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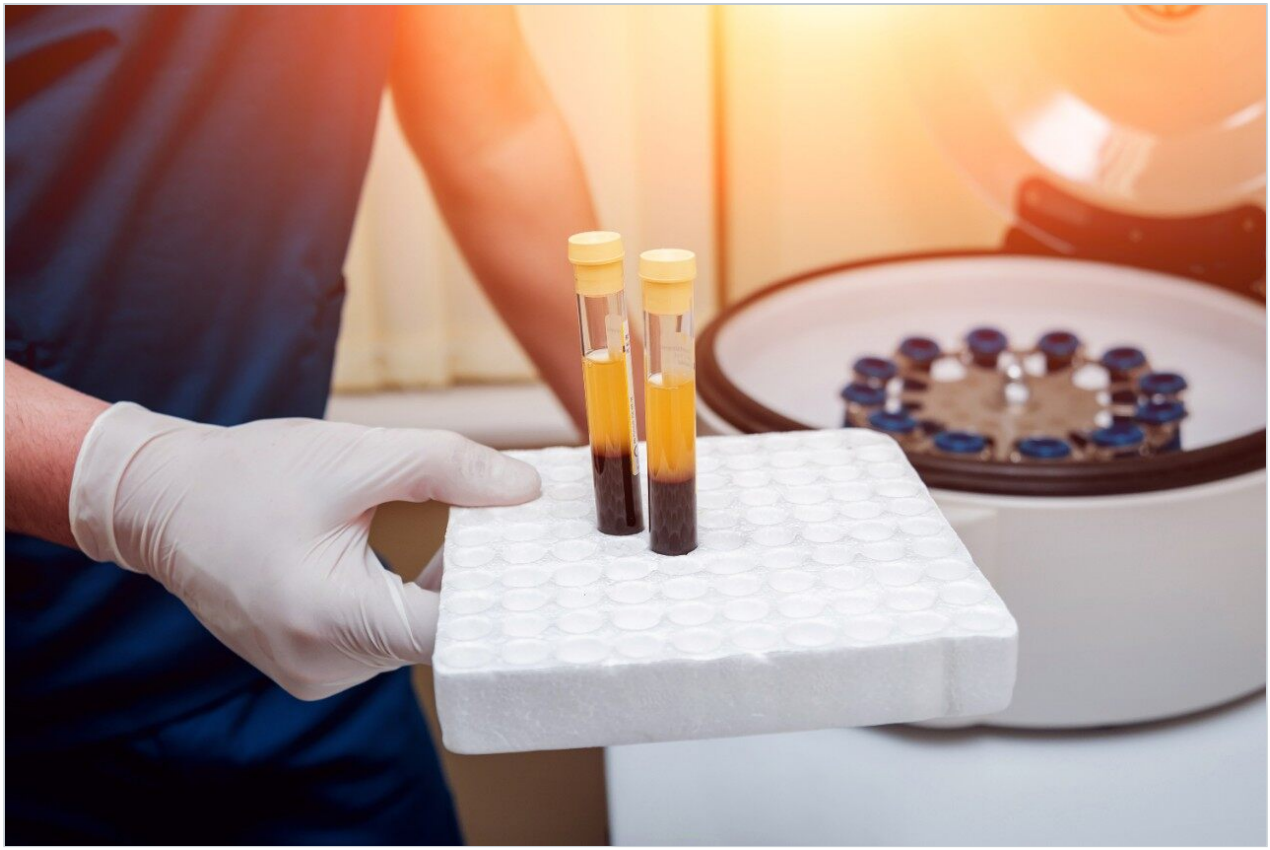
A Rapid And Sensitive SPE-UPLC-MS/MS Method For Determination Of Ropinirole In Human Plasma

Erin E. Chambers, Diane M. Diehl, Jeffrey R. Mazzeo

Waters Corporation



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Abstract

Ropinirole is a potent drug with a low oral dose range. A bioanalytical method needs to be developed that can reach a lower limit of quantitation of 0.005 ng/mL. We describe here a method which increases the productivity and efficiency of pk laboratories. The method minimizes solvent composition and time as well as being fully automatable.

Benefits

- A rapid and sensitive method
- Enables researchers to obtain higher quality data faster in order to make critical project decisions

Introduction

Ropinirole (marketed as Requip in the US and as Adartrel in Europe) is a non-ergoline dopamine agonist used in the treatment of Parkinson's disease, and is also the only medication in the United States with a Food and Drug Administration-approved indication for the treatment of restless leg syndrome. It is extensively metabolized by the liver to inactive metabolites through N-despropylation and hydroxylation, primarily by the specific P450 enzyme CYP1A2. Monitoring authorities require that the safety and efficacy of drugs and active metabolites be assessed. Many of the challenges of developing a bioanalytical method revolve around working in complex biological matrices and meeting the rigorous criteria set forth in the FDA Guidance for Industry for Bioanalytical Method Validation. Methods need to be acceptable in terms of linearity, sensitivity, accuracy and precision, selectivity, stability, and carryover. In addition, sample preparation needs to be as thorough and selective as possible to ensure a robust final method. It is important to obtain the cleanest final extracts and to minimize or eliminate matrix effects. We employ mixed-mode Solid-Phase Extraction (SPE) for this purpose as it relies on both reversed phase and ion exchange to more selectively separate analytes from matrix components.

Solution

A rapid and sensitive method for the determination of ropinirole in human plasma has been developed. The required LLOQ of 0.005 ng/mL was achieved through the combination of sample concentration using Oasis MCX µElution 96-well plate and the speed and sensitivity of UltraPerformance Liquid Chromatography and

Mass Spectroscopy (UPLC-MS/MS). Standard curves were linear over the range 0.005 to 10 ng/mL.

Sample preparation of human plasma was carried out with Oasis MCX, as both ropinirole and its internal standard, citalopram, are basic compounds, with pka's of 9.7 and 9.5, respectively. The 96-well µElution plate format was employed to facilitate direct injection and to obtain the analyte concentration necessary to meet the required LLOQ of 0.005 ng/mL. Structures for the analytes are shown in Figure 1.

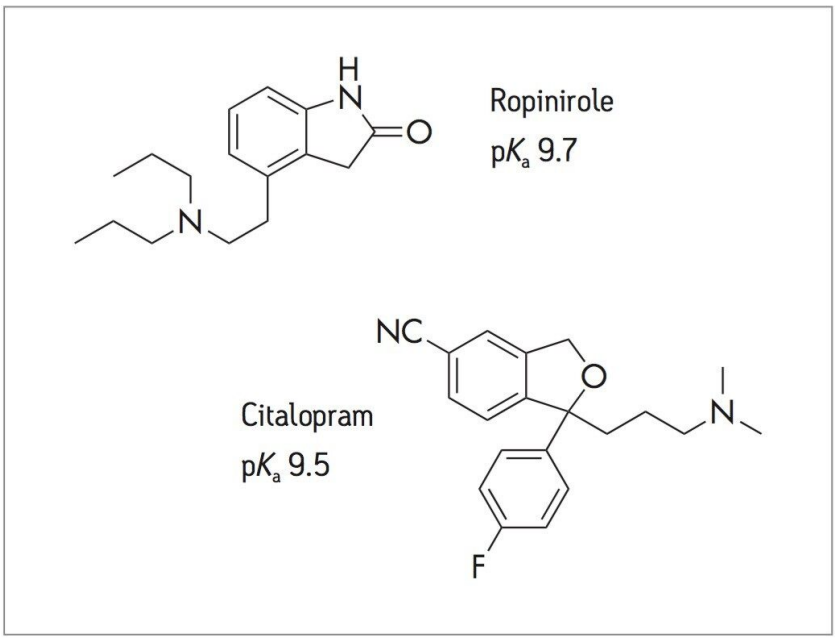


Figure 1. Structures and pK_as of ropinirole and citalopram.

In addition to optimized sample preparation, chromatography was optimized for speed, sensitivity, and resolution. The technology of UPLC takes advantage of smaller chromatographic particles to improve these important parameters. UPLC peak widths, on the order of 2-3 seconds, necessitated the use of a detector capable of high speed data acquisition without loss of data quality. Adequate characterization of chromatographic peaks is critical for accurate quantitation, we therefore utilized the Waters Quattro Premier tandem mass spectrometer because of its superior performance in these areas.

Experimental

ACQUITY UPLC Conditions

Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Part number:	186002350
Mobile phase A:	10 mM NH_4COOH, pH 9
Mobile phase B:	MeOH
Flow rate:	0.5 mL/min
Injection volume:	8 µL
Column temperature:	45°C
Sample temperature:	15°C
Sample diluent:	100% MeOH + 5% NH_4OH
Strong needle wash:	60:40 ACN:IPA + 0.5% HCOOH (1200 µL)
Weak needle wash:	95:5 H_2O:MeOH (500 µL)

Gradient

Time(min)	A%	B%	Curve
0.0	95	5	6
2.0	2	98	6
2.5	2	98	6
2.6	95	5	6
3.0	95	5	6

Waters Quattro Premier Conditions

Desolvation temperature:	350°C
Cone gas flow:	50 L/Hr
Desolvation gas flow:	750 L/Hr
Collision cell pressure:	2.6 x 10($^{-3}$) mbar
MRM transitions monitored:	Ropinirole *m/z* 261.2 > 113.95 (ESI+)
	Citalopram (IS) *m/z* 325.2 > 108.85 (ESI+)

For both analytes, the optimal cone voltage was 40 V. For ropinirole and citalopram, the collision energies were 18 eV and 25 eV, respectively.

Standard Solutions

Stock solutions (1 mg/mL) of each analyte were prepared by dissolving the appropriate amount of compound in MeOH. Working solutions of ropinirole were then prepared by diluting the appropriate volume of stock solution with 50:50 MeOH:H_2O v/v to give the following concentrations:

0.1, 0.2, 0.4, 2, 20, 100, and 200 ng/mL.

An appropriate volume of citalopram was diluted with 50:50 MeOH:H_2O v/v to give an internal standard working solution of 20 ng/mL. The above concentrations were necessary for a 50 µL per mL spike volume.

Sample Preparation Procedure

Calibration curves for the determination of ropinirole in human plasma were prepared at the following concentrations: 0.005, 0.01, 0.02, 0.1, 1, 5, and 10 ng/mL.

The internal standard, citalopram was spiked to a final concentration of 1 ng/mL.

Bulk human plasma was fortified as follows for each concentration point: 250 µL internal standard (20 ng/mL) and 250 µL ropinirole standard solution were added to 5 mL human plasma; this was then diluted with 4.5 mL 4% H_3PO_4 in water. The plasma was acidified to disrupt protein binding and diluted to improve flow through the SPE device as well as enhance contact time with the sorbent. These solutions represent the

pretreated (acidified and diluted) plasma solutions which are ready for solid-phase extraction. The analytes are added as part of the dilution for accuracy and simplicity. Concentrations above refer to concentration in the plasma, not the diluted solution.

Plasma blanks were prepared by mixing equal volumes plasma and 4% H_3PO_4 in water.

Extraction Procedure

Solid-phase extraction was performed with the Oasis MCX µElution 96-well plate for increased sensitivity, through concentration, without drying down and reconstituting the extract.

Oasis MCX µElution 96-well plate (Part Number: 186001830BA): Condition the wells with 200 µL MeOH and then equilibrate with 200 µL water.

Load 600 µL diluted plasma sample.

Wash with 200 µL 2% HCOOH in water.

Wash with 200 µL MeOH.

Elute with 1 x 25 µL 5% NH_4OH in MeOH.

The Oasis µElution plate eluates were injected directly, without dilution, onto the ACQUITY UPLC BEH C_{18} column.

SPE Recovery

Recoveries of ropinirole and citalopram were assessed by the comparison of peak areas of extracted samples at 0.1, 1, and 10 ng/mL to post-extracted spiked plasma extracts at the same concentrations. Blank plasma was extracted through the plate, dried down, and reconstituted with 50 µL of MeOH containing 0.1, 1, or 10 ng/mL ropinirole and 1 ng/mL citalopram.

The extracted samples (plasma spiked with analytes extracted through the plate) were also dried down and reconstituted in 50 µL of MeOH. A consistent volume of a constant solvent must be used for both post-extracted spiked and actual extracted samples in order to accurately calculate recovery. The average area counts for each analyte from 8 replicates of the extracted sample were divided by the average area counts in 8 replicates of the post spiked standard. The result was then multiplied by 100 to obtain a percentage recovery for each analyte.

Calibration Curves

Calibration curves were generated using the Waters Quanlynx Application Manager for MassLynx Software by plotting the peak area ratio of ropinirole to the internal standard for each calibration concentration. The linear regression was constructed from 0.005 – 10 ng/mL (excluding the origin) and a weighting of $1/x^2$ was applied.

Results and Discussion

The assay was determined to be linear over the required range of 0.005 to 10 ng/mL. For each day of analysis, calibration curves were analyzed in duplicate or triplicate. All calibration curves had an r2 > 0.998 and all calibration points were within the FDA defined criteria for acceptance of ±15% of their theoretical concentration. A representative standard curve is shown in Figure 2.

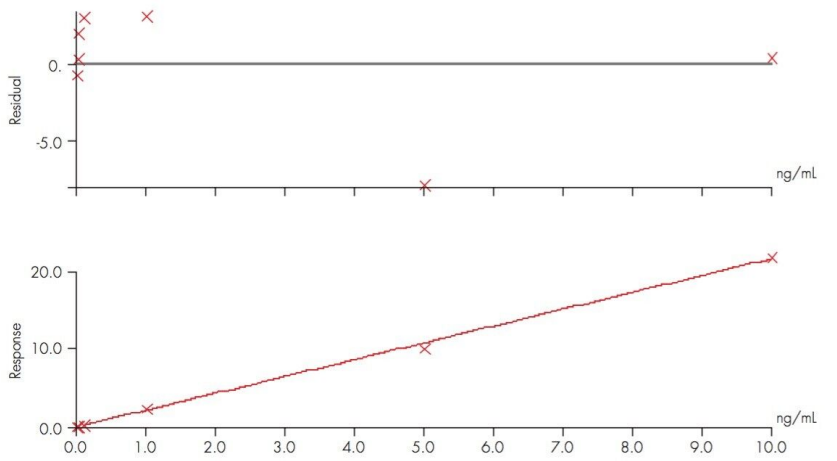


Figure 2. Representative standard curve demonstrating method linearity from 0.005 to 10 ng/mL

Correlation Coefficient: r = 0.999054, r^2 = 0.998108

*Calibration Curve: 2.20969 * x + 0.00746661*

Curve Type: Linear, Origin: Exclude, Weighting: $1/x^2$, Axis trans: None

SPE recoveries for both citalopram and ropinirole were > 90% for all concentrations. A representative chromatogram of ropinirole and its internal standard at 1 ng/mL is shown in Figure 3.

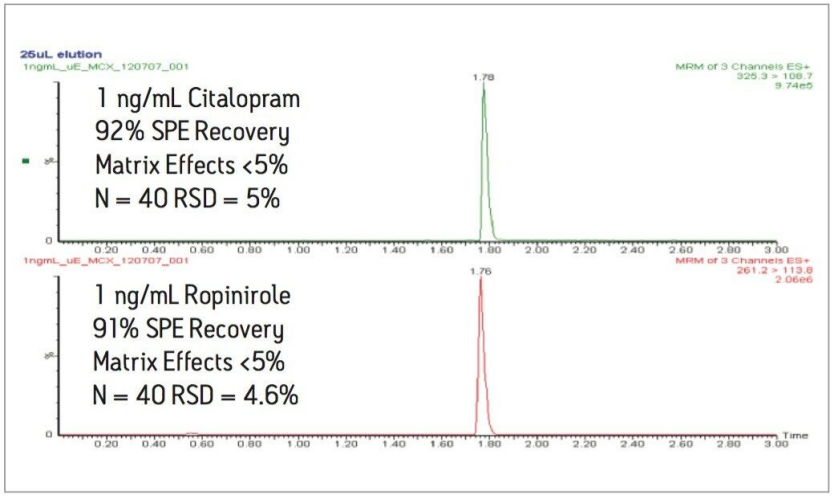
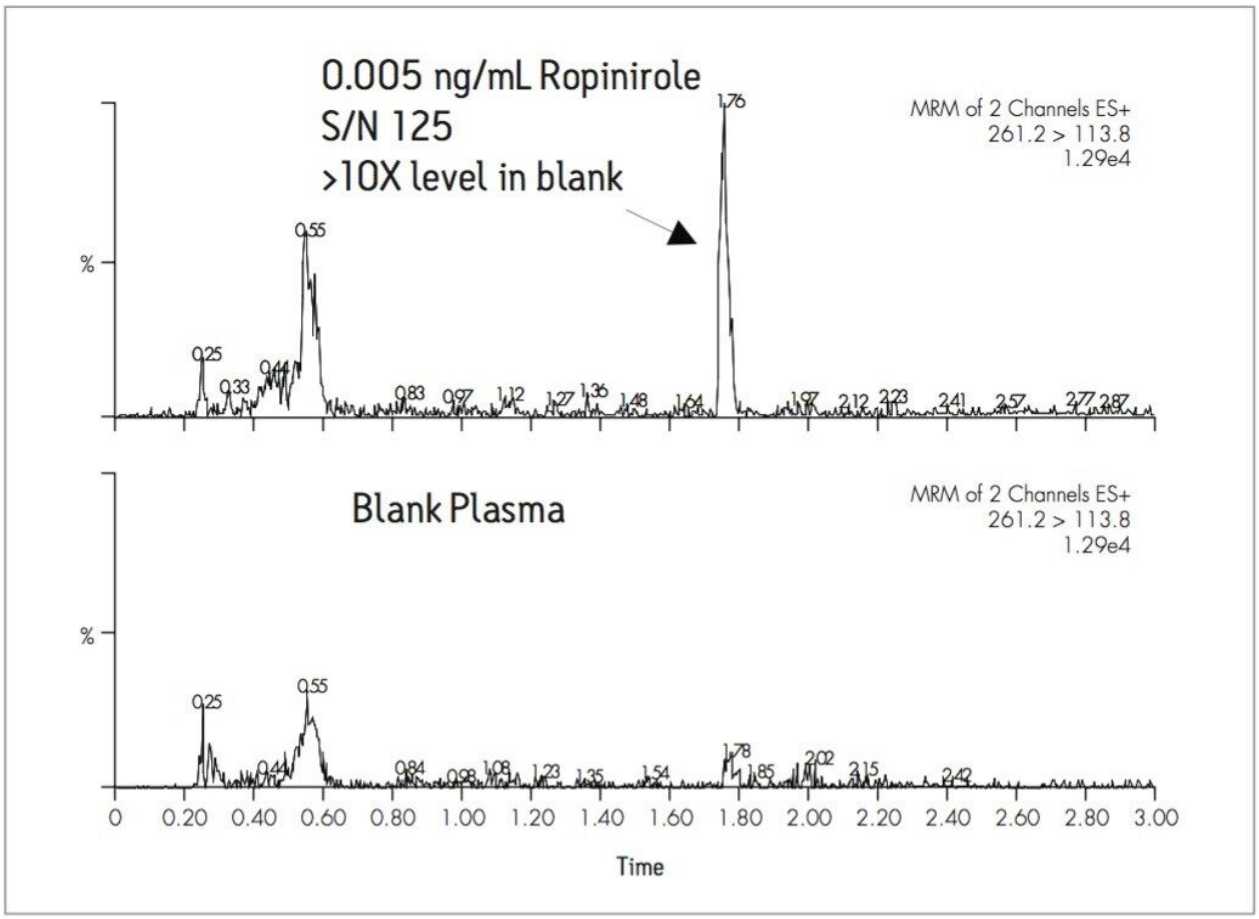


Figure 3. A 1 ng/mL sample of ropinirole and citalopram in human plasma, prepared with Oasis MCX µElution plate SPE.

Due to the relatively low dose regime for ropinirole, a lower limit of quantitation in the pg/mL range was required. The combination of UPLC Technology and Oasis µElution SPE provided the sensitivity necessary to achieve a 0.005 ng/mL LLOQ. Chromatography at the LLOQ for a sample prepared by µElution is shown in Figure 4.

For UPLC, the recommended starting point for strong needle wash composition is to make it the same organic strength as the end of your gradient. In this case, that would have been 98% MeOH, 2% aqueous. We found it necessary to modify the strong needle wash from 98% MeOH to 60:40 ACN:IPA with 0.5% HCOOH to eliminate carry-over.



Conclusion

A rapid and sensitive method for the determination of ropinirole in human plasma has been developed. The method achieves a S/N of over 100:1 at the required LLOQ of 0.005 ng/mL. The method meets the FDA requirements for linearity and excellent recovery for both analytes is achieved.

The combination of the Oasis MCX µElution plate for easy sample preparation and the speed and sensitivity of the UPLC/Quattro Premier platform facilitated rapid development of a robust bioanalytical method. This combination also enables researchers to obtain higher quality data faster in order to make critical project decisions.

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