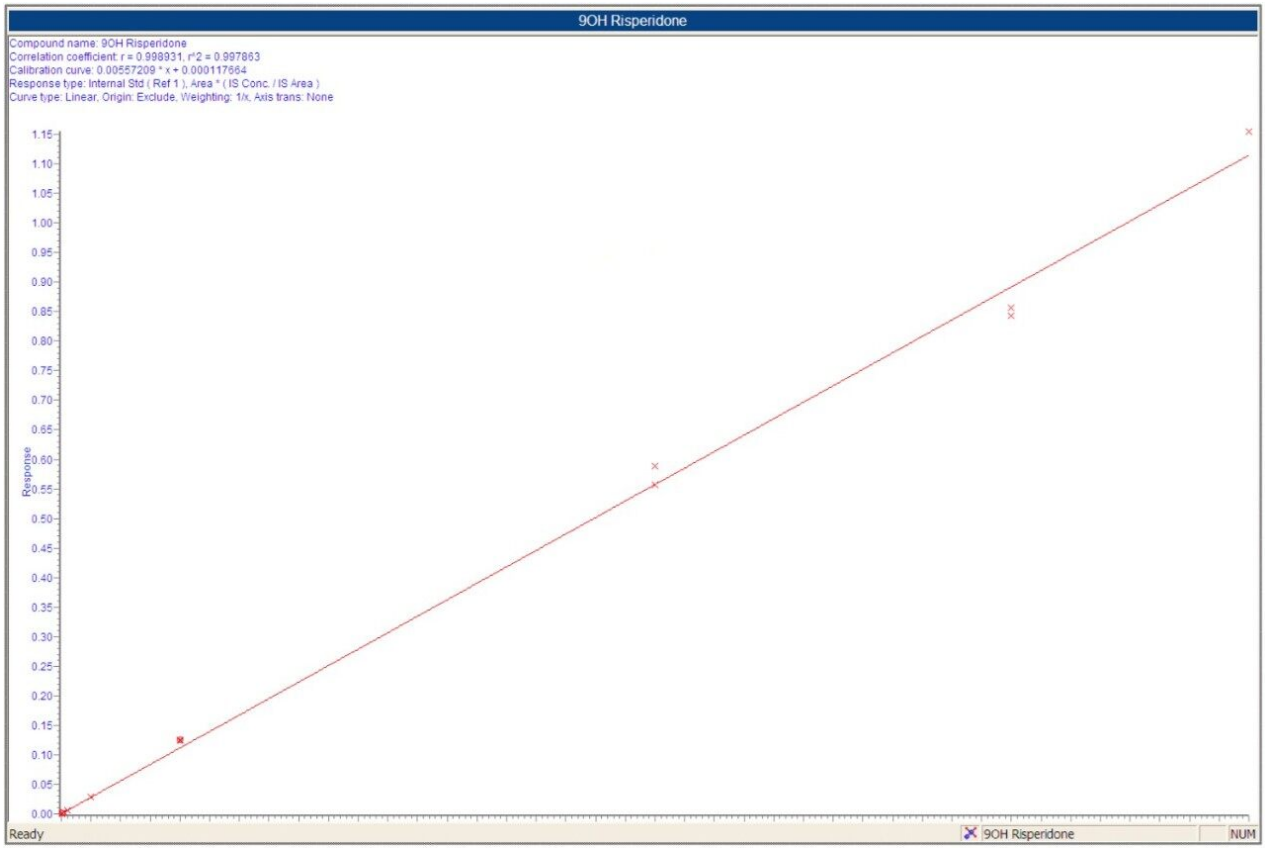

Figure 2. Example of risperidone calibration.

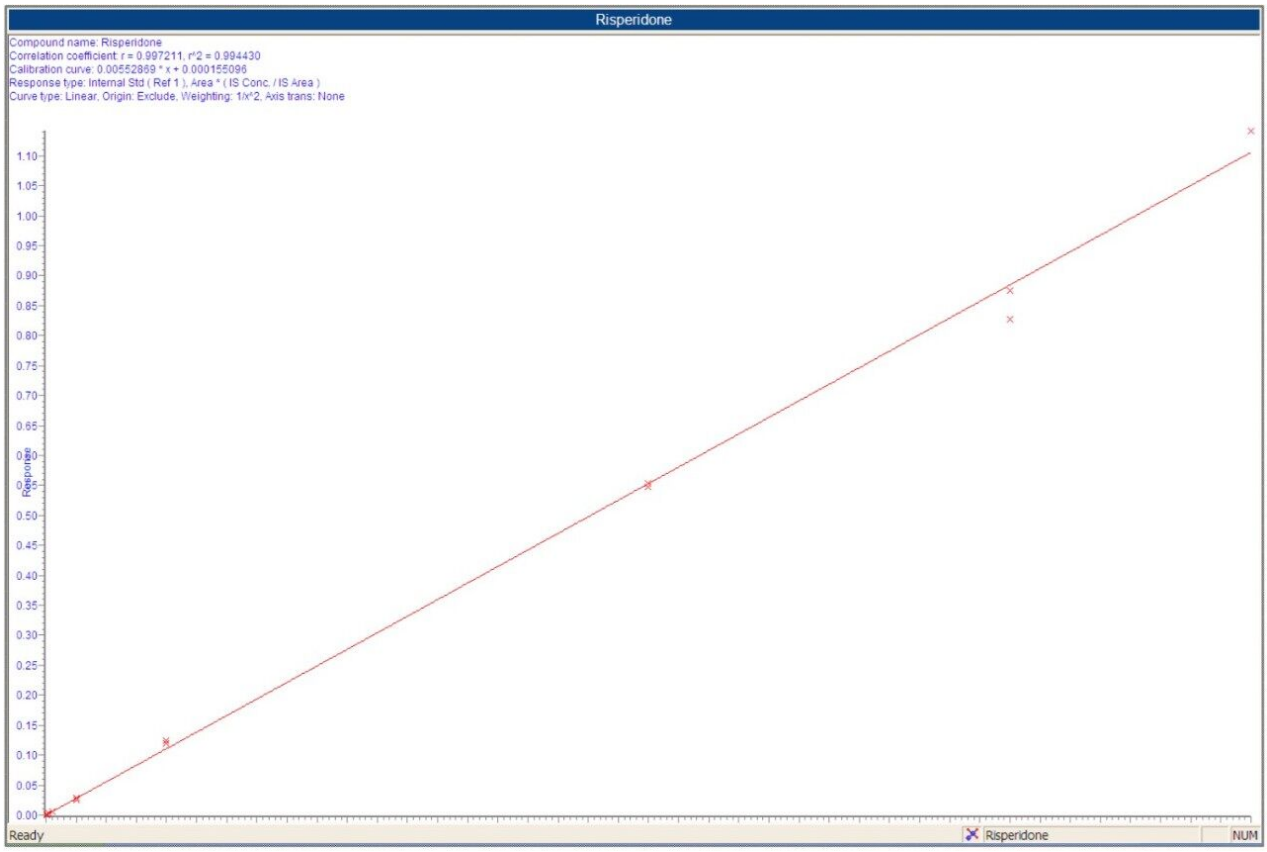

Figure 3. Example of 9-OH risperidone calibration.

Results and Discussion

Sensitivity

The sensitivity (LLOQ) of the assay was confirmed to be 0.1 ng/mL, and was assessed by the accuracy and precision of six replicate LLOQ QC samples (Figure 4). Inter-batch accuracy and precision were 99.8% and 8.9%, respectively, for risperidone, and 95% and 5.5%, respectively, for 9-hydroxyrisperidone.

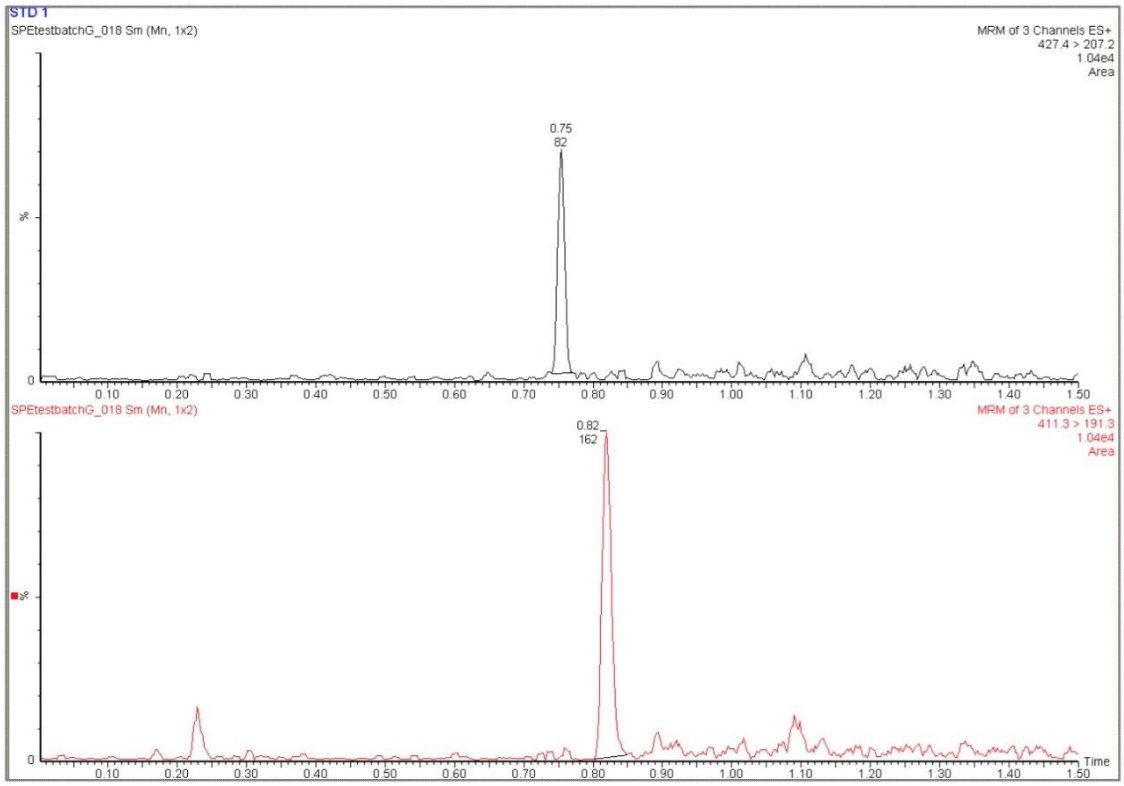

Figure 4. 0.1 ng/mL risperidone (bottom) and 9-OH risperidone (top) plasma extracts.

Accuracy and Precision

Intra-batch accuracy and precision was assessed by the analysis of six replicates at each QC level within each validation batch.

Inter-batch accuracy and precision was assessed by the analysis of each QC level (six replicates) within three validation batches.

The intra- and inter-batch accuracy and precision for both risperidone (Figure 5) and 9-hydroxyrisperidone (Figure 6) exceeds the FDA requirements for method validation.

	Intra Batch			Inter Batch
Theorectical Conc. Of Risperidone (ng/ml)	Batch A	Batch B	Batch C	
0.1				
%CV	**4.72**	**13.27**	**6.48**	**8.65**
%Accuracy	**106.0**	**98.0**	**108.0**	**104.0**
0.3				
%CV	**2.44**	**9.09**	**7.00**	**10.10**
%Accuracy	**109.3**	**91.7**	**100.0**	**99.0**
70				
%CV	**2.69**	**4.89**	**7.45**	**5.43**
%Accuracy	**101.0**	**97.0**	**102.0**	**100.0**
150				
%CV	**6.48**	**3.96**	**7.14**	**6.96**
%Accuracy	**104.1**	**97.3**	**107.0**	**102.5**
200				
%CV	**5.99**	**4.83**	**8.42**	**7.16**
%Accuracy	**104.1**	**95.7**	**100.6**	**100.1**

Figure 5. Intra- and inter-accuracy and for risperidone.

	Intra Batch			Inter Batch
Theorectical Conc. Of 9OH Risperidone	Batch A	Batch B	Batch C	
0.1				
%CV	**5.32**	**7.14**	**16.49**	**10.42**
%Accuracy	**94.0**	**98.0**	**97.0**	**96.0**
0.3				
%CV	**4.52**	**6.29**	**5.05**	**8.22**
%Accuracy	**110.7**	**95.3**	**99.0**	**101.3**
70				
%CV	**2.42**	**6.57**	**7.32**	**5.75**
%Accuracy	**106.0**	**101.0**	**103.0**	**103.0**
150				
%CV	**4.73**	**3.14**	**7.76**	**6.11**
%Accuracy	**104.6**	**102.9**	**110.1**	**105.9**
200				
%CV	**3.28**	**2.65**	**6.03**	**4.90**
%Accuracy	**103.3**	**97.0**	**97.2**	**99.3**

Figure 6. Intra- and inter-accuracy and for 9-OH risperidone.

Carryover

The carryover for the UPLC method was assessed by the duplicate injections of extracted plasma blanks directly after a high concentration (200 ng/mL) standard (Figure 7). The results showed that there was no detectable carryover of risperidone and 9-hydroxyrisperidone, and negligible levels of clozapine were observed in some of the samples (< 0.04% carryover) (Figure 8).

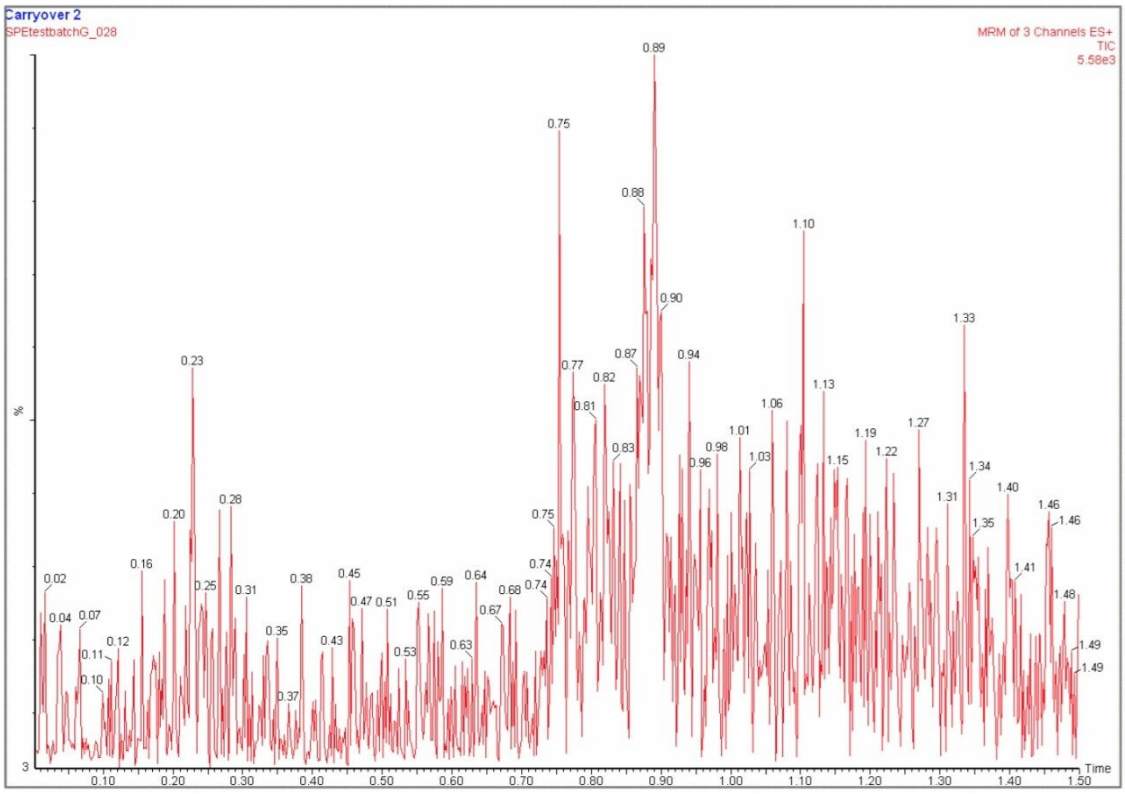

Figure 7. Example of a carryover blank injection.

	Risperidone		
	Batch A	Batch B	Batch C
LLOQ	225.71	116.00	116.64
	214.36	118.51	151.68
Mean	220.04	117.26	134.16
Carryover 1	0.00	0.00	0.00
Carryover 2	0.00	0.00	0.00
Mean	0.00	0.00	0.00
% Carryover	**0.00**	**0.00**	**0.00**

	Clozapine		
	Batch A	Batch B	Batch C
LLOQ	240373	153193	155405
	270207	177036	146456
Mean	255290	165115	150930
Carryover 1	132.21	55.94	0.00
Carryover 2	71.15	70.55	0.00
Mean	101.68	63.25	0.00
% Carryover	**0.04**	**0.04**	**0.00**

	9OH Risperidone		
	Batch A	Batch B	Batch C
LLOQ	136.08	100.58	67.86
	144.39	111.05	73.11
Mean	140.24	105.82	70.48
Carryover 1	0.00	0.00	0.00
Carryover 2	0.00	0.00	0.00
Mean	0.00	0.00	0.00
% Carryover	**0.00**	**0.00**	**0.00**

Figure 8. Carryover results.

Selectivity

The selectivity of the method was assed by the analysis of blank plasma extracts from six different, non-

pooled, human plasma sources (Figure 9).

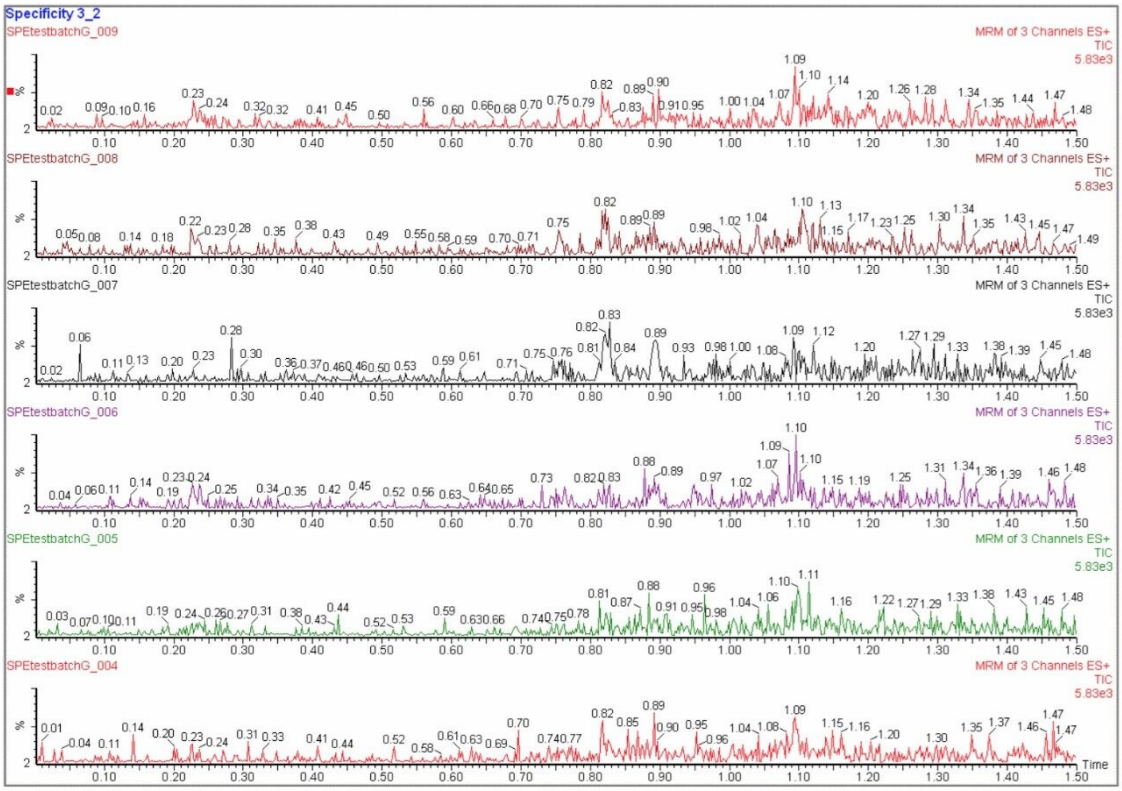

Figure 9. Example of selectivity TIC from six individual plasma sources.

After analysis, all six sources of plasma tested had no risperidone, 9-hydorxyrisperidone, or clozapine peaks present.

Stability

The following stability experiments were carried out:

– 24 hour extract stability, 4 °C

– Freeze/thaw stability, –20 °C

– Room temperature matrix stability

All stability experiments passed the relevant FDA guidelines.* Plasma samples and plasma extracts were stable for 24 hours at room temperature and at 4 °C, respectively. Plasma samples were stable for three freeze/thaw cycles at –20 °C.

**Raw data available upon request*

Recovery

Recovery of risperidone, 9-hydroxyrisperidone, and clozapine was assessed by the comparison of peak areas of extracted low-, mid- and high-concentration QC samples (0.3, 70, and 160 ng/mL, respectively) to post-spiked plasma extracts at the same concentrations, assuming 100% recovery (Figure 10). All recoveries were found to be >90% across the validation range.

	% Recovery		
	Low QC	Mid QC	High QC
Risperidone	**95**	**94**	**99**
9OH Risperidone	**92**	**96**	**100**
Clozapine	**90**	**95**	**93**

Figure 10. Percent recovery results.

Ion Suppression

Ion suppression is generally caused by endogenous matrix components that are not removed during sample preparation, and co-elute with the peaks of interest and compete for ionization in the ion source.

Ion suppression was assessed from six individual sources of human plasma both qualitatively (using a T-infusion experiment, Figure 11) and qualitatively (by comparison of post-spiked plasma extracts at the LLOQ (0.1 ng/mL) to post-spiked solutions containing no matrix components, Figure 12).

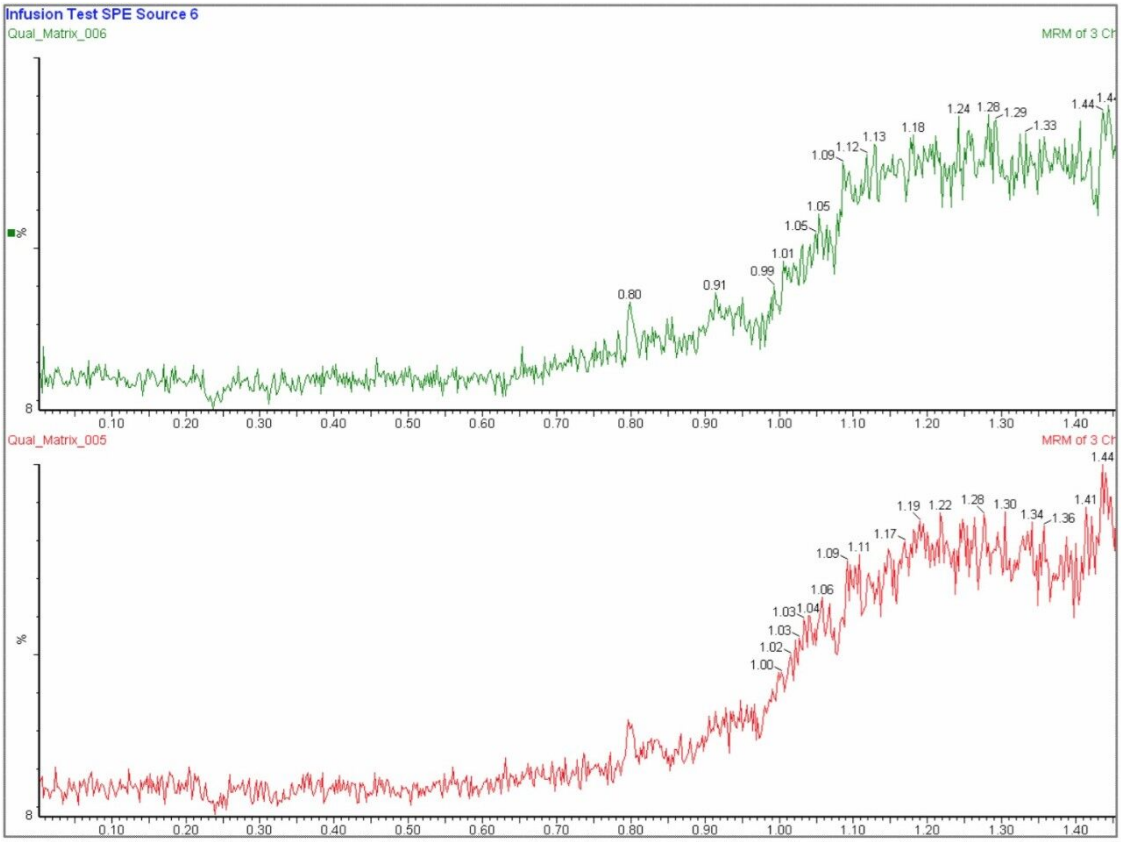

Figure 11. Example of a qualitative ion suppression experiment SPE extract (top) vs. blank solution (bottom).

	Risperidone		
Matrix Source	To	Mean	% Diff
100% Recovery Soln	176.19	159.85	N/A
	143.52		
1	201.769	184.494	13.4
	167.219		
2	177.267	169.048	5.4
	160.829		
3	163.801	167.746	4.7
	171.691		
4	150.273	158.838	-0.6
	167.403		
5	195.369	177.947	10.2
	160.525		
6	153.351	157.639	-1.4
	161.927		

	9OH Risperidone		
Matrix Source	To	Mean	% Diff
100% Recovery Soln	111.51	96.80	N/A
	82.10		
1	76.556	85.535	-13.2
	94.513		
2	93.267	94.262	-2.7
	95.257		
3	103.112	93.833	-3.2
	84.553		
4	81.845	87.855	-10.2
	93.864		
5	94.030	102.032	5.1
	110.034		
6	96.437	103.488	6.5
	110.538		

Figure 12. Quantitative ion suppression results.

Results from the qualitative matrix effect tests showed no signs of a matrix effect and was confirmed by the quantitative tests that showed negligible levels of ion suppression.

Conclusion

A UPLC-MS/MS method has been validated for the determination of risperidone and 9-hydroxyrisperidone concentrations in human plasma, meeting the FDA Guidance for Industry for Bioanalytical Method Validation. The combination of the Oasis MCX µElution plate for easy sample preparation with the powerful ACQUITY UPLC/Quattro Premier XE platform allowed a fast and robust method to be developed. The sample preparation effectively removed all matrix components from the plasma extract that could lead to ion suppression. Low UPLC system volumes and innovative 1.7 µm particles enabled fast LC method development and, due to the narrow peaks produced and the resulting increase in signal-to-noise, a small sample volume of 100 µL could be used.

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ACQUITY UPLC System <https://www.waters.com/514207>

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