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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ROPINIROLE HCL IN TABLETS BY RP-HPLC

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Abstract
A simple and precise and accurate RP - High performance liquid chromatography (Reverse Phase - HPLC) method has been developed for the estimation of Ropinirole in tablet formulations. The separation was achieved on a C_{18} (250 x 4.6 mm) 5 - micron Hypersil BDS using a mobile phase consisting of a degassed mixture of 0.05 M glacial acetic acid (2.85 mL of glacial acetic acid in 1000 mL of water) and acetonitrile (50:50) with a flow rate of 1.0 mL/min. The mobile phase showed the most favorable chromatographic parameter for analysis. The detection of the constituent was done using UV detector at 250 nm. The retention time of ropinirole was found to be 3.987 minutes. The method was validated for system suitability, precision, accuracy, linearity, robustness. The linear range for ropinirole was 4 – 12 µg / ml. The method is validated for accuracy, precision, linearity, specificity and robustness in accordance with ICH guidelines and revealed that the method established is specific, accurate, rapid, precise, reliable and reproducible for the method has been successfully used to analyze commercial solid dosage forms which are locally available 0.25 mg ropinirole tablets and its percentage recovery was found to be 99.82%.
***Keywords*:** Ropinirole Hydrochloride (RPR), High Performance Liquid Chromatography (HPLC), Mobile phase, Validation.

1. Introduction

Ropinirole (RPR) is a non-ergoline dopamine agonist, chemically it is 4-[2-(dipropylamino) ethyl] -1, 3-dihydro-2H-indol-2-one monoHCL, is a specific D2 and D3 receptor non-ergoline dopamine agonist that is probably equally effective as L-dopa in mild, early Parkinson's disease[1] . Very few high-performance liquid chromatographic (HPLC) methods in plasma were developed using ultraviolet[2,3] , electrochemical [4] or mass spectrometric [5] detection. Capillary zone electrophoresis was used for the determination of the dissociation constants of RPR. It is not official in any pharmacopoeia. UV, LC-MS [6], Visible Spectrophotometric, Spectroflourimetric methods [7-10] have been reported for the estimation of RPR in dosage forms and in human plasma, but these methods are costly and require sophisticated equipments for the processing of drug. The present study is designed to develop a simple, precise and accurate reverse phase HPLC method with good recoveries and shorter retention time for the estimation of Ropinirole in the tablet formulations.

2. Materials and Methods

2.1 Materials: Ropinirole, Acetonitrile HPLC Grade- (E. Merck Ltd. Mumbai, INDIA), Glacial acetic acid, Water HPLC Grade- (E. Merck Ltd. Mumbai, INDIA)

2.2 Instrument: Highperformanceliquid chromatography (HPLC): Model - shimadzu-LC-10ADVP, Pump -LC-10ATVP, operates back pressure 5000 psi.
Detector - UV-Visible, **Analytical Balance –** Afcoset, **Vacuum filter -** BV– 40**,** Smart Labtech Pvt. Ltd., **Sonicator -** Fast clea.

2.3 Chromatographic Conditions: Chromatographic separation was performed at ambient temperature on a reverse phase C_{18} (250 x 4.6 mm) 5-micron Hyper-sil BDS column with use of a filtered and degassed mobile phase consisting of Acetonitrile: 0.05M Glacial Acetic Acid containing (50:50 v/v).The flow rate of mobile phase was adjusted to 1.0 mL/min. The UV detector wavelength was set at 250 nm. The injection volume of the standard and sample solutions was 20 mcL. Run time was 8 min.

2.4 Procedure

2.4.1 Preparation of 0.05 M Glacial Acetic Acid: Accurately measured volume of 2.85 mL

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of glacial acetic acid (0.05 M) was transferred to a 1000 mL volumetric flask containing 500 mL of distilled water and sonicate for 5 min and final volume was made with distilled water. The solution was filtered through 0.45 µm membrane filter.

2.4.2 Preparation of Mobile Phase: A mixture of Acetonitrile: 0.05M Glacial Acetic Acid (50:50) previously filtered through 0.45µm membrane filter was used as a mobile phase.

2.4.3 Preparation of Standard Stock Solution: 25 mg of Standard Ropinirole was accurately weighed and transferred into a 25 mL of clean and dry volumetric flask. Dissolve and dilute to volume with mobile phase to give a solution containing 1 mg/mL.

2.4.4 Preparation of Working Standard Solution: From the prepared stock solution further dilution were made in order to get concentrations of 4, 6,8,10, 12 ppm for the construction of calibration curve

2.4.5 Preparation of Sample Solution: Twenty tablets were weighed and finely powdered. Accurately 612.35 mg of tablet powder equivalent to 1 mg of Ropinirole was weighed and transferred to a 10 mL volumetric flask containing 5 mL of mobile phase and sonicate for 30 minutes and the volume was made with mobile phase. The solution was filtered through 0.45 µm membrane filter.

5 mL of the above solution was transferred into a clean and dry 50 mL volumetric flask and volume was made with mobile phase to give a solution containing 10 ppm.

2.5 Method Development: It was done by using different made of C_{18} stationary phase like Thermo column, octadecyl silane and Hypersil BDS with different ratios of mobile phase (Acetonitrile: 0.05M Glacial Acetic Acid) that is 60:40, 40:60, 50:50 on each of the above mentioned column. It was found that C_{18} (250 x 4.6 mm) 5-micron Hypersil BDS with 50:50 ratio of mobile phase offered more advantageous than others. Because with this combination the peak shape of Ropinirole was found to be good and has optimum plate count and tailing. Individual drug solution of 20 mcL was injected into the column at ambient temperature at a concentration of 10 ppm and it was chromatographed for 8 min using mobile phase at a flow rate of 1.0 mL/min and the UV spectra of ropinirole was recorded at a wavelength of 250 nm. The retention time of ropinirole was found to be 3.987 minutes. The overlain of spectra is shown in Fig.2.

3. Method validation

To confirm its suitability for its intended purpose, the method was validated in accordance with ICH guidelines, for system suitability, linearity, specificity, precision, accuracy, and limit of detection, limit of quantification, robustness, and solution stability. All the validation studies were performed by replicate injections of sample and standard solutions.

3.1 Linearity and Range: Linearity was established for the analyte peaks from 40 -120% of the test concentrations and were subjected to linear least square regression analysis. The calibration and linearity equation relating Y (drug: peak height ratio) to X (concentration µg ml^{-1}) was fitted and correlation coefficient (r^2), slope, intercept were calculated. The intra and inter day linearity values were established. The values were tabulated in table no 1.

3.2 System Suitability: To know reproducibility of the developed RP - HPLC method, system suitability performance parameters were determined by injecting standard solutions. Parameters such as retention time Rt), peak area, number of theoretical plates (N), tailing factor (As) were determined. The results were shown in Table I, indicating good performance of the system.

System repeatability was determined by ten replicate injections of a standard solution, the relative standard deviation (RSD) of retention time and peak area of ropinirole was less than 2.0 %.

3.3 Detection and Quantitation limit: Detection and Quantitation limit were calculated by the method based on the standard deviation (σ) and slope (S) of the calibration plot, using the formulae LOD = 3.3 σ/ S and LOQ = 10 σ/ S. and were mentioned in table no.1

3.4 Specificity of Ropinirole: Specificity was assessed by comparison of chromatograms obtained from tablets and blank solution and from the drug standards. Because the retention times of ropinirole from standard solution and from dosage forms were identical & no-co-eluting peaks from diluents were observed [fig.2 (a); fig.2 (b); fig.2(c)], the method was specific for quantitative estimation of drug in commercial formulations (table no.2).

3.5 Accuracy: Accuracy was determined by recovery study. It was carried out by taking the known amount of drug corresponding to 80%, 100% and 120% of the label claim of ropinirole.

$$\%\text{Recovery} = [(c_t - c_u)/ c_a] \times 100.$$

Where c_t is the total conc. of the analyte found; c_u is the conc. of the analyte present in

formulation; and c_a is the conc. of the pure analyte added to the formulation. The values were tabulated in the table no.3, indicating good accuracy of the method.

3.6 Precision: Precision was determined by analyzing variation of results within the same day (intra day) and variation of results between days (inter day). Intra-day and inter-day precision and accuracy were evaluated by analyzing quality-control samples containing low, medium, and high concentrations of ropinirole 4, 8, 12 ppm. For intra-day variation, sets of five replicates of the three concentrations were analyzed on the same day; for inter-day variation, five replicates were analyzed on three different days. The values were listed in the table. No.4

3.7 Robustness: In this we determined effect of variation of organic phase composition in mobile phase using 90% and 110%, effect of variation of flow rate using 0.9 mL/min and 1.1 mL/min and effect of variation of temperature using 30^o C. For all the above system suitability parameters were evaluated and found to be within the limits, for all % RSD was less than 2.0 %.

4. Result and Discussion

The method was carried out using C_{18} Hyper-sil BDS (250mm x 4.6mm) 5-mcm. The mobile phase was 0.05 M Glacial acetic acid: Acetonitrile (50:50) with a flow rate of 1.0 mL/min and detection using a UV detector at 250 nm. The retention time of ropinirole was found to be 3.987 min and % Recovery for ropinirole was 99.82.

It shows that the method is accurate and free from interference of the excipients. The low value of standard deviation obtained confirms the precision of the method. A linear relationship was obtained in the range of 4-12 µg / ml for ropinirole. When pharmaceutical preparations containing ropinirole was analysed, the results obtained by the proposed method has good agreement with the labeled amount.

The robustness of the method showed that there were no marked changes in the chromatographic parameters, which demonstrates that the method developed is robust and the data from system suitability studies indicate conformity to compendial requirement.

5. Conclusion

A new, reversed-phase HPLC method has been developed for the analysis of ROPINIROLE in marketed tablets. It was shown that the method is accurate, repeatable, linear, precise, specific, and selective, and therefore reliable. The run time is relatively short, i.e. 3.987 min, which enables rapid quantitation of many samples in routine and quality-control analysis of the tablet formulation. The same solvent was used through out the experimental work and no interference from any excipient/ placebo was observed. The method could therefore find practical application as a quality-control tool for estimation of drug in tablet dosage forms.

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Table no 1. Linearity and System Suitability

Method characteristic	Ropinirole
Linearity Range (µg / ml)	4-12
Regression equation	Y= 4.4675X+5.4148
Retention time	4.037
Correlation coefficient(r^2)	0.9974
SD of intercept	0.146
%RSD of peak areas	0.513
Theoretical plates	2744.549
Tailing factor	1.069
LOD (ng / ml)	0.1078
LOQ (ng / ml)	0.3268

*Mean of ten observations

Table no 2. Specificity of the method

S No.	Sample name	Average Retention time (minutes)[a]
1	blank	2.9 (aprox.)
2	Standard	4.037
3	Sample	4.020

Average of four replicate injections of four samples

Table 3: Results of Accuracy of Ropinirole

Drug	% of spiked Level	*Amount of drug added (mg)	*Amount of drug found (mg)	%*Recovery	Statistical Analysis of % Recovery	
					SD	%RSD
Ropinirole	80% Sample	0.796	0.7943	99.88	0.147	0.15
	100% Sample	0.9956	0.9896	99.393	0.454	0.46
	120%Sample	1.1843	1.18066	99.69	99.69	0.25

*Mean of five observations

Table no. 4 Precision of Ropinirole

Drug amount Con (µg / ml)	Intra-day [a]		Inter-day[b]	
	Mean ± SD	%RSD	Mean ± SD	%RSD
4	3.95 ± 0.022	0.5569	4.032 ± 0.064	1.5873
8	7.96 ± 0.014	0.1758	8.034 ± 0.036	0.4480
12	11.942 ± 0.028	0.2344	12.048 ± 0.049	0.4067

a. Intra-day accuracy and precision were determined by five replicate analyses for each concentration
b Inter-day accuracy and precision were determined by fifteen replicate analyses (day 1, $n = 5$; day 2, $n = 5$; day 3, $n = 5$) for each concentration

Table no: 5 Results for the assay of drugs.

Formulation	Active ingredient (Label claim mcg)	Amount found * ± SD	%purity* ±SD
Ropin	0.25	0.247 ± 0.003	98.96 ± 1.42
Requip	0.25	0.256 ± 0.0026	102.64 ± 1.06

- -- a mean of 5 injections of each formulation

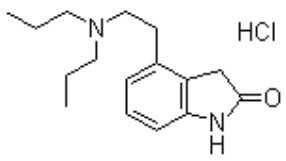

Fig. 1. Structure of Ropinirole (RPR)

Fig. 2

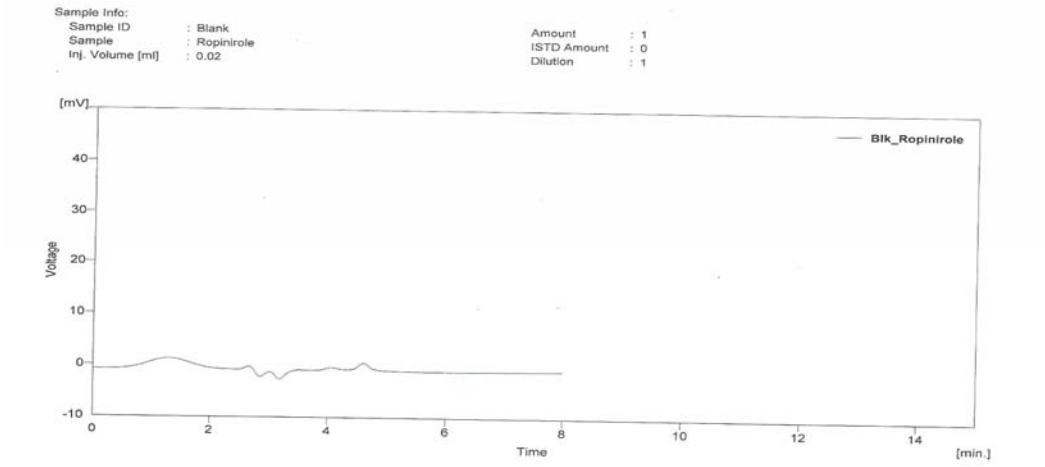

Fig.2 (a). Chromatogram of blank

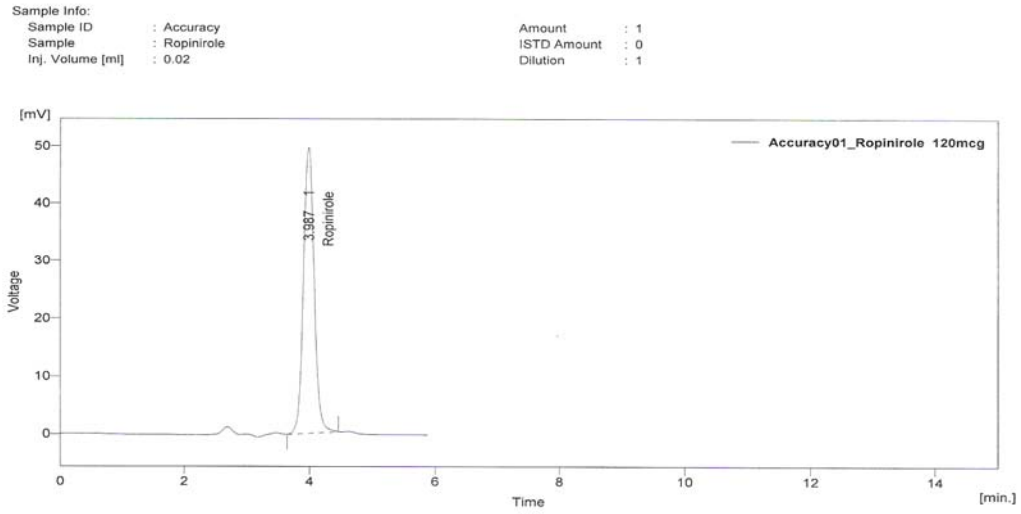

Fig.2 (b). Chromatogram of standard Ropinirole

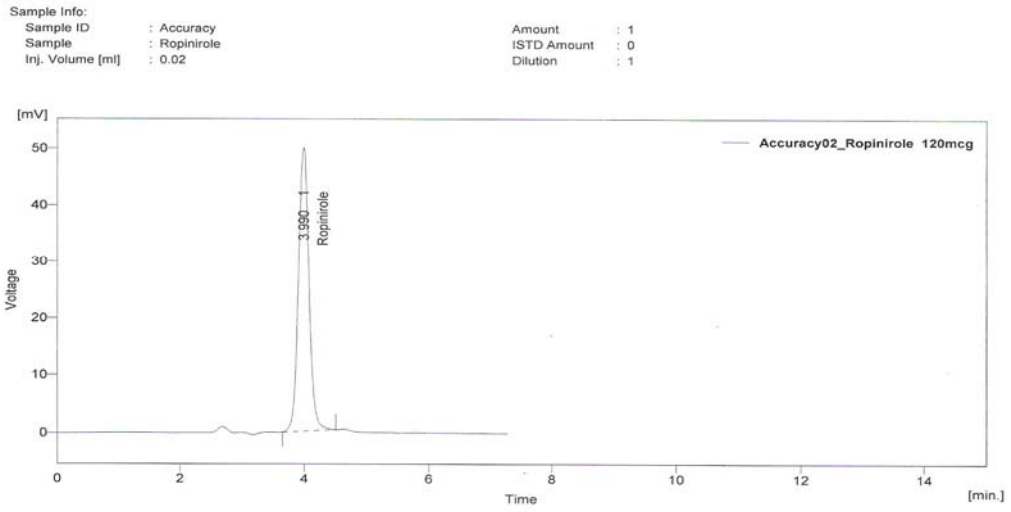

Fig.2 (c). Chromatogram of sample Ropinirole