A VALIDATED METHOD FOR THE ANALYSIS OF RISPERIDONE IN HUMAN PLASMA USING UPLC MSMS

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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 SPE vs. UPLC™ for - Sensitivity - Selectivity - 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 run times to be developed. A fast tandem mass spectrometry (MS/MS) method can be achieved; however, there are potential challenges with accurate quantification.

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 (LOQ) or interferences from co-eluting drug metabolites can give falsely elevated responses for the drug substance.

Improved sample preparation can reduce these effects, but it is often necessary to develop longer chromatographic methods to separate the drugs from matrix components. This results in reduced sample throughput.

In this project we have utilized Ultra Performance LC™ (UPLC™) coupled to a Waters Quattro Premier™ tandem quadrupole MS, ESI+ for the analysis of Risperidone and 9-OH Risperidone in human plasma. The use of Ultra Performance LC™ (UPLC™) coupled to a Waters Quattro Premier™ tandem quadrupole MS, ESI+ was selected as the technique of choice for the analysis of Risperidone and 9-OH Risperidone in human plasma.

METHODS

HPLC Conditions

- Waters Alliance™ 2695 HPLC System
- Waters Xterra® MS C18, 2.1 x 50mm, 3.5µm
- Column Temperature: 50°C
- Total run time: 1.5min
- Gradient:

<table>
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<th>%A</th>
<th>%B</th>
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<tr>
<td>3.5</td>
<td>50</td>
<td>50</td>
<td>11</td>
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- Flow rate: 0.6 mL/min
- Mobile Phase A: 2mM ammonium acetate in water, pH 9.0
- Mobile Phase B: 100% methanol
- Injection Volume: 5µL

MS Conditions

- Waters Quattro Premier™ tandem quadrupole MS, ESI+
  - Capillary Voltage: 1.0kV
  - Source temperature: 125°C
  - Cone Gas Flow: 50 L/H
  - Desolvation temperature: 350°C
  - Collision Cell Pressure: 3.0x10⁻² mbar

Concentration ranges from 0.1-200ng/mL

- Linear range of 0.1-200ng/mL
- Protein Precipitation (PPT)
  - Water Oasis® MCX 30mg 96 well plate
  - Wash 1: 1ml 2% formic acid in water
  - Equilibrate with 1ml water
  - Condition with 1ml methanol

- Dilute extract with 500ul of water prior to injection

UPLC Conditions

- Waters ACQUITY™ UPLC™ System
- 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: 0.3 mL/min

Concentration ranges from 0.1-200ng/mL

- Linear range of 0.1-200ng/mL
- Protein Precipitation (PPT)
  - Water Oasis® MCX 30mg 96 well plate
  - Wash 1: 1ml 2% formic acid in water
  - Equilibrate with 1ml water
  - Condition with 1ml methanol
  - Elute 500ul of water prior to injection

- Dilute extract with 500ul of water prior to injection

- Blank PPT Extract
- Blank SPE Extract

Sample Preparation

- Sample: 100ul plasma (Na Heparin as anti coagulant) spiked with Risperidone, 9-OH Risperidone and internal standard
- Dilute extract with 500ul of water prior to injection

CONCLUSIONS

- 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 ±5% and ±5% respectively with correlation coefficients (r²) of 0.998.

- Figure 1 is an example of a SPE extract vs. 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 (Hilguard Mode) Column 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 results in a three fold 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.

- Significant reductions in LC run time can be achieved using UPLC resulting higher sample throughput, while maintaining resolution.

- 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.

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Figure 6. shows the robustness of the UPLC chromatography after more than 400 injections, to test the robustness of the column, it was constantly used under the UPLC conditions (pH9.0 @ 50°C), the system back pressure throughout the validation ranged from 800 to 1100bar. These back pressures are the result of running the 1.7µm particle at these optimum linear velocity for optimum performance. This is only possible when using the UPLC system and cannot be achieved using traditional HPLC technology.

Figure 7. shows the stability of UPLC column after >600 injections.