International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (4): 250-259
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved

A Simple and Improved HPLC Method Development and Validation and Stability Studies for Estimation of Ropinerole in Tablet Dosage Forms

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Received: 09 Jul 2019 / Accepted: 11 Aug 2019 / Published online: 1 Oct 2019
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Abstract
A simple and accurate, precise method was developed for the estimation of the Ropinerole in bulk and pharmaceutical dosage form. The chromatography was performed by running through X-Bridge C18 Column which is having 250X4.6 mm I.D with 5 µm particle size. Column temperature was maintained at ambient, with a mobile phase phosphate buffer: Acetonitrile at the ratio of 60:40 v/v. The flow rate was 1 ml/min and the UV detections was carried out at 210-300 nm. Retention time of Ropinerole were found to be 2.29 mins. The percentage purity of the Ropinerole were found to be 99.76% respectively. The average percentage recovery of Ropinerole was found to be 98.94%. The LOD values of Ropinerole was 0.03 µg/ml and LOQ values were 0.09 µg/ml. The intraday and inter day precision (%RSD) was found to be 0.86 & 0.87 and repeatability was found to be 0.440 less than, the method was validated as per ICH guidelines. The percentage recovery was in good agreement and the method is simple, specific, precise, and accurate for the determination of Ropinerole which can be applied for the routine quality control analysis. The statistical parameters and recovery studies were carried out and reported.

Keywords
Ropinerole, Parkinson's disease, Development, Validation, HPLC.

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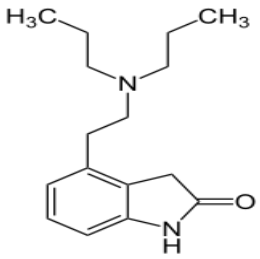
INTRODUCTION
Ropinerol is a dopamine agonist of the non-ergo line class of medications and it acts as a D_2, D_3, and D_4 dopamine receptor agonist with highest affinity for D_2. It is used in the treatment of Parkinson's disease and restless legs syndrome.



Chemical Structure: Ropinerole

Mechanism of action: Ropinerole is a non-ergo line dopamine agonist. The precise mechanism of action of ropinerole as a treatment for Parkinson's disease is unknown, although it is thought to be related to this ability to stimulate dopamine D_2 receptors within the caudate putamen in the brain. Ropinerole acts as a D_2, D_3, and D_4 dopamine receptor agonist with highest affinity for D_2. It is weakly active at the 5H, and receptors and is said to have virtually no affinity for the 5-HT_1, GABA, and β –adrenoreceptors.

Pharmacokinetic-Pharmacodynamic actions of Ropinerole:

This Parkinson's disease mainly on neuroprotective interventions. Drugs that have been used for the therapy are levodopa, usually combined with a peripheral decarboxylase inhibitor, synthetic dopamine receptor agonists, and antimuscarinic drugs, amantidine, monoamine Oxidase-B(MAO-B) inhibitors and catechol-o-methyl transferase (COMT) inhibitors.

MATERIALS AND METHODS

Reagents & Chemicals

Ropinerole, Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate, Phosphate buffer, Ortho phosphoric acid, Milli Q water and Acetonitrile (HPLC grade, Loba Chem). Mobile phase was filter through a 0.45µ membrane filter were used for the preparation of sample Solutions. All chemicals were of an analytical grade and used as received.

Instrumentation

Chromatographic separation was achieved by using a Waters 2489 UV 2695 pump, Waters 2998 PDA 2695 pump Software Empower2 photodiode array detector was used.

Chromatographic conditions

A Symmetry C-18 (Make: Waters, 250 mmx4.6 mm I.D; particle size 5µm) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 20µL. The photodiode array detector was set to a wavelength of 290nm for the detection and Chromatographic runtime was 10minutes.

Preparation of Standard

25mg of Ropinerole Working standard was accurately weighed and transferred into a 25 ml volumetric flask and about 20 ml of diluent was added to it and sonicated to dissolve drug completely and volume was made up to the mark with the same solvent which gave Stock solution of 1000 ppm. 1 ml of the above stock solution was pipetted into a 10ml volumetric flask and was diluted up to the mark with diluents to prepare 100ppm solution. Further 1 ml of prepared 100 ppm solution was pippetted into a 10ml volumetric flask and was diluted up to the mark with diluents which gave 10ppm Ropinerole working standard solution. The solution was mixed well and filtered through 0.45µm filter.

Buffer preparation

About 6.8 grams of Potassium di hydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. The pH was adjusted to 3.0 with Orthophosphoric acid.

Mobile phase preparation

400ml (40%) of above buffer and 600 ml of Acetonitrile HPLC (60%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

Diluent Preparation: Mobile phase as diluent.

Sample Preparation

20 tablets of marketed drug were weighed, and the average weight was calculated. The sample equivalent to 25 mg of Ropinerole was accurately Weighed and transferred into a 25 ml volumetric flask. About 20 ml of diluent was added and sonicated to dissolve drug completely and the volume was made up to the mark with diluent which gave stock solution of 1000ppm. The solution was mixed well and filtered through 0.45µm filter. 1 ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent to prepare 100ppm solution. Further 1 ml of prepared 100ppm solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent which gave 10 ppm Ropinerole working

standard solution. It was mixed well and filtered through 0.45µm filter.

METHOD DEVELOPMENT

To develop a suitable and robust RP-HPLC method for the determination of Ropinerole different mobile phases were employed to achieve the best separation and resolution. The method development was started with AGILENT 250mm Column with the mobile phase composition of Methonol and water with the ratio of 80:20 v/v. The flow rate is maintained 0.5ml/min. At the first trail, low response of peak was identified. At the second trial, the mobile phase composition of acetonitrile only and maintained flow rate at 0.5ml/ min. Here we got tailing of peak. For the next trail 3, analysis was run through C18 X-Bridge Column with 4.6X150mm I.D with mobile phase ratio (acetonitrile: water) 50:50v/v with flow rate 1ml/ min. We observed tailing of peaks. Trail 4, flow rate was maintained 1.0ml/ min. mobile phase composition acetonitrile: acetate buffer was kept in the ratio of 50:50 v/v. R_t for Ropinerole 2.25 mins, but we got broad and tailing peak observed and combined other peakes. At last trail that was optimized trail, mobile phase composition phosphate buffer: acetonitrile (60:40 v/v) flow rate 1.0ml/ min. Retention times for Ropinerole was 2.29 mins. At this trail the separation was completely done with sharp and good peak shape.

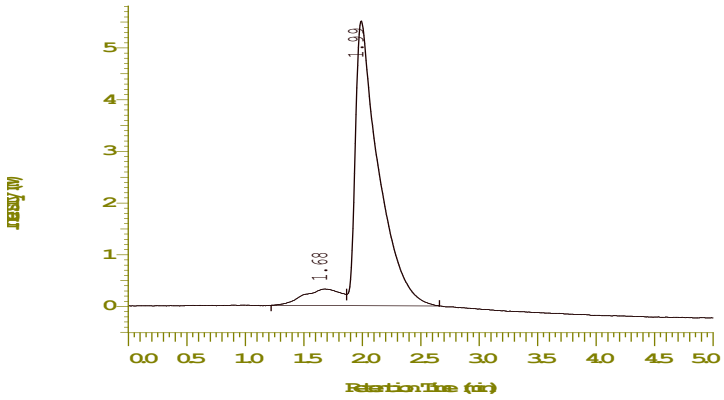


Figure 1: Chromatogram for Ropinerole at Trail 1

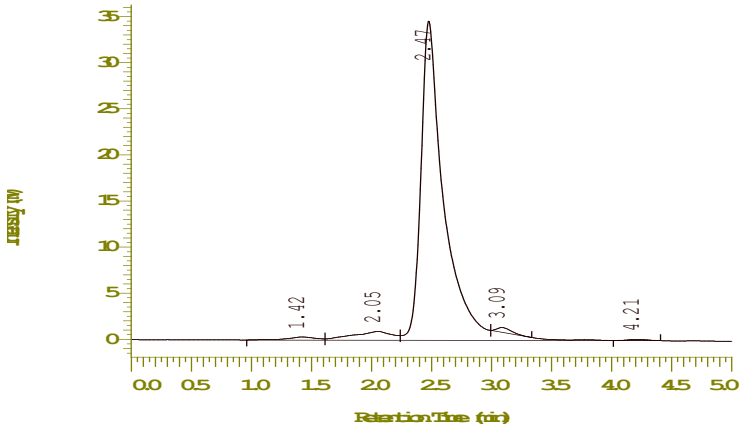


Figure 2: Chromatogram for Ropinerole at Trail 2

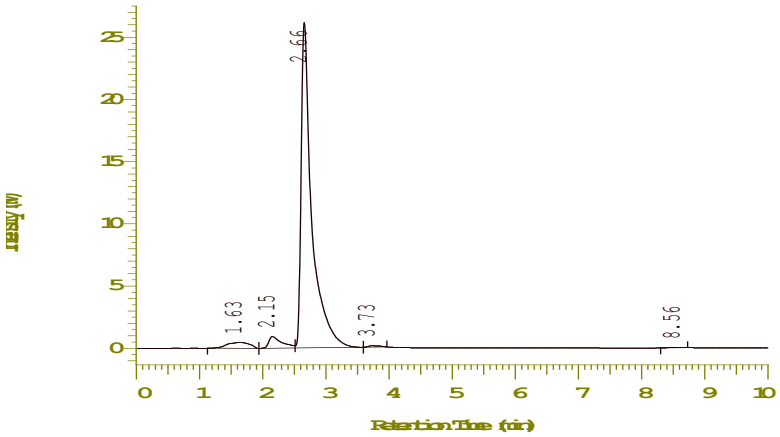


Figure 3: Chromatogram for Ropinerole at Trail 3

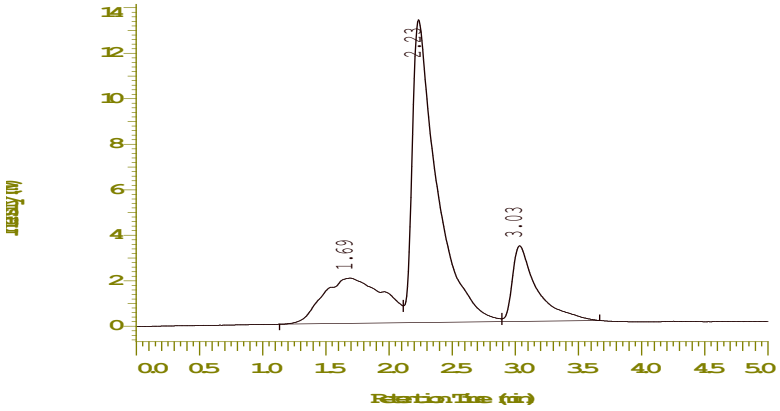


Figure 4: Chromatogram for Ropinerole at Trail 4

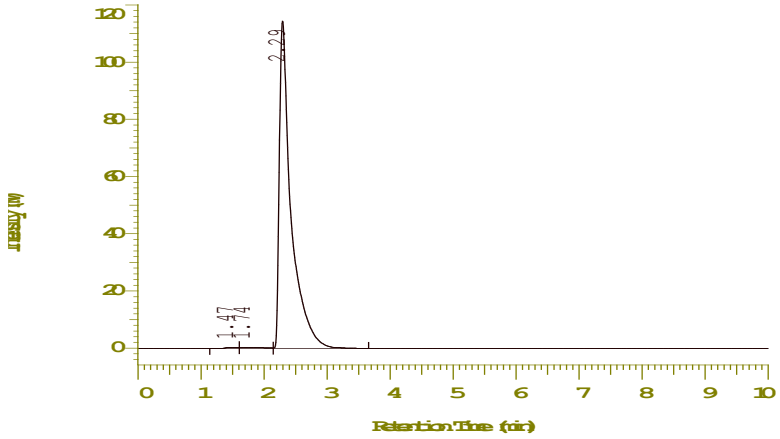


Figure 5: Optimized Chromatogram for Ropinerole at Trail 5

METHOD VALIDATION

System Suitability

To demonstrate system suitability, the standard solution prepared as per method. Weighed accurately 10 mg of Ropinerole working standard was weighed and transferred to 10 ml clean and dry volumetric flask. Add 7ml of diluent and sonicate to dissolve it completely and dilute it with diluent. From the above solution, transfer 0.9 ml of stock solution into 10 ml of flask and again dilute it with diluent. This solution is injected six replicate injections into the HPLC system as per methodology. The system suitability parameters were evaluated from the standard solution and found to be within the acceptance criteria. The % RSD for Ropinerole peak areas from six replicate injections of standard

solution was found to be within the limits. The results are summarized in Table-1 and Figure 6.

Table 1: System Suitability for Ropinerole

S.No	Name of the Compound	$R_t$mins	Peak Area	USP Theoretical Plate Count	USP Tailing Factor	%RSD
1.	Ropinerole	2.96	1025457	2085	0.37	0.86

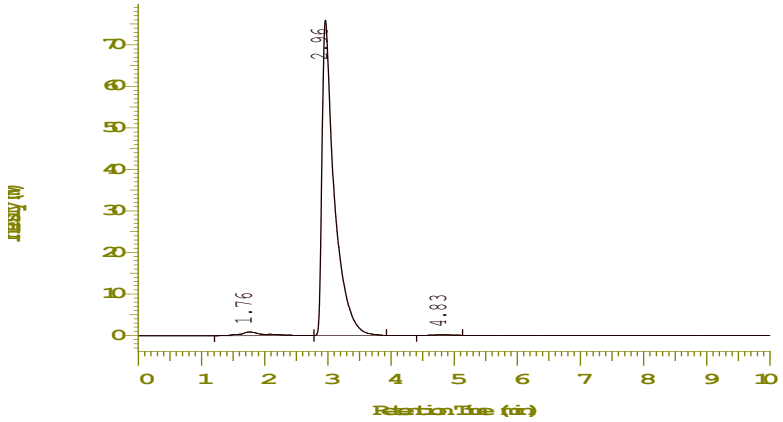


Figure 6: System Suitability Chromatogram for Ropinerole

Specificity

Blank interference

The specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. No interferences were found in Ropinerole retention time.

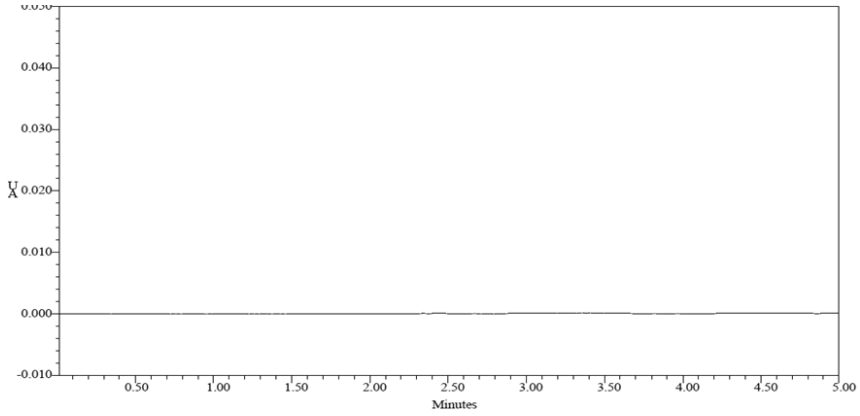


Figure 7: A typical HPLC Chromatogram showing the no interference of diluent

Establishment of Limit of Detection and Limit of Quantification:

A study was conducted to establish the limit of detection (LOD) and limit of quantification (LOQ) of Ropinerole based on slope method. Prepared a series of solutions from 10% to 50% of standard concentration of Ropinerole. These solutions were injected into the HPLC system as per methodology. Plotted a graph by taking concentration on X-axis and area on Y-axis, calculated the standard error and slope of the calibration curve. The predicted LOQ concentration and LOD concentration are calculated by using formula given below. The results are summarized in the Table 3-4.

$$LOQ = \frac{10 \times \sigma}{S} \qquad LOD = \frac{3.3 \times \sigma}{S}$$

σ = Standard Error of the calibration curve
S = Slope of the calibration curve

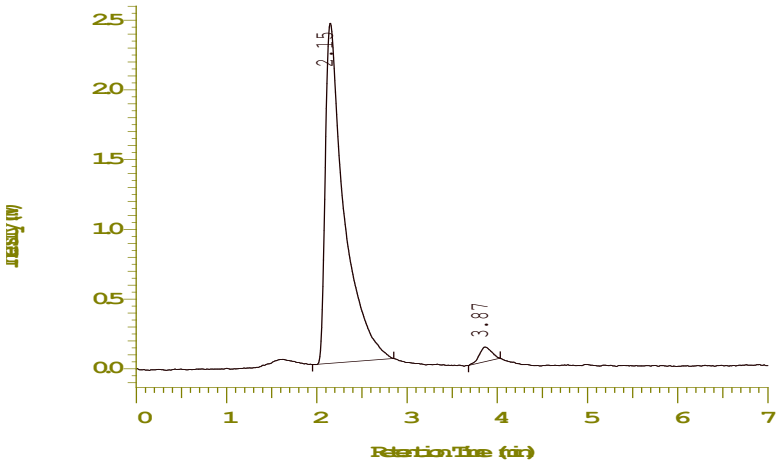


Figure-8: Chromatogram for LOD and LOQ

Table 2: Results of LOD and LOQ of Ropinerole

Sl. No	Rt	Theoretical Plates	% Area	Tailing factor
1	2.95	2951	99.91	0.63
2	3.87	1934	0.09	0.21

Discussion:
The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.03 & 0.09 µg/ml respectively.

Linearity:
Linearity is carried out under LOD-LOQ establishment experiment, the same linearity establishment data can be used to deduce the linearity from 10 ppm to 50 ppm for Ropinerole. A graph was plotted to concentration in ppm on X-axis versus response on Y-axis. Calculated % y-intercept and correlation coefficient. The calibration curve for Ropinerole are shown as in the Figures 9.

Table 3: The results and the linearity graph of Ropinerole

Name of the Level	Ropinerole			
	Concentration In ppm	Theoretical plates	%Area	R_t mins
Level - 1	0	0	0	0
Level - 2	10	2149	0.06	2.96
Level - 3	20	1934	0.06	2.96
Level - 4	30	2583	99.37	2.97
Level - 5	40	2086	99.96	2.96
Level - 6	50	2963	99.97	2.96
Correction Coefficient				2.96

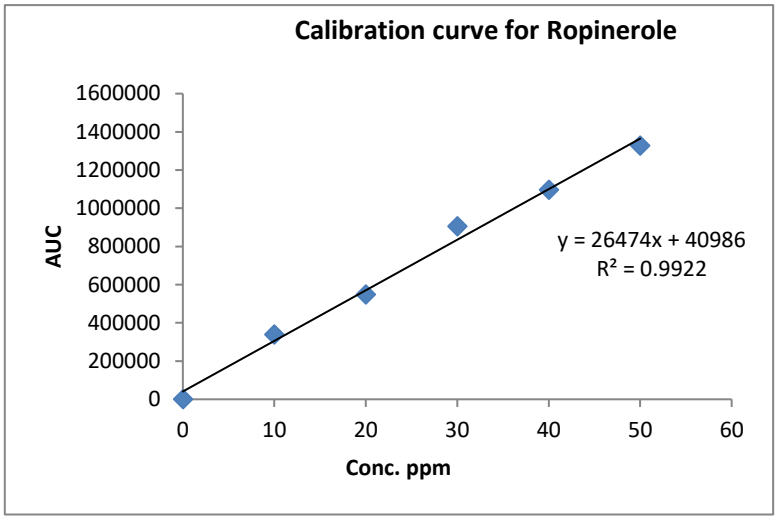


Figure9: Calibration Curve for Ropinerole

Precision:

Repeatability

The standard solution was injected for the five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injection was found to be within specified limits.

Table 4: Results showing Repeatability of Ropinerole

S.No.	Injections	Area Response	R_t(mins)
1.	I	1025457	2.96
2.	II	1003224	2.94
3.	III	995798	2.97
4.	IV	992259	2.95
5.	V	998740	2.97
Average		1003096	2.958
Standard Deviation		13131.13	0.013038
% RSD		1.309061	0.440784

Accuracy

The study was performed for 80 %, 100 % and 120 % for Ropinerole. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. The results were summarized below.

Table 5: Results showing for Accuracy of Ropinerole

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	8	10	99.13	Mean= 98.94667%
S_2 : 80 %	8	10	98.79	S.D. = 0.171561
S_3 : 80 %	8	10	98.92	% R.S.D.= 0.1733
S_4 : 100 %	10	10	99.72	Mean= 99.76%
S_5 : 100 %	10	10	99.81	S.D. = 0.045826
S_6 : 100 %	10	10	99.75	% R.S.D.= 0.0459
S_7 : 120 %	12	10	99.36	Mean= 99.37667%
S_8 : 120 %	12	10	99.28	S.D. = 0.105987
S_9 : 120 %	12	10	99.49	% R.S.D. = 0.1066

Robustness:
Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table 11, % RSD < 2%) the developed RP-HPLC method for the analysis of Ropinerole(API).

Table 6: Results showing for Robustness of Ropinerole

Change in parameter	% RSD
Flow (1.1 ml/min)	0.06
Flow (0.9 ml/min)	0.04
Temperature (27^{0}C)	0.08
Temperature (23^{0}C)	0.11
Wavelength of Detection (213 nm)	0.03
Wavelength of detection (209 nm)	0.02

Intra-assay & inter-assay:
The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Ropinerole revealed that the proposed method is precise

Table 7: Results of intra-assay & inter-assay

Conc. Of Ropinerole(API) (µg/ml)	Observed Conc. Of Ropinerole (µg/ml) by the proposed method			
	Intra-Day Assay		Inter-Day Assay	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	10.01	0.86	10.03	0.87
30	30.02	0.30	30.03	0.32
100	99.97	0.13	99.95	0.11

Assay
Standard preparations were made from the API and sample preparation are from formulation. Both the sample and standards are injected six homogenous samples. Drug in the formulation was estimated by taking the standard as the reference.

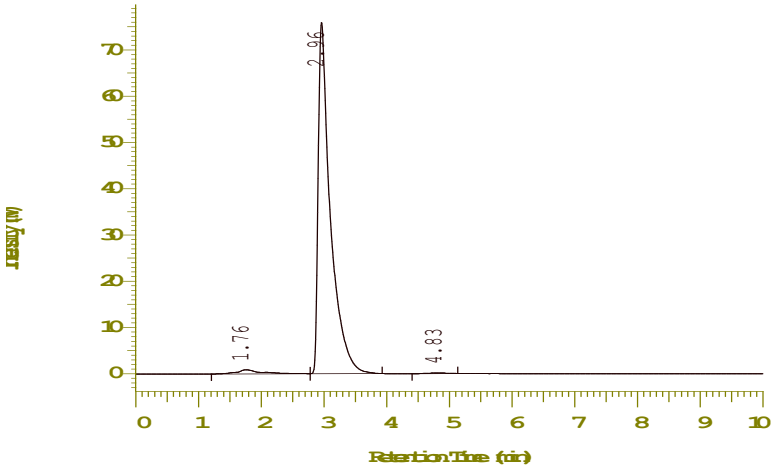


Figure 10: Assay of Ropinerole

Table 8: Result of assay of Ropinerole tablets

Brand name of tablets	Labeled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
ROPIN	150	149.34 (±0.06)	99.56 (±0.48)

STABILITY RELATED IMPURITY STUDIES:

Following protocol was strictly adhered to for forced degradation of Ropinerole Active Pharmaceutical Ingredient (API).

Table 9: Results of force degradation studies of Ropinerole api.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	43.75	54.61	98.36
Basic Hydrolysis (0.I M NaOH)	24Hrs.	43.32	55.02	98.32
Thermal Degradation (50 ^{0}C)	24Hrs.	97.39	----------	97.39
UV (254nm)	24Hrs.	95.19	04.34	99.53
3 % Hydrogen peroxide	24Hrs.	19.75	80.28	99.03

CONCLUSION

A simple, economic, accurate and precise RP-HPLC method was successfully developed. In this method, it was carried out by using symmetry C18, (250× 4.6mm) with 5µm particle size. Injection volume of 20µl is injected and eluted with the mobile phase A as buffer of KH_2PO_4, pH 3.0 adjusted with dilute ortho phosphoric acid and buffer and acetonitrile as mobile phase B over gradient program, which is pumped at a flow rate of 1.0 ml/min. Detection, was carried out at 211 nm. The two compounds are well resolved from each peak and there is no interference from blank. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of Selectivity, accuracy, linearity, precision, robustness, stability of solution and mobile phase stability.

For Selectivity, the chromatograms were recorded for standard and sample solutions of the above experiment was carried out at the wavelength of 211 nm, ambient temperature, Phosphate Buffer, Acetonitrile in 60:40 used as mobile phase, the experiment was carried out at the pH 2.9

The above conditions were preferred because the peak shape resolution & absorbance were good.

The Ropinerole was subjected to hydrolytic (acidic, alkaline and neutral) oxidation, photlytic and thermal stress conditions, all stability related impurity studies of Ropinerole were carried out according to ICH guidelines.

The RP-HPLC method described here had given all the validation parameters and stability studies of results.

The developed method was validated for parameters as per ICH viz, linearity, precision, accuracy specificity and robustness.

The %recovery was found to 98.9446,99.76,99.376 and %RSD was found to be 0.1733,0.0459,0.1066 respectively.

In this experiment the precision(%RSD) for repeatability was found to be 0.440784 and the results of Intra & Inter day assays ((10,30,100 µg/ml)) are found to be 0.86,0.36,0.13 and 0.87,0.32,0.11 respectively.

The calibration curve showed good linearity in the range of 0-50 µg/ml, for Ropinerole (API) with correlation coefficient (r^2) of 0.992 (Fig. 4). A typical calibration curve has the regression equation of y = 26474x + 40981 for Ropinerole.

For Robustness the %RSD with the change in parameters like flowrate 1.1 ml/min ,0.9 ml/min, the %RSD was found to be 0.06,0.04, with change in the temperature as 27^{0}C, 23^{0}C the %RSD was found to be 0.08, 011 respectively and at 213nm, 209nm it was found to be 0.03 and 0/02 respectively.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.03 & 0.09 µg/ml respectively. The % purity of ROPIN tablets containing ROPINEROLE was found to be 99.56%.

The results of the stress studies indicated the specificity of the method that has been developed. Ropinerole was degraded in acidic, basic & 3 % hydrogen peroxide & stable at thermal & light stress conditions.

Hence, the chromatographic method developed for Ropinerole are rapid, simple, sensitive, precise, and

accurate. Therefore, the proposed method can be successfully applied for the routine analysis of in API and Pharmaceutical dosage form.

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