

A Sensitive Method for the Determination of Ropinirole in Human Plasma by LC-MS-MS

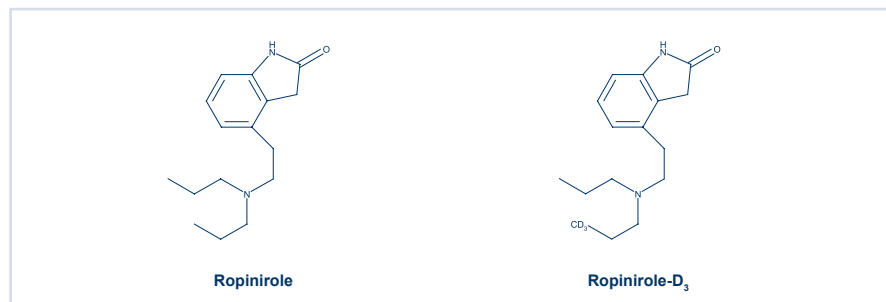
Authors:

William S. Edgemond, Kalyn Sowell, Christopher J.L. Buggé, and David B. Garcia
(CEDRA Corporation, Austin TX)

Introduction:

Ropinirole is used in the treatment of Parkinson's disease and for the treatment of Restless Legs Syndrome. An LC-MS-MS method for the quantitation of ropinirole in human plasma was developed and validated.

Ropinirole is a non-ergoline dopamine receptor agonist that exhibits a high affinity for the D₂ and D₃ receptors but little or no affinity for the D₁ receptor. Ropinirole is rapidly absorbed and well tolerated after oral administration. Ropinirole is effective in reducing motor complications. Also, ropinirole was effective in treating resting tremor in early Parkinson's disease, in reducing periodic leg movements and in improving sleep efficiency in patients with restless legs syndrome. These positive effects of ropinirole in Parkinson's disease are believed to be due to stimulation of the post-synaptic dopamine D₂-type receptor. A rugged and sensitive method for the analysis of ropinirole for pharmacokinetic investigation was developed and validated. This presentation provides details for the quantitation of ropinirole in human plasma.



Objective:

- Develop and validate a sensitive method for the quantitation of Ropinirole.
- Develop a rugged method that is simple to extract.
- Develop method suitable to analyze pharmacokinetic samples

Methodology:

Chemicals

Ropinirole hydrochloride and ropinirole-D₃ hydrochloride were purchased from Toronto Research Chemicals. All other chemicals were AR grade and solvents were HPLC grade or better.

Sample Preparation

Stock solutions of ropinirole were prepared in acetonitrile/water 1:1, as were combined intermediate, calibration spiking and internal standard solutions. Quality Control (QC) solutions were prepared in human K₂-EDTA plasma and stored in 0.5-mL aliquots at -20° C. QC samples were prepared from different weighings of drugs than the calibration spiking standards.

Extraction

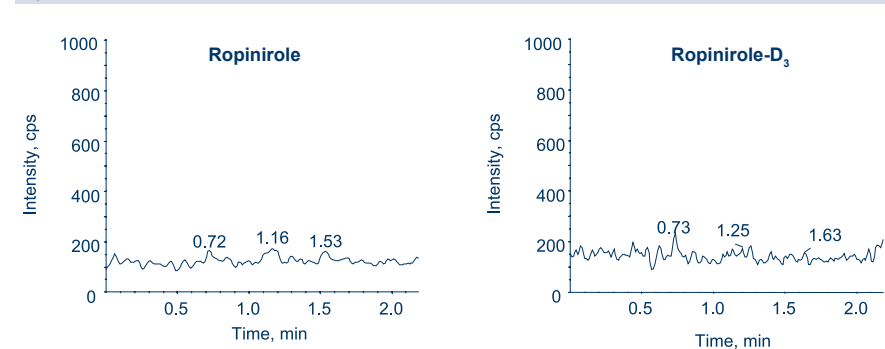
To all samples (K₂-EDTA plasma, 0.5 mL) except blank-blank samples, ropinirole-D₃ internal standard solution is added. After adding 0.200 mL of 1.0 M sodium carbonate, the samples were extracted with 3.0 mL of ethyl acetate/cyclohexane, 9:1. After centrifuging the samples, the organic layer was removed. The organic layer was dried with a gentle stream of nitrogen at 40 °C. The samples were reconstituted in 0.150 mL of mobile phase.

Sample Analysis by LC-MS-MS

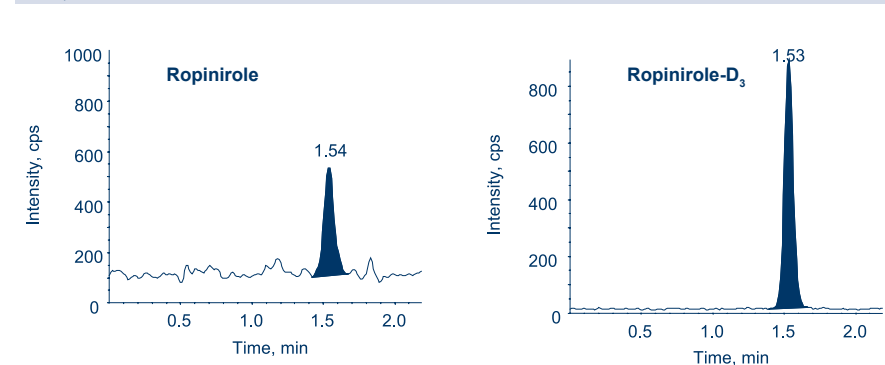
A SCIEX API-5000 LC-MS-MS in positive IonSpray MRM mode analyzed the extract. The analytes were resolved under isocratic conditions with a Thermo Scientific Hypersil GOLD PFP (3 μ, 4.6 × 50 mm) column and a mobile phase of 10 mM ammonium acetate in methanol/water, 1:1. The flow rate was 1.0 mL/min. Ropinirole (m/z 261 → 114) and the internal standard ropinirole-D₃ (m/z 264 → 117) eluted at approximately 1.5 minutes.

Representative Chromatograms:

Blank



LLOQ



Results:

Standard Precision and Accuracy

Mean of Three Validation Runs

Amount Added, ng/mL	10	20	50	100	200	500	900	1000
Mean Found, ng/mL	10.1	20.4	45.1	102	203	504	917	1010
CV (%)	3.0	6.7	2.2	2.1	1.5	0.5	3.8	0.6
% Bias	1.0	2.0	-9.8	2.0	1.5	0.8	1.9	1.0

LLOQ and QC Precision and Accuracy

Mean of Three Validation Runs

Amount Added, ng/mL	10	30	180	750	5000
Mean Found, ng/mL	9.39	30.9	187	786	4900
CV (%)	7.6	5.7	3.2	2.2	1.2
% Bias	6.1	3.0	3.9	4.8	-2.0
n	17	18	18	18	6

Stability

Concentration, pg/mL	750	30.0
Mean % Change		
BTS, 24 hrs @ 22°C	4.2	-2.1
FTS, 5 cycles	7.4	6.1
XTS, 71 hrs @ 22°C	3.5	1.4
LTS, 75 days @ -20°C	-7.3	-14.0

The isocratic chromatography conditions provided a quick and reliable way to measure ropinirole, resulting in a run time of 3 minutes or less. The recovery of ropinirole and ropinirole-D₃ was 90% and 94% respectively. Three validation runs were performed on separate days. The average precision (CV) and accuracy (%bias) across all levels of the QC range for ropinirole were within ±8.0%. The precision and accuracy at the LLOQ were within 9.0%. No chromatographic interferences or matrix effects from six different lots of plasma were observed indicating the specificity of the method. Stability of ropinirole in plasma was established for 24 hours at room temperature (BTS), 5 cycles of freezing and thawing (FTS), 80 days of storage at -20 °C (LTS), and 67 hours in the final extract (XTS).

Conclusion:

This method was successfully validated. The method proved rugged and sensitive in the determination of the concentrations of ropinirole in over 3400 human plasma samples generated from clinical trials.