A NEW VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF ROPINIROLE IN BULK AND PHARAMACEUTICAL DOSAGE FORM

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ABSTRACT : A simple, accurate, economic, rapid and precise RP-HPLC method has been developed and validated for determination of Clonazepam in bulk and pharmaceutical dosage form. The RP-HPLC separation was achieved on Symmetry ODS RP C18,5mm, 15mm x 4.6mm i.d. column using mobile phase 0 Methanol : Phosphate buffer (pH=2.80) in the ratio of 73:27v/v and pH-2.80 adjusted with orthophosphoric acid at flow rate of 1.0 ml/min at ambient temperature. The retention times were 3.545 min for Clonazepam. Calibration plot is linear over the concentration range 0-70 µg/ml for Clonazepam. Quantification was achieved with UV detection at 242nm over the Beer-Lambert's range. The proposed method has been validated statistically as per the ICH guidelines for linearity, accuracy, precision, specificity, LOD and LOQ. The above method developed and validated successfully for the quantitative routine analysis of Clonazepam in bulk and pharmaceutical dosage form. The proposed method was validated as per the ICH and USP guidelines.

Key Words: RP-HPLC, Method Development, Validation, Accuracy, Precision, ICH Guidelines.

I.INTRODUCTION

Ropinirole, sold under the brand name Requip among others, is a medication used to treat Parkinson's disease (PD) and restless legs syndrome (RLS). In PD the dose needs to be adjusted to the effect and treatment should not be suddenly stopped. It is taken by mouth. Ropinirole[1] is a selective dopamine receptor agonist used in the therapy of Parkinson disease. Ropinirole therapy is associated with low rate of transient serum enzyme elevations during treatment and has been implicated in rare cases of acute liver injury. Ropinirole, also known as ReQuip, is a non-ergoline dopamine agonist used in Parkinson's disease and restless legs syndrome. It is manufactured by GlaxoSmithKline Pharmaceuticals. Ropinirole was initially approved in 1997 by the FDA for the management of Parkinson's disease. In 2005, it was the first drug approved in the US for the management of primary moderate to severe restless legs syndrome. In 2008, the extended-release capsules of Ropinirole[2] were approved, allowing for less frequent dosing, therefore increased compliance, and offering a similar side effect profile and efficacy to previous formulations of Ropinirole. Ropinirole is a member of indolones and a tertiary amine. It has a role as a dopamine agonist, an antiparkinson drug, a central nervous system drug and an antidyskinesia agent. The IUPAC Name of Ropinirole is 4-[2-(dipropyl amino) ethyl]-1, 3-dihydroindol-2-one. The Chemical Structure of Ropinirole is

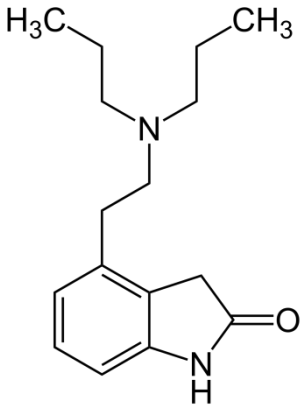

Fig.1. Chemical Structure of Ropinirole

The literature review[25-30] reveals that these different dosage forms of Ropinirole is analyzed by a different method including UV, IR, HPLC, LCMS, and such more technique are useful in different types of analysis in the different

dosage form. But now a day most important and better techniques in HPLC by using HPLC simple Reverse Phase Chromatographic method develop for the determination of active form of Ropinirole, while it is better to find a method for analysis.

II. MATERIALS AND METHODS

Table-1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	**HPLC** with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	**ELICO SL-159 UV – Vis spectrophotometer**
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	**Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm**
6.	P^H Analyzer **(ELICO)**
7.	Vacuum filtration kit **(BOROSIL)**

Table-2: List of Chemicals used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	Sd fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	Sd fine-Chem ltd; Mumbai
7.	Ethanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai
8.	Octanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai

Method Development:

HPLC Instrumentation & Conditions:

The HPLC system employed was waters 717, empower2 as Software with Isocratic with UV-Visible Detector (L-2400).

Standard & sample preparation of UV analysis:

Take 25 mg of Ropinirole API into 25 ml volumetric flask, shake appropriately to break up, at that point make-up the volume with diluent. Move 0.1 ml of the above arrangement into a 10ml volumetric flask blend well and make-up the volume which gives 10 ppm.

The standard and test stock arrangements were arranged independently by dissolving standard and test in a dissolvable in portable stage weakening with a similar dissolvable for UV examination.

To choose the wavelength for discovery and evaluation of Ropinirole, ELICO SL-159 make UV – Vis

spectrophotometer model UV-2450 was utilized, so a similar lambda max can be fixed in HPLC UV locator for assessing the Ropinirole The ideal recognition wavelength was controlled by running UV range of arrangement arranged for the dissolvability ponders with water and natural dissolvable organization in the middle of 200 and 330 nm. The most extreme absorbance was seen at around 280-300 nm.

Initialization of the Instrument

The HPLC instrument was switched on. First the column[3] was stabilized with mobile phase for 120 minutes. The mobile phase was run to find the peaks or identification of peaks. After 20 minutes the standard drug solution was prepared and injected in HPLC system.

Mobile Phase Preparation

This has been done by taking 800 ml of 0.025M KH2PO4 with pH of 3.5 with OPA and 200 of acetonitrile in a measuring cylinder. Than mixed with stirrer and sonicated to evaporate the dissolved gas, sonicated for 15 mins. Finally taken to reservoir for HPLC[4] run and required amount kept to use as diluent for sample preparation.

Standard Preparation:

To prepare the API solution for analysis, we took 10 mg of API into a 10ml volumetric flask, and made it 1000 ppm by adjusting the volume with diluent. For the final concn. We took 0.1 ml from stock to a 10 ml volumetric flask and made to 10 ppm by filling the volumetric flask with diluent to mark of volumetric flask[5].

Method Validation:

Specificity: Specificity of the method can be termed as the absence of any interference at a retention time of samples. Specificity[6] was performed by injecting blank and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of analytes.

Linearity and Range: A series of standard solutions 0-14µg/ml of Ropinirole were prepared. An aliquot of 10µl of each solution was injected 1 time for each standard solution, and peak area was observed. The plot of average peak area versus the concentration[7] is plotted, and from this, the correlation coefficient[8] and regression equation were generated. The calibration[9] data of Ropinirole is given in Table 4, while Fig. 4 represents overlain represent linearity[10] graph of drug respectively.

Limit of Detection: It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The limit of detection[11] (LOD) of the drug was derived by calculating the signal to noise ratio (S/N, i.e., 3.3. Limit of detection can be calculated using the following equation as per ICH guidelines. LOD = 3.3 x σ/S Where, σ = the standard deviation of response and S = Slope of calibration curve.

Limit of Quantification: It is the lowest concentration of an analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. The limit of quantification[12] (LOQ) of the drugs was derived by calculating the signal to noise ratio (S/N, i.e., 10 for LOQ) using the following equation as per International Conference on Harmonization (ICH) guidelines[13]. LOQ = 10 ×σ/S Where, σ = the standard deviation of response and S = Slope of the calibration curve[14-15].

Method Precision: The method was validated regarding intra-day[16] and inter-day precision[17]. The intra-day and inter-day study were performed by injecting 8, 10 and 12µg/ml of Ropinirole solutions three times for each aliquot. The % RSD[18] for the precision study was found less than 2% as shown in Table 5.

Accuracy (% Recovery): The recovery[19-20] of an analytical method is determined by applying the method to analyze samples to which known amounts of analyte have been added. The recovery is calculated from the test results as the percentage of analyte recovered by the assay[21]. The known amounts of standard solutions of Ropinirole (8, 10 and 12 µg/ml) were added to a pre quantified test solutions of Ropinirole. Each solution was injected in triplicate, and the recovery was calculated by measuring peak areas. Results are shown in Table 7.

Robustness: The robustness[22-23] of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was studied by changing flow rate (±0.1ml/min-1), mobile phase composition (±1ml/min-1) and the change in wavelength (±1ml/ min-1). After each changes sample solution was injected and system suitability parameters[24] were observed. The results were shown in Table 8.

System Suitability

System suitability[25] is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factor, resolution, precision, capacity factor time and theoretical plates. System suitability parameter Results were reported in Table-9.

Estimation of Ropinirole in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms were taken and the I.P. technique was taken after to decide the normal

weight. Above measured tablets were at long last powdered and triturated well. An amount of powder comparable to 25 mg of medications were exchanged to 25 ml volumetric jar, make and arrangement was sonicated for 15 minutes, there after volume was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with mobile phase. The arrangement was separated through a film channel (0.45 µm) and sonicated to degas[26]. The arrangement arranged was infused in five repeats into the HPLC framework and the perceptions were recorded.

A copy infusion of the standard arrangement was likewise infused into the HPLC framework and the pinnacle zones were recorded. The information is appeared in Table-10.

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of medication acquired with test readiness
AS = Peak Area of medication acquired with standard readiness
WS = Weight of working standard taken in mg
WT = Weight of test taken in mg
DS = Dilution of Standard arrangement
DT = Dilution of test arrangement
P = Percentage virtue of working standard

III.RESULTS AND DISCUSSION

UV Analysis:

The assimilation band 287 nm was chosen as the wavelength for this examination work. The UV range[27] of Ropinirole is appeared in figure 2.

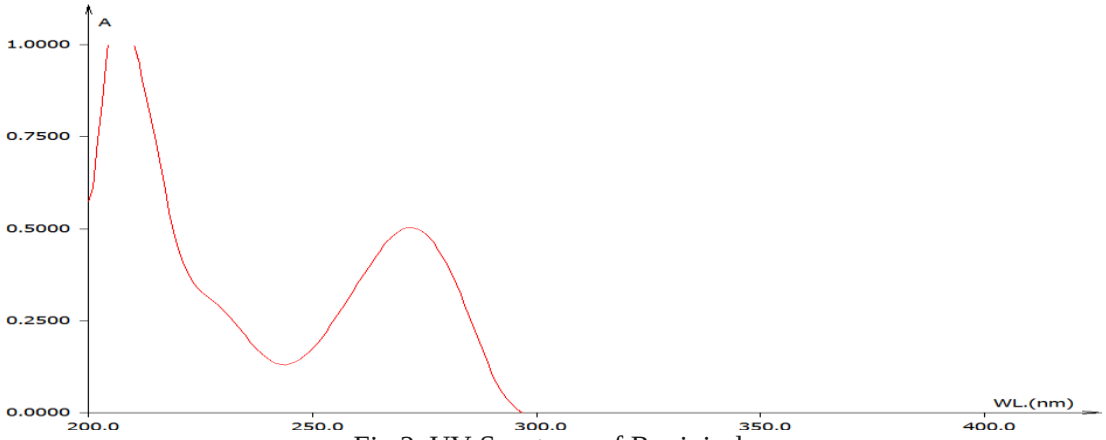

Fig.2. UV Spectrum of Ropinirole

Method Development:

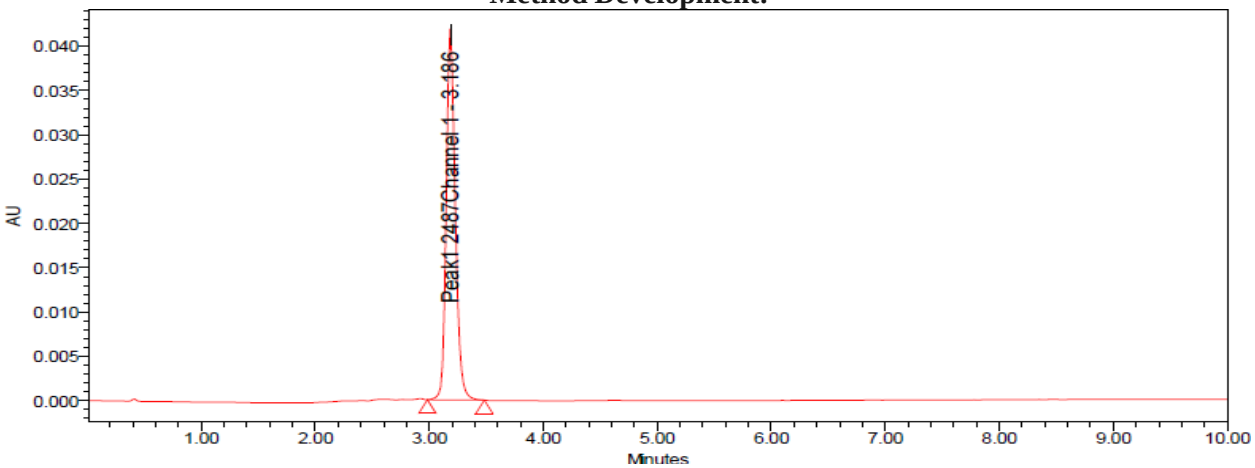

Fig.3. Optimized Chromatographic Condition of Ropinirole

Summary of final Chromatographic Conditions: The final conditions obtained from experiments have been summarized as below:

Table-3: Summary of final Chromatographic Conditions

Column	Waters ODS C18, 5µm, 25cmx4.6mm, i.d.
Mobile phase	Potassium Dihydrogen Phosphate buffer (0.025M, pH: 3.5) : Acetonitrile = 80:20
Wavelength	287 nm
Flow rate	1.0 ml/ min.
Sampling System	Automatic
Injection Volume	10µl
Run Time/ Stop Time	7minutes
Concentration of Sample	10ppm

Method Validation:

Linearity and Range

The standard solutions of Ropinirole the concentration range of 0µg/ml to 14µg/ml were obtained by taking (0.6ml, 0.8ml, 1.0ml, 1.2ml, 1.4 ml) of Ropinirole stock solution (1000ppm) to the series of 10 ml volumetric flask. The solutions were filtered[28] through a 0.45µm membrane filter and degassed by ultrasonication. The final resulted solutions were injected into HPLC the system. The run time/stop time maintained was 7 mins and the various types of peak areas were measured.

Table-4: Calibration Data for Ropinirole

S.No.	Conc. (µg/ml)	Mean Peak Area
1	0	0
2	6	194804
3	8	246214
4	10	327612
5	12	391983
6	14	451497

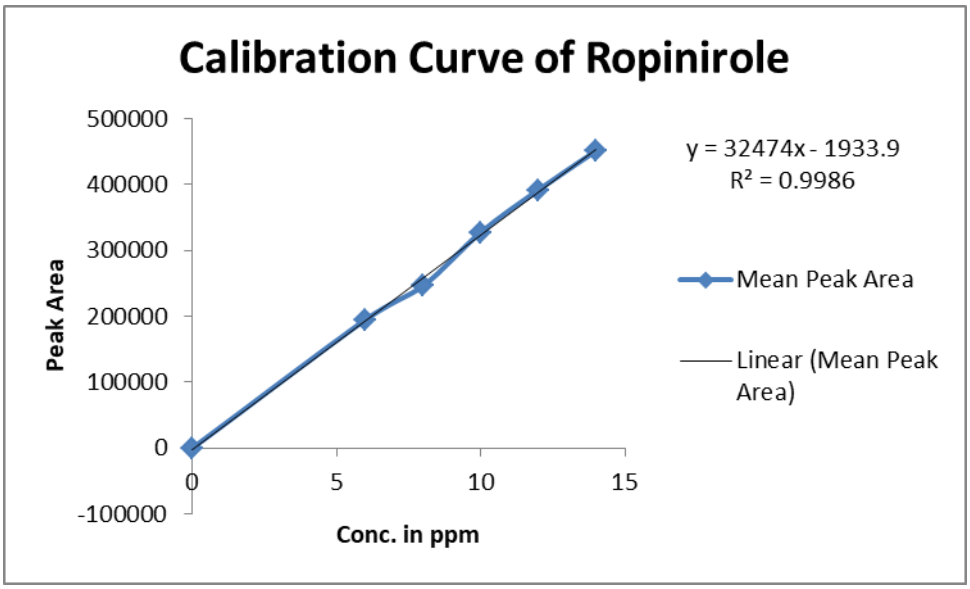

Fig.4. Calibration Curve of Ropinirole

Result & Discussion: The calibration curve showed good linearity in the range of 0-14µg/ml, for Ropinirole (API) with correlation coefficient (R2) of 0.998. A typical calibration curve has the regression equation of y = 32474x + 1933 Ropinirole.

Precision

Repeatability: The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug Ropinirole (API). The percent relative standard deviations were calculated for Ropinirole.

Table-5: Repeatability Results of Precision

HPLC Injection Replicates of Ropinirole	Retention Time	Peak Area
Replicate – 1	3.115	221617
Replicate – 2	3.113	220686
Replicate – 3	3.118	221456
Replicate – 4	3.108	225036
Replicate – 5	3.104	224821
Replicate – 6	3.097	225776
Average		223232
Standard Deviation		2213.404
% RSD		0.99152630

Result & Discussion: The repeatability study which was conducted on the solution having the concentration of about 10µg/ml for Ropinirole (n=6) showed a RSD of 0.991526304% for Ropinirole It was concluded that the analytical technique showed good repeatability.

Intermediate Precision:

Intra-assay & inter-assay:

This is done by taking three different concn. Of the API and injected at 3 different time in a day to obtain untraday results. For interday the same concen. Were injected in a particular time for 3 different days. The same replicated for 6 times to find %RSD and it should be less than2.

Table-6: Results of Intermediate Precision of Ropinirole

Conc. of Ropinirole (API) (µg/ml)	Observed Conc. of Ropinirole (µg/ml) by the Proposed Method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	8.06	1.08	7.86	1.05
10	10.26	0.97	10.13	0.94
12	12.51	0.92	11.09	0.96

Accuracy:

Recovery study: for the recovery studies 3 different level of API has been taken and 3 replicates of injections has been carried out. From areas obtained from the respective injections, the concentrations were calculated from the calibration curve equation obtained from linearity. % recovery should be in between 98-102 and %RSD should be less than 2 as per guidelines.

Table-7: Accuracy Readings

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	7.995217	261656	99.94021	Mean= 99.9339%
S_2 : 80 %	8	7.979635	263050	99.74544	S.D. = 0.18541
S_3 : 80 %	8	8.00929	263123	100.1161	R.S.D.= 0.1855326
S_4 : 100 %	10	9.8495	323761	98.495	Mean= 99.998%
S_5 : 100 %	10	10.06136	326752	100.6136	S.D. = 1.310544
S_6 : 100 %	10	10.08908	327652	100.8908	% R.S.D.= 1.310544
S_7 : 120 %	12	11.92316	387212	99.35966	Mean= 100.135%
S_8 : 120 %	12	12.04738	391246	100.3948	S.D. = 0.684346
S_9 : 120 %	12	12.07833	392251	100.6527	% R.S.D. = 0.6834206

Method Robustness:

It has been obtained by slight change in flow rate, Temperature, and lambda max in the optimized conditions. The results obtained are below:

Table-8: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.09
Flow (0.9 ml/min)	0.56
Temperature (27^0C)	0.12
Temperature (23^0C)	0.17
Wavelength of Detection (284 nm)	0.42
Wavelength of detection (280 nm)	0.46

LOD & LOQ:

The LOD and LOQ were calculated by the use of the equations

$$LOD = 3.3 \times \sigma / S$$

and

$$LOQ = 10 \times \sigma / S$$

Where,

σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

Results:

The Minimum conc. at which the analyte can be detected (LOD) & quantified (LOQ) were 0.001 & 0.003 µg/ml respectively.

SST Parameter

The SST parameters are the parameters based on the idea that the instrument, electronics, operations and the samples to be analyzed constitute as an integral system which can be examined. It is an integral part of analytical procedures. Finally SST parameters are established. The obtained data is shown in the following table-6.25.

Table-9: Results of System Suitability Parameter

S.No.	Parameter	Ropinirole
1	Retention time	3.085
2	Theoretical plates	4187
3	Tailing factor	1.16
4	Peak Area	2051315
5	Resolution	3.41

Estimation of Ropinirole in Pharmaceutical Dosage Form

Table-10: Recovery Data for estimation of REQUIP Tablets

Brand name of Linagliptin	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
REQUIP	4mg	3.90 (±0.06)	99.68 (±0.49)

The assay of REQUIP tablets containing Ropinirole was found to be 99.68 %.

IV.SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Ropinirole, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Ropinirole it is evident that most of the HPLC work can be accomplished in the wavelength range of 200-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay and stability related impurity studies which can help in the analysis of Ropinirole in different formulations. Finally a sensitive & selective RP-HPLC method has been developed & validated for the analysis of Ropinirole API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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