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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF ROPINIROLE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Ropinirole HCL is an orally administered, non-ergoline dopamine agonist indicated for the treatment of signs and symptoms of idiopathic Parkinson's disease. A simple, selective, precise, accurate and cost effective reverse phase HPLC method has been developed and validated for estimation of Ropinirole in extended release tablet dosage form. In the chromatographic conditions, Phenomenex Luna C8 (250 X 4.6 mm, 5 µm) stationary phase with mobile phase consisting of potassium phosphate buffer (pH 7.5 ± 0.05): Methanol (25: 75 v/v) was used at a flow rate of 1.0 mL/min. and column temperature was maintained at 35°C. Ropinirole was detected at 250 nm. The chromatographic procedure separated Ropinirole and potential interfering peaks in an analysis time of 20 min. with Ropinirole eluting at about 15 min. The assay method was found linear in the concentration range of 50% to 150% of assay working concentration (0.016 mg/mL) with a correlation coefficient of 0.9999. The percentage recovery of assay was found between 100.3 and 100.5. The developed method was validated with respect to specificity, linearity, accuracy, precision, sensitivity, robustness and solution stability as per ICH guidelines. The proposed method can be used for routine analysis of Ropinirole formulations in quality control laboratories.

KEY WORDS

Ropinirole, HPLC, Validation, Dissolution, Extended Release

INTRODUCTION:

Ropinirole acts as a D2, D3, and D4 dopamine receptor agonist with highest affinity for D2. It is weakly active at the 5-HT2, and α2 receptors and is said to have virtually no affinity for the 5-HT1, GABA, mAChRs, α1, and β-adrenoreceptors.[2,7] Ropinirole is metabolized primarily by cytochrome P450 CYP1A2 to form two metabolites; SK&F-104557 and SK&F-89124, both of which are renally excreted,[5] and at doses higher than clinical, is also metabolized by CYP3A4. At doses greater than 24 mg, CYP2D6 may be inhibited, although this has only been tested in vitro.[1]

Reverse Phase HPLC:

In this chromatographic technique, the stationary phase is non-polar, and the mobile phase is polar, non-polar compounds are retained for longer periods as they have more affinity towards the stationary phase. Hence, polar compounds travel faster and are eluted first.[3]

Steps involved in development of RP-HPLC method:

Selection of chromatographic method:

The proper selection of methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and stability. The drug selected is polar and ionic hence, reverse phase chromatography was used because of its simplicity and suitability.[4]

Selection of stationary phase:

Matching the polarity of sample and stationary phase and using a mobile phase of different polarity will achieve a successful separation.[5]

Selection of mobile phase:

Reverse phase bonded packing, when used in conjunction with highly polar solvents; approach is ideal and is a universal system for liquid chromatography. Mobile phase may be either single liquid or combination of liquids, which are compatible with sample, column and instrument.[6]

Selection of suitable detector:

Detector is the eye of HPLC system that measures the compounds after their separation on the column. There are basically two types of detectors- the bulk property detectors and solute property detectors. Detectors, in order of their popularity are UV, fluorescent, conductivity, polarimeter and refractive index detectors. UV detector is the first choice because of its convenience and applicability in case of most of the samples. The latest versions of equipment's are available with photo diode- array detectors (PAD or DAD).

Method optimization:

During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and shape, plate count, asymmetry, capacity, elution time, detection limits, limit of quantitation, and overall ability to quantify the specific analyte of interest. Literature survey reveals that, analytical methods were reported for the determination of Ropinirole alone or in combination with other drugs in active pharmaceutical ingredients, oral and parenteral formulations. These methods used different detection techniques like spectrophotometry[4-8], HPTLC[9-10] and HPLC[11-13]. In the current work we have made an attempt to develop simple, robust, cost effective and high throughput analytical method for the determination of Ropinirole in tablet dosage form. The method uses UV detection with a run time of 20 min. The method has several advantages like simple mobile phase, low injection volume, less run time over the reported methods. The developed method was validated as per international conference on harmonization (ICH) Q2 (R2) guidelines.[9]

MATERIALS AND METHODS

Drug profile of Ropinirole

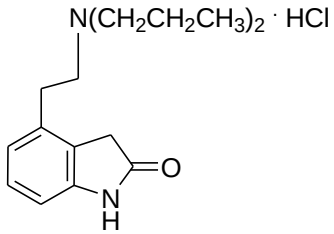

Fig.1.Structure of Ropinirole HCl

IUPAC Name:4-[2-(dipropylamino) ethyl]-1,3-dihydro-2H-indol18 2-one

Chemical formula: $C_{16}H_{24}N_2O$•HCl

Molecular weight: 296.84 (260.38 as the free base)

Description: White to off-white powder.

Solubility: Freely soluble in water, soluble in methanol, slightly soluble in ethanol

Category: Non-ergoline dopamine agonist parkinson's disease

λ_{max}: 250 nm

Drugs Used:

Table 1: List of Drugs Used

S. No.	Drugs	Manufacturer
1.	Ropinirole HCL	Glenmark generics Ltd
2.	Ropark 2 mg Commercial Tablets	Sun pharma

Reagents Used:

Table 2: List of Reagents Used

S. No.	Chemicals	Manufacturer Name	Grade
1	Water	Merck	HPLC
2	Methanol	Merck	HPLC
3	Acetonitrile	Merck	HPLC
4	Potassium phosphate	Merck	G.R
5	Trimethylamine	Merck	G.R

Equipment and Apparatus Used:

Table 3: Equipment and Apparatus Used

S. No.	Instrument Name	Model Number	Software	Manufactures Name
1	HPLC	Alliance UV-Visible detector-2487	Empower	Waters
2	U.V Double beam spectrophotometer	SL 210	-	ELICO
3	Digital weighing balance (Sensitivity 5 mg)	BL-200H	-	SHIMADZU
4	PH-meter	LI-120	-	ELICO
5	Sonicator	3305013	-	SISCO

Preparation of mobile phase:

A combination of mobile phase consisting of potassium phosphate buffer (pH 7.5 ± 0.05): methanol (25: 75 v/v) was mixed and degassed in ultrasonic water bath for 5 minutes and finally filtered through 0.45µm membrane filter. This prepared solution was used as mobile phase.

Diluent:

HPLC grade water and acetonitrile in the ratio of 50:50 (v/v) was used as diluent.

Preparation of standard solution: (16µg/ml)

Accurately weighed working standard equivalent to 20 mg of Ropinirole into 100 mL volumetric flask, add 70 mL of diluent and dissolve, further make up the volume with diluent. Further dilute 2 mL to 25 mL with diluent.

Preparation of sample solution: (16 µg/ml)

Crushed to powder (20 tablets), weighed and transferred the tablet powder equivalent to 20 mg of Ropinirole into 100 ml volumetric flask, added 70 ml of diluent and sonicated for 15 min. and finally made the volume with diluent. Further filtered the solution through nylon 66-membrane filter. 2ml of the above solution was additionally diluted to 25 ml with diluent.

Wavelength selection:

About 0.25 mg/mL of Ropinirole solution was accurately prepared by dissolving the standard in water. The Ropinirole solution was scanned in the 200-400 nm UV region. The wavelength maximum (λ_{max}) was observed at 250 nm and this wavelength was adopted for absorbance measurement.

Optimized chromatographic conditions:

Column:

Phenomenex Luna C8 (250 X 4.6 mm, 5 µm

Column temperature: Ambient

Wave length: 250 nm

Mobile phase ratio:

Potassium phosphate Buffer: Methanol (25: 75 v/v)

Flow rate: 1.0 min/ml

Injection volume: 20 µl

Run time: 20 minutes

Validation of developed RP-HPLC method:

As per the International conference on harmonization (ICH) guidelines the method validation parameters such as linearity, precision, accuracy, system suitability, limit of detection and limit of quantitation were optimized.

Assay

Sample and standard solutions were injected into the chromatographic system and measured the area for Ropinirole and calculated the % assay by using below formula

Calculation:

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where:

Avg. wt. = average weight of tablets

P = percentage purity of working standard

LC = label claim of Ropinirole mg

RESULTS AND DISCUSSION:

Optimized method:

It was performed on Phenomenex Luna C8 (250 X 4.6 mm, 5 µ) with a mobile phase composition of potassium phosphate buffer: methanol (25: 75 v/v) at a flow rate of 1.0 min/ml. 20 µl of sample was injected and the run time was 20 minutes.

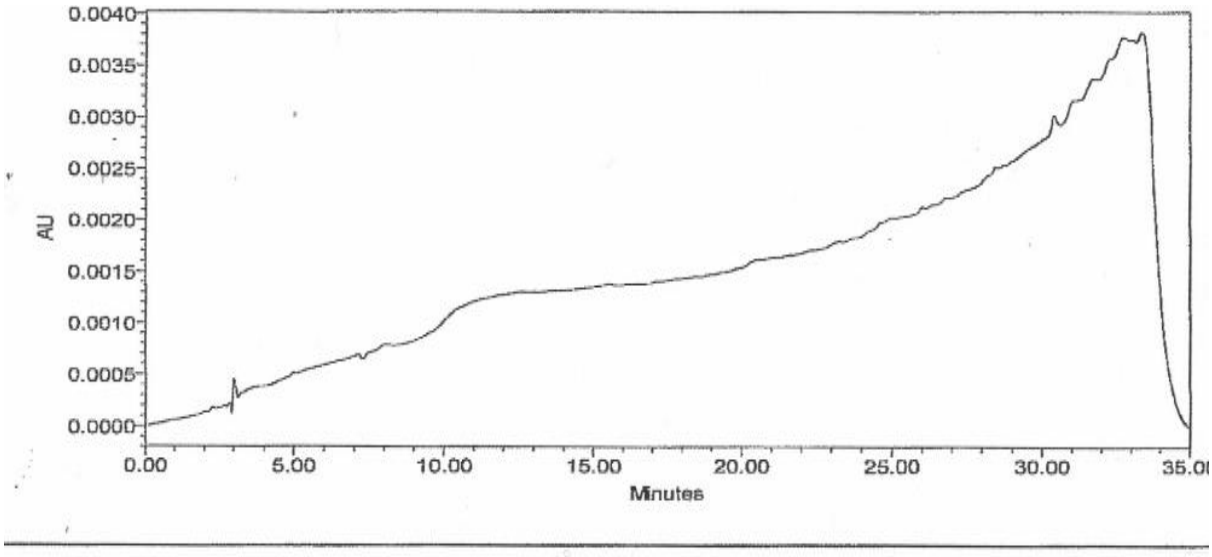

Fig.2: Chromatogram showing blank preparation (mobile phase)

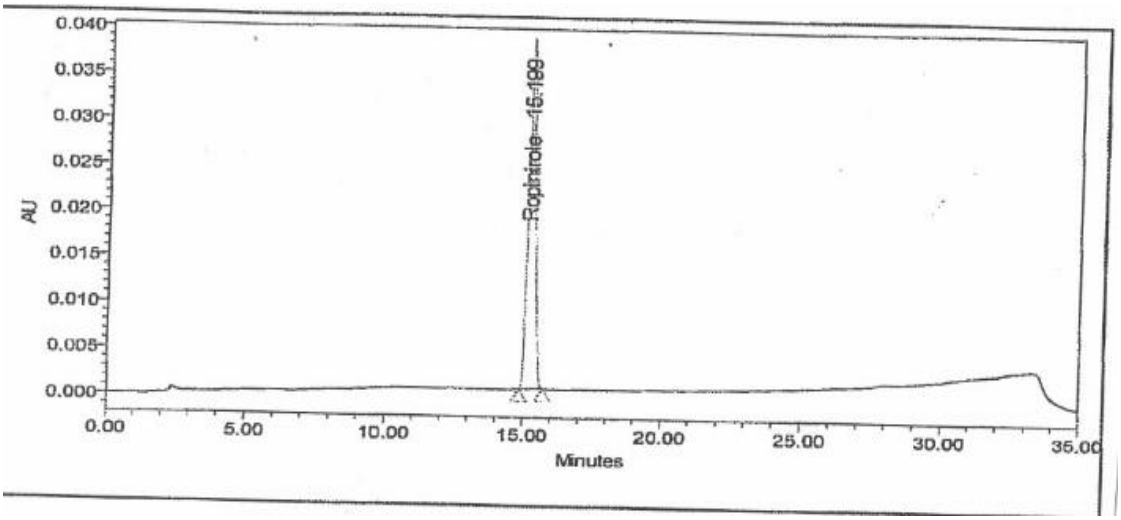

Fig.3: Chromatogram of Ropinirole HCl standard peak

Linearity:

8.0, 13.0, 16.0, 19.0, 24.0 µg/ml was injected into the chromatographic system and peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and Y-axis peak area) and the correlation coefficient was calculated.

Acceptance criteria:

Correlation coefficient should be not less than 0.999.

Table 4: Showing the results for the Linearity

Conc.(µg/ml)	RT	Area
8.132	15.122	333782
13.02	15.124	538825
16.26	15.131	666206
19.56	15.124	803332
24.43	15.132	1001123
Co efficient of correlation(R^2)		0.999

Precision:

The standard solution (16 µg/ml) was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria:

The %RSD for the area of five standard injections results should not be more than 2.

Table 5: Overlain peak results of method Precision

Injections	Conc.(µg/ml)	RT	Area
1	16	15.122	667859
2	16	15.122	667478
3	16	15.126	668287
4	16	15.122	667102
5	16	15.123	666752
Mean			667680
SD			586.80
% RSD			0.1

Accuracy:

The standard solution of concentration 50,100 and 150 µg/ml were injected into chromatographic system. Calculated the individual % recovery and mean % recovery of Ropinirole.

Acceptance criteria:

The % recovery for each level should be between 98.0 to 102.0%.

Table 6: Showing accuracy results for Ropinirole HCl

S. No	Conc (µg/ml)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean% recovery
1	8.132	1103356	8	8.02	100.3%	
2	16.26	2276356	16	16.08	100.5%	100.5%
3	24.43	3302058	24	24.06	100.3%	

System suitability:

The standard I solution was injected one time and standard II solution was injected 5 times.

Table 7: Showing system suitability results for Ropinirole HCl

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	9656	1.1
2	1.0	9822	1.2
3	1.2	9866	1.2

Limit of detection (LOD)

From the above preparation 1ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluents.

Table 8: Showing results for Limit of Detection

Drug Name	y-Intercept	Slope(s)	LOD(µg/ml)
Ropinirole	1963	42966	0.909

Limit of quantification (LOQ)

From the above preparation 0.5ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluent.

Table 9: Showing results for Limit of Quantitation

Drug name	y-Intercept	Slope(s)	LOQ(µg/ml)
Ropinirole	1963	42966	2.711

Assay:

The developed and validated method was applied to the determination of Ropinirole HCl in marketed tablets containing 2 mg of drug per tablet. Three injections of sample were injected into chromatographic system. Assay % was calculated by using the formula mentioned above and it was found to be 99.6%.

Table 10: showing the results of assay

S. No	Name	RT	Area
1	Ropinirole HCl	15.126	668152
2	Ropinirole HCl	15.123	669156
3	Ropinirole HCl	15.128	665665

CONCLUSION:

A simple, rapid, accurate and precise RP-HPLC method was developed for the determination of Ropinirole HCl in pure form and in tablets. The analytical conditions and solvent system developed provided a good separation for Ropinirole HCl within a short analysis time. The method was validated and demonstrated a wide linear dynamic range, a good precision and accuracy. Thus, the method can be proposed for routine analysis laboratories and for quality control.

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