



RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF ROPINIROLE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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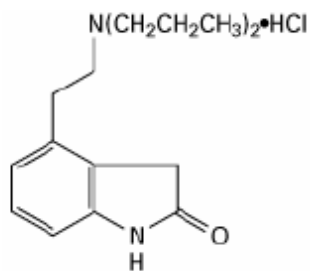
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ABSTRACT

A simple and accurate RP-HPLC method has been developed for the estimation of Ropinirole hydrochloride in bulk and pharmaceutical dosage forms using C₁₈ column 250 x 4.6 mm i.d, 5µm particle size in isocratic mode, with mobile phase comprising of buffer (pH 6.0) and Acetonitrile in the ratio of 50:50 v/v. The flow rate was 0.5ml/min and detection was carried out by UV detector at 245nm. The retention time for Ropinirole Hydrochloride was found to be 4.867 min. The proposed method has permitted the quantification of Ropinirole hydrochloride over linearity in the range of 5-50µg/ml and its percentage recovery was found to be 99.3-100.4%. The intra day and inter day precision were found 0.27% and 0.26% respectively.

Keywords: Ropinirole HCL, HPLC, Isocratic.

INTRODUCTION



Parkinson's disease is a progressive, neurodegenerative disorder primarily affecting dopaminergic neuronal systems, with impaired motor function as a consequence. It is manifested by the 'cardinal signs' of bradykinesia, rigidity of the extremities, resting tremor and later in the disease, postural reflex impairment. Ropinirole hydrochloride is an orally administered non-ergoline dopamine agonist. Chemically it is hydrochloride salt of 4-[2-(di propyl amino) ethyl]-1, 3-dihydro-2H-indol-2-one, with empirical formula of C₁₆H₂₄N₂O.HCl and molecular weight of 296.84. It is a white to pale greenish-yellow powder with a melting range of 243° to

250°C and a solubility of 133 mg/ml in water. It has high relative *in vitro* specificity and acts by binding with higher affinity to D₃ than to D₂ or D₄ receptor subtypes. The mechanism of Ropinirole hydrochloride induced postural hypotension is presumed to be due to a D₂ -mediated blunting of the noradrenergic response to standing and subsequent decrease in peripheral vascular resistance. Literature review reveals that very few analytical methods were evoked for the estimation of Ropinirole hydrochloride in human plasma by modern analytical instrument like LC-MS/MS¹, Stability indicating assays⁵, Establishment of impurity profile was carried out by HPLC⁶ and estimation of drug content in bulk and pharmaceutical dosage forms by HPLC³, HPTLC⁴ and spectro photometric methods² were reported. We here in report a simple and reliable RP-HPLC for the estimation of Ropinirole hydrochloride in bulk and pharmaceutical dosage forms.

EXPERIMENTAL

Reagents & chemicals

Pure standard of Ropinirole hydrochloride (99.65%) was obtained as gift sample from Inventis drug delivery systems Pvt. Ltd, Hyderabad along with certificate of analysis (COA). HPLC grade Acetonitrile (Qualigens), HPLC grade water, Potassium di hydrogen phosphate (S.D. Fine Chemicals) H_3PO_4 (Qualigens), Ropitar tablets (Torrent Pharmaceuticals) and Parkirop tablets (Cadila Pharmaceuticals Ltd.), Electronic analytical balance (DONA), Micro pipette (In labs, 10-100 μ l) were employed in the study. All the glassware employed in the work cleaned with hot water followed acetic anhydride then acetone and dried in hot air oven when ever required. Working environment was maintained in between 18-22 $^{\circ}$ c. However, the chemical structure and purity of the sample obtained were confirmed by TLC, IR, Melting point, DSC, and XRD studies.

HPLC apparatus and chromatographic conditions

The HPLC system (Shimadzu co, Tokyo, Japan) consisted of a Shimadzu model LC-10 ATVP, a Shimadzu model SPD-6AV variable wavelength detector (Possessing deuterium lamp with a sensitivity of 0.005 AUFs and adjusted to an absorbency of 245nm), a Shimadzu model C-R5A chromatograph integrator module (chart speed at 10mm/min and an attenuation 0), a Shimadzu model SIL-6A auto injector and a Shimadzu module SCL-6A system controller. Isocratic elution of mobile phase (50:50 v/v of buffer pH 6.0 and Acetonitrile) with flow rate of 0.5 ml/min was performed on C_{18} ODS analytical column (thermo hypresil, 5 μ m; 250x4.6mm i.d with C_{18} insert (100 Å), waters limited) as pre

column to protect the analytical column from strongly bonded material. Integration of the detector out put was performed using the Shimadzu class Vp soft ware to determine the peak area. The contents of the mobile phase were 50:50 v/v Buffer pH 6.0 and Acetonitrile. They were filtered through 0.45 μ m membrane filter and degassed by sonication before use. The flow rate of mobile phase was optimized to 0.5 ml / min which yield a column back pressure of 43-45 kg/cm 2 . The run time was set at 10 min and column temperature was maintained at ambient. The volume of injection was 20 μ l, prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The eluent was detected at 245 nm.

Procedure

Preparation of mobile phase

Buffer pH 6.0 and Acetonitrile in the ratio of 50:50 v/v were employed as a mobile phase and Buffer solution was prepared as directed by the procedure of Indian pharmacopoeia (1996).

Preparation of stock solution of Ropinirole hydrochloride

A stock solution was prepared by dissolving 50mg of Standard Ropinirole hydrochloride in a 100 ml volumetric flask containing 70 ml of methanol (HPLC grade) and sonicated for about 15 min and the volume made to the mark with methanol. Daily working standard solutions of Ropinirole hydrochloride were prepared by suitable dilution of the stock solution with the mobile phase where, ten sets of analyte solution were prepared in the mobile phase containing Ropinirole hydrochloride at a concentration of 5-50 μ g/ml. Each of the these dilutions (20 μ l) was injected six times in to the column, with flow

rate of 0.5 ml/min and peak area of each of the drug concentrations, retention times were recorded.

Construction of linearity

The concentrations of analyte were prepared from the stock solution by taking suitable volume (0.1 - 1 ml) and diluted up to 10 ml to get the desired concentrations for linearity in the range of 5-50 µg/ml. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilutions was injected five times into the column. The calibration curve for Ropinirole hydrochloride was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). It was found to be linear in the concentration range 5-50 µg/ml with good correlation in between concentration and mean peak area. The values were shown in Table1.

Estimation of Ropinirole hydrochloride in Tablet dosage forms

20 tablets were weighed to obtain the average tablet weight and were powdered by trituration. A sample of the powdered tablets claimed to contain 50 mg of active ingredient, was mixed with 30 ml of methanol. The mixture was allowed to stand with intermittent sonication to ensure complete solubility of drug. Further the resulting solution was passed through 0.45 µm membrane filter followed by adding of methanol to obtain a stock solution of 0.5mg/ml. An aliquot of this solution (0.5 ml)

Method validation

Linearity

The linearity for the detection of Ropinirole hydrochloride was 5-50µg/ml with ($R^2=0.997$; $y = 156.3x + 70.59$) the

was transferred to a volumetric flask and made up to a sufficient volume with mobile phase to get desired concentration of 25 µg/ml. The prepared dilution was injected five times into the column to obtain chromatogram. From that peak area, the drug content in the tablets was quantified.

RESULTS AND DISCUSSIONS

Method development

Buffer pH 6.0 and Acetonitrile in the ratio of 50:50 v/v were employed as a mobile phase. The present RP – HPLC method for the quantification of Ropinirole hydrochloride in bulk and pharmaceutical dosage forms, revealed as simple, accurate and precise method with significant shorter retention time of 4.867min. The typical chromatogram of Ropinirole hydrochloride was shown in Fig.1 and Fig.2.

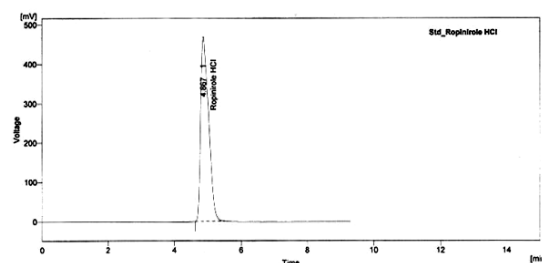


Fig.1: A Typical Chromatogram of Ropinirole Hydrochloride

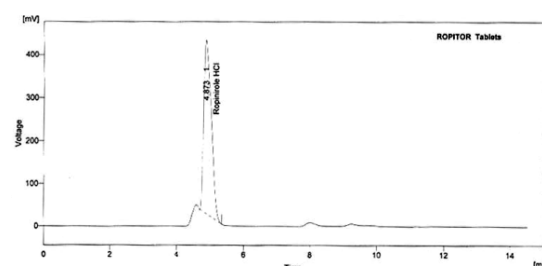


Fig.2: A Typical Chromatogram of Ropinirole Hydrochloride Tablets

coefficients of variation based on mean peak area for five replicate injections were found to be 0.07% to 0.47%. Results were shown in table1 and statistical data of calibration curves were shown in table2.

Table 1: Concentration Vs mean peak area of Ropinirole hydrochloride

Concentration ($\mu\text{g} / \text{ml}$)	Mean Peak Area	%RSD
5	786	0.29
10	1538	0.22
15	2539	0.47
20	3246	0.07
25	4026	0.05
30	4977	0.06
35	5367	0.05
40	6535	0.06
45	7008	0.06
50	7741	0.07

$$y = 156.31x + 70.591, R^2 = 0.9972$$

Table 2: Statistical data of calibration curves of Ropinirole hydrochloride

Parameters	Ropinirole hydrochloride
Linearity	5—50 $\mu\text{g}/\text{ml}$
Regression equation	156.3x + 70.59
Standard deviation of slope	0.034
Relative standard deviation of slope (%)	0.352
Standard deviation of intercept	0.153
Correlation coefficient (r^2)	0.997

Precision

The intraday and inter-day variations of the method were determined using five replicate injections of three concentrations and analysed on the same day and three

different days over a period of two weeks.

The result revealed the precision with %RSD (0.27% and 0.26%) respectively for intra day and inter day. Results were shown in **table 3**.

Table 3: Precision of method

Concentration of Ropinirole hydrochloride($\mu\text{g}/\text{ml}$)	Observed Concentration*			
	Intra day	%RSD	Inter day	%RSD
5	5.02	0.27	4.98	0.17
10	9.98	0.24	10.03	0.23
15	15.04	0.23	15.02	0.26

*Mean of five values

Accuracy

To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre-analysed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated by injecting the Ropinirole hydrochloride about five times, at three different concentrations equivalent

to 80, 100, and 120% of the active ingredient, by adding a known amount of Ropinirole hydrochloride standard to a sample of known concentration and calculating the recovery of Ropinirole hydrochloride with RSD (%), and % recovery for each concentration. The mean % recoveries were in between 99.3-100.4% and were given in **table 4**.

Table 4 : Recovery of the method

Drug Labeled claim (2mg)	Amount Added (mg)	Amount Present (mg)	Mean Amount Found(n=5)*	Mean % Recovery
Ropinirole hydrochloride	8	10.00	9.93±0.245	99.3
	10	12.00	12.05±0.340	100.4
	12	14.00	13.95±0.315	99.64

*Mean of five values

Assay

The assay for the marketed tablets (PARKIROP, ROPITAR) was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug

content was found to be 99.5 and 100.5% of the labeled claim. No interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. The results were shown in **table 5**.

Table 5 : Estimation of amount present in tablet dosage form

Brand Name Of The Tablet	Labeled Claim(mg)	Amount Estimated*(mg)	Mean ±SD	%Purity ±SD
PARKIROP	2	1.99	1.99±0.04	99.5±2.09
ROPITAR	1	1.04	1.04±0.02	100.4±1.9

*Mean of five values

System suitability

To know reproducibility of the method system suitability test was employed to establish the

parameters such as tailing factor, theoretical plates, limit of detection and limit of quantification and the values were shown in **table 6**.

Table 6 : System suitability parameters

Parameter	Ropinirole Hydrochloride
Retention time	4.867
Theoretical Plates	4803
Tailing factor	0.9
Linearity Range (µg /ml)	5-50
Limit Of Detection (LOD) (µg /ml)	0.045
Limit Of Quantitation (LOQ) (µg /ml)	0.151
Relative standard deviation (RSD)	0.538

Ruggedness

Ruggedness of the method (intermediate precision) was estimated by preparing six dilutions of the Ropinirole hydrochloride as

per the proposed method and each dilution injected in duplicate using different column and analyst on different days. The results were shown in **table 7**.

Table7 : Ruggedness of Ropinirole hydrochloride

S. No.	Labeled claim(mg)	Amount estimated*(mg)	Mean \pm SD	%RSD
Set-1	2	2.03	2.03 \pm 0.03	1.5
Set-2	2	1.97	1.97 \pm 0.02	1.02

*Mean of six values

Robustness

Robustness of the proposed method was estimated by changing mobile phase composition from buffer: Acetonitrile 50:50v/v to buffer: Acetonitrile 55:45 v/v, changing the column brand, changing the flow rate from 0.5 ml to 0.7ml/min, changing the pH (\pm 0.2), changing the temperature

(\pm 5⁰c) and changing the wave length (\pm 5nm) and System suitability parameters were found to be within acceptable limits. Results were shown in **table 8** indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions.

Table 8 Robustness of Ropinirole Hydrochloride

Parameter	Variation	System Suitability		
		Theoretical Plates	Tailing Factor	% RSD
Standard	-	4803	0.9	0.3
Flow rate	0.5-0.7	3959	0.85	0.1
Wave Length	-5nm	5965	0.73	0.25
	+5nm	6239	0.72	0.32
Mobile Phase	50:50 to 55:45	3583	0.99	0.21
Temperature	-5°C	3446	0.9	0.13
	+5°C	3739	1.1	0.15
pH	-0.2 units	5823	0.7	0.48
	+0.2 units	6232	0.5	0.16

Detection and quantification limits

Limits of Detection (LOD) and Quantification (LOQ), the limits of detection and quantification were calculated by the method based on the standard deviation (σ) and the

slope (S) of the calibration plot, using the formulae $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$.

Specificity

The specificity test of the proposed method demonstrated that the excipients from tablets

do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method.

CONCLUSION

The results of the study reveal that the proposed RP-HPLC method for the estimation of Ropinirole hydrochloride is simple and accurate in bulk and pharmaceutical dosage forms.

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