Chapter VII

Ropinirole Hydrochloride

7.1. LITERATURE REVIEW OF ROPINIROLE HYDROCHLORIDE

1. Armagan Onal et al reported a Simple and reproducible spectrophotometric methods have been developed for determination of cabergoline (CAB) and Ropinirole hydrochloride (ROP), in pharmaceutical preparations and these were based on the reactions between the drug substances and ion pair agents such as methyl orange (MO), bromocresol green (BCG) and bromophenol blue (BPB) producing yellow colored ion pair complexes in acidic buffers, after extracting in dichloromethane, which are Spectrophotometrically determined in the Beer's law range of 1.0 to 35 µg/mL.

2. Azeem et al reported an isocratic RP-HPLC stability indicating assay for estimation of Ropinirole in the bulk and pharmaceutical preparations on a C_{18} column (250 mm × 4.6 mm i.d, 5-µm) with methanol and 0.05 M ammonium acetate buffer (pH 7) in the ratio of 80:20 (v/v) as mobile phase, pumped in to column at a flow rate of 1 mL/min and detected at wave length of 250 nm. The method was linear over the concentration range 0.2–100 µg/ mL (r = 0.9998), with limits of detection and quantitation of 0.061 and 0.184 µg/mL, respectively.
3. Coufal P et al reported an isocratic Capillary liquid chromatography (CLC) method used for the quantification of Ropinirole and its five related impurities. The separation of the six substances was achieved on a C_{18} column with a mobile phase of acetonitrile and 2-(N-morpholino) ethane sulfonic acid adjusted to pH 6.0 in the ratio of 55:45 v/v.

4. Jignesh Bhatt et al reported a rapid LC–MS/MS method for the determination of Ropinirole in human plasma using Es-citalopram oxalate as an internal standard by solid phase extraction. The proposed method was validated with linear range of 20–1200 pg/mL and the extraction recoveries for Ropinirole and internal standard were 90.45 and 65.42%, respectively. The % R.S.D of intra-day and inter-day assay was lower than 15%.

5. Onal reported a RP-HPLC/UV method for the determination of Ropinirole in tablets. Separation achieved on a Luna CN column (250 × 4.6 mm I.D, 5 µm) with mobile phase of acetonitrile and 10 mM nitric acid (pH 3.0) in the ratio of (75:25,*v/v*) and the UV detection at 250 nm. The method was linear over the concentration range of 0.5–10.0 µg/mL with good recoveries 99.75–100.20% and the relative standard deviations of intra and inter-day assays were 0.38–1.69 and 0.45–1.95%, respectively.
6. Sahasrabuddheya B et al reported detection of three impurities in Ropinirole hydrochloride at levels 0.06–0.15% by RP-HPLC. Based on the IR, NMR and MS spectral data, the structures of these impurities were characterized as 4-[2-(propylamino) ethyl]-1,3-dihydro-2H-indol-2-one hydrochloride (impurity-A), 5-[2-(diropylamino)ethyl]-1,4-dihydro-3H-benzoxazin-3-onehydrochloride (impurity-B) and 4-[2-(diropylamino) ethyl]-1H-indol-2,3-dione hydrochloride (impurity-C).
7. Susheel JV et al reported UV and HPTLC method for the determination of Ropinirole in tablet dosage form with detection wave lengths 250 nm and 254 nm, respectively. The linearity range both UV and HPTLC methods were found be 5-30 mg/mL and 40 and 120 mg/mL, respectively.

8. William S. Edgemond et al reported an isocratic chromatography method of Ropinirole, eluted in a run time of 3 minutes or less. The method was found to be more specific and the recovery of Ropinirole and ropinirole-D3 was 90% and 94% respectively. The precision and accuracy at the LLOQ were within 9.0%. Stability of Ropinirole in plasma was established for 24 hours at room temperature (BTS), 5 cycles of freezing and thawing (FTS), 80 days of storage at -20 °C (LTS).

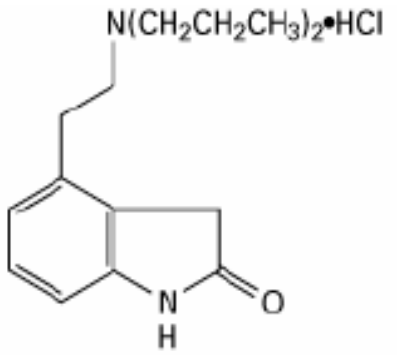
9. Zeynep Aydogmus et al reported three sensitive spectrophotometric and Spectrofluorimetric methods for the determination of Ropinirole hydrochloride in tablets. UV method was developed for the detection of Ropinirole at 250 nm in the concentration range 2.5–24 µg/mL and spectrofluorimetric method was developed to detect Ropinirole at 842 nm in the concentration range 0.6–8 µg/mL and another fluorimetric method developed for the detection of Ropinirole at 525 nm with excitation at 464 nm in chloroform in concentration range of 0.01–1.3 µg/mL.

7.2. DRUG PROFILE OF ROPINIROLE HYDROCHLORIDE

7.2.1. Introduction

Ropinirole hydrochloride is an orally administered Non ergot-derivative dopamine receptor agonist[1-9]. It is used for the treatment of the signs and symptoms of idiopathic parkinsonian syndrome, Used as an adjunct to levodopa for the symptomatic management of parkinsonian syndrome in patients with advanced disease[10, 11] and also used for the Symptomatic management of moderate-to-severe primary restless legs syndrome[12-18] (Ekbom syndrome).

7.2.2. Chemistry

Chemical name	Hydrochloride salt of 4-[2-(dipropylamino) ethyl]-1, 3-dihydro-2H-indol-2-one monohydrochloride
Empirical formula	$C_{16}H_{24}N_2O.HCl$.
Molecular weight	296.84 (260.38 as the free base).
Chemical structure	 **Fig 7.2.1: Chemical structure of RPR**

7.2.3. Properties

Ropinirole hydrochloride is a white to pale greenish-yellow powder with a melting range of 243° to 250°C.

7.2.4. Solubility: 133 mg/mL in water.

7.2.5. Category: Dopamine Agonist, Anti parkinson Agent, Anti dyskinetic, Central Nervous System Agents

***7.2.6. Dose*:** 0.25 mg 3 times daily

7.2.7. Mechanism of Action: Ropinirole binds the dopamine receptors D_3 and D_2. Although the precise mechanism of action of Ropinirole as a treatment for Parkinson's disease is unknown, it is believed to be related to its ability to stimulate these receptors in the striatum. This conclusion is supported by electrophysiologic studies in animals that have demonstrated that Ropinirole influences striatal neuronal firing rates via activation of dopamine receptors in the striatum and the substantia nigra, the site of neurons that send projections to the striatum.

7.2.8. Adverse effects

Adverse events commonly reported during dopaminergic therapy (nausea, dizziness), as well as visual hallucinations, hyperhidrosis, claustrophobia, chorea, palpitations, asthenia, and nightmares. Additional symptoms reported for doses of 24 mg or less or for overdoses of unknown amount included vomiting, increased coughing, fatigue, syncope, vasovagal syncope, dyskinesia, agitation, chest pain, orthostatic hypotension, somnolence, and confusional state.

7.2.9. Interactions

Some of these drug interactions are

- Ciprofloxacin
- Estrogens
- Dopamine antagonists, including neuroleptics (phenothiazines, butyrophenones, thioxanthenes) and metoclopramide.

7.2.10. Contraindications

Ropinirole (HCl) is contraindicated in conditions like Hypersensitivity, Pregnancy, and lactation

7.2.11. Precautions

Ropinirole may cause fainting, known as syncope. These episodes occurred in people with Parkinson's disease and in people with restless legs syndrome (RLS). Make sure you discuss this with your healthcare provider before starting Ropinirole. This is especially true for people with blood vessel or heart disease (cardiovascular disease).

7.2.12. Available marked formulations

Brand Name	Available Dosage Form	Available Strengths	Dosage Frequency
Parkirop (Cadila)	Tablet	0.5mg, 1mg, 2mg	Usual dose ranges from 3-9 mg daily, maximum dose24mg/day
Ropitar (torrent)	Tablet	0.25mg, 0.5mg, 1mg, 2mg	

7.3. AUTHENTICATION OF ROPINIROLE HYDROCHLORIDE

The obtained sample was authenticated by recording the following

- Infra red spectrum
- Thermo gram
- UV Spectrum

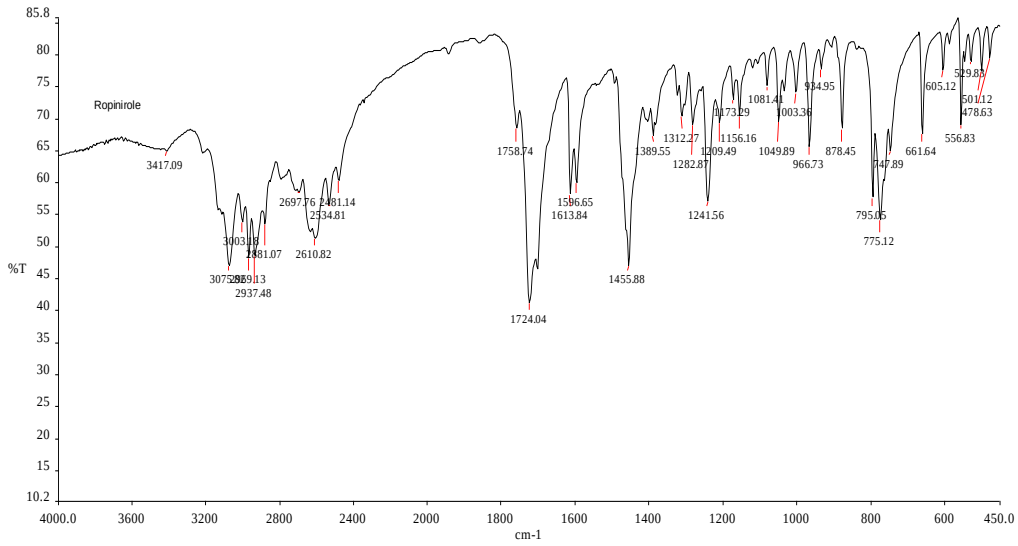


Fig 7.3.1: Infra red spectrum of RPR

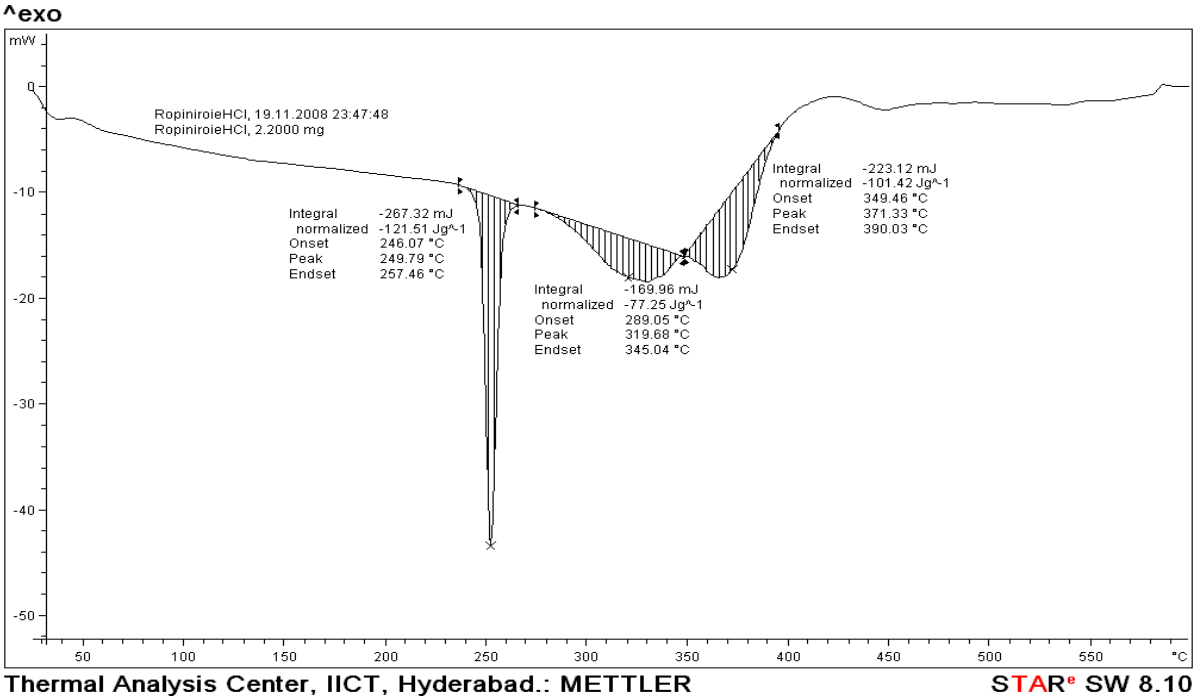


Fig 7.3.2: DSC Spectrum of RPR

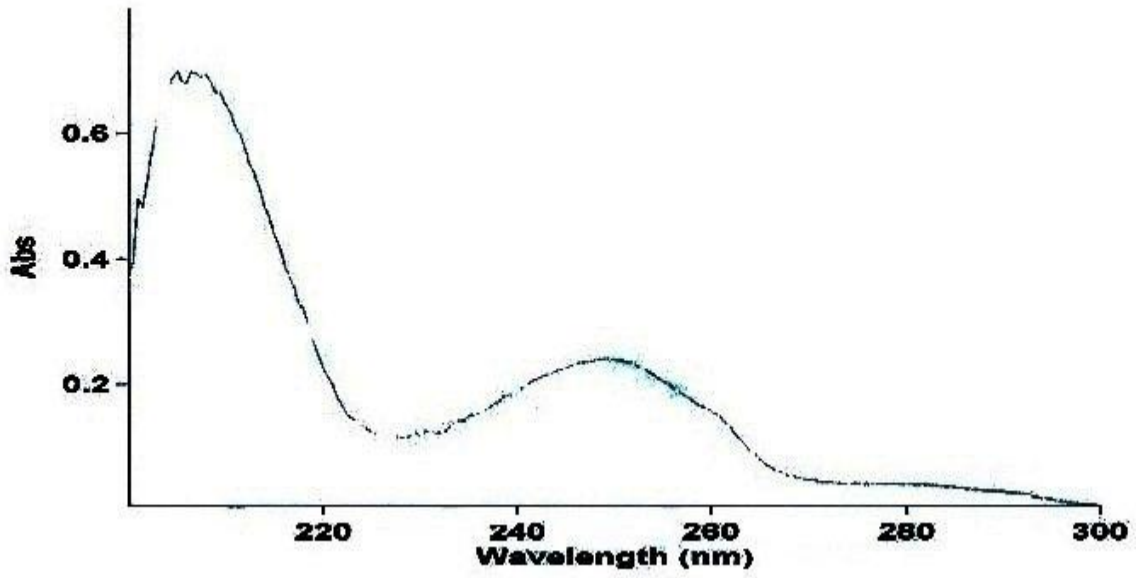


Fig 7.3.3: UV spectrum of RPR

7.4. RP-HPLC Method Development and Validation of Ropinirole Hydrochloride in Bulk and Pharmaceutical Dosage Forms

7.4.1. Introduction

Ropinirole hydrochloride (RPR) is an orally administered non ergoline dopamine agonist and majorly used in the treatment of Parkinson's disease. 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. Chemically it is hydrochloride salt of 4-[2-(dipropyl amino) ethyl]-1, 3-dihydro-2H-indol-2-one, with empirical formula of $C_{16}H_{24}N_2O.HCl$ 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 D_3 than to D_2 or D_4 receptor subtypes. The mechanism of RPR induced postural hypotension is presumed to be due to a D_2 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 RPR in human plasma by LC/MS/MS[19], Spectrophotometric method[20], estimation of drug content in bulk and pharmaceutical dosage forms by HPLC[21,22], stability indicating assays[23]and establishment of impurity profile HPLC[24] were reported. We here in report a simple and reliable RP-HPLC for the estimation of RPR in bulk and pharmaceutical dosage forms.

7.4.2. Experimental

7.4.2.1. Materials & Supplies

Pure standard of RPR (99.65%) was obtained as gift sample from Inventis drug delivery systems Pvt. ltd, Hyderabad along with certificate of analysis

(COA). Acetonitrile and water (HPLC grade, Qualigens), Potassium dihydrogen phosphate (S.D. Fine Chemicals), Phosphoric acid (Qualigens), Ropitar tablets (Torrent Pharmaceuticals) and Parkirop tablets (Cadila Pharmaceuticals Ltd.), Electronic analytical balance (DHONA), Micro pipette (In labs, 10- 100µL) were employed in the study. All the glassware employed in the work cleaned with hot water followed acetic anhydride then acetone and dried when ever required.

7.4.2.2. HPLC apparatus and chromatographic conditions

A Shimadzu model LC-10 ATVp HPLC system (Shimadzu co, Tokyo, Japan) consisted of SPD-6AV variable wavelength detector (Possessing deuterium lamp with a sensitivity of 0.005 AUFs and adjusted to an absorbency of 245nm), C-R5A chromatograph integrator module (Chart speed at 10mm/min and attenuation 0), SIL- 6A auto injector and SCL-6A system controller. The separation was achieved in isocratic elution mode using a mobile phase composed of 50:50 v/v of buffer pH 6.0 and Acetonitrile, pumped with a flow rate of 0.5 mL/min into C_{18} ODS analytical column (Thermo hypresil, 250x4.6mm i.d, 5µm) with C_{18} insert (100 A^0, waters limited) as pre column to protect the analytical column. Integration of the detector output was performed using the Shimadzu class Vp soft ware to determine chromatographic parameters. The contents of the mobile phase filtered through 0.45 µm 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 as ambient. The volume of injection was 20 µL, prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The eluent was detected at 245 nm.

7.4.3. Method development

7.4.3.1. Optimization of chromatographic conditions

Initial stages of method development the chromatographic conditions were optimized by performing different trails and the details of chromatographic conditions were shown in table 7.4.1.

7.4.1. Optimized chromatographic conditions

Drug	RPR
Column	Column 250 x 4.6 mm i.d, 5µm particle size
Flow rate	0.5 mL/min
UV detection wave length	245 nm
Mobile phase	Buffer and Acetonitrile in the ratio of 50:50 (v/v) with p^H 6.0, adjusted with phosphoric acid
Column temperature	Ambient
Volume of injection	20 µL
Mode of operation	Isocratic

7.4.3.2. Preparation of mobile phase

Buffer and Acetonitrile (pH adjusted to 6.0 with phosphoric acid) in the ratio of 50:50 v/v were employed as a mobile phase.

7.4.3.3. Preparation of stock solution

A stock solution was prepared by dissolving 50.0 mg of RPR in a 100 mL volumetric flask containing 70.0 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 RPR were prepared by suitable dilution of the stock solution with the mobile phase. Ten sets of solutions were prepared in

the mobile phase containing RPR at a concentration of 5-50 µg/mL. Each of these dilutions (20µL) was injected six times in to the column, peak area and retention times were recorded.

7.4.3.4. Construction of linearity

The concentrations for linearity 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 in the linearity range of 5-50 µg/mL. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilutions was injected six times into the column. The calibration curve for RPR 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.

7.4.3.5. Estimation of RPR 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) 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 in to the column to obtain chromatogram. The typical chromatograms of RPR was shown in fig 7.4.1 and fig 7.4.2. From that peak area, the drug content in the tablets was quantified.

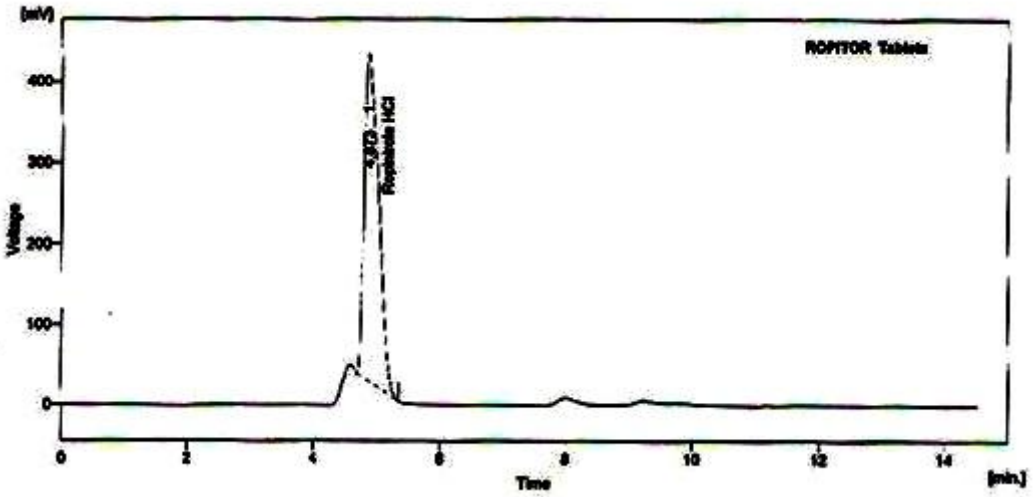


Fig 7.4.1: A typical chromatogram of RPR tablets

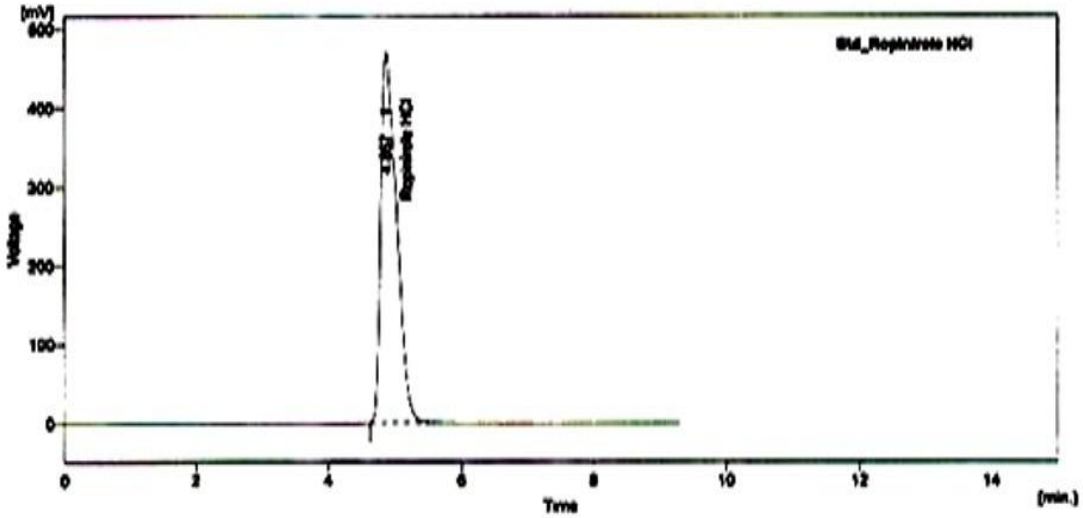


Fig 7.4.2: A typical chromatogram of RPR

7.4.4. Method validation

7.4.4.1. Linearity

The linearity for the detection of RPR was 5-50µg/mL with (R^2=0.997; y = 156.3x + 70.59) the 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 table 7.4.2 and statistical data of calibration curves were shown in table 7.4.3. The linearity curve was shown in fig 7.4.3.

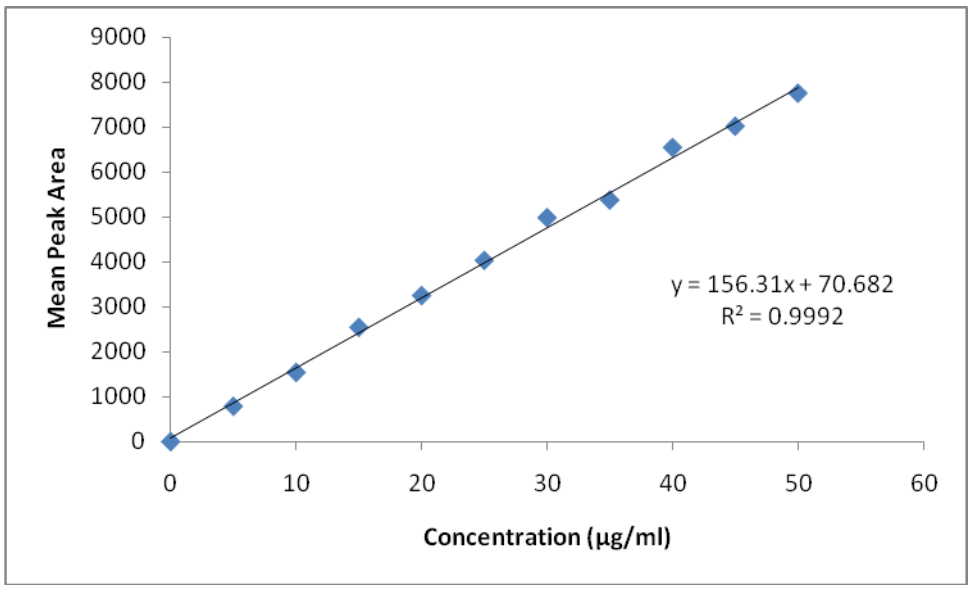


Fig 7.4.3: Calibration curve of RPR

Table 7.4.2: Concentration Vs Mean Peak area of RPR

Concentration (µg / 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 + 70.682, R² = 0.9992

Acceptance Criteria: Correlation Coefficient should not be less than 0.9990.

Table 7.4.3: Statistical Data of Calibration Curves of RPR

Parameters	Value
Linearity	5—50µg/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

7.4.4.2. 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 intraday and inter day. Results were shown in table 7.4.4.

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Table 7.4.4: Precision of RPR

Concentration of RPR (µg/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

Acceptance Criteria: RSD should not be more than 1.0%

7.4.4.3. 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 RPR about five times, at three different concentrations equivalent to 80, 100, and 120% of the active ingredient, by adding a known amount of RPR standard to a sample of known concentration and calculating the recovery of RPR with RSD (%), and % recovery for each concentration. The mean % recoveries were in between 99.3-100.4% and were given in table 7.4.5.

Table 7.4.5: Recovery Studies of RPR

Drug Labeled claim (2mg)	Amount Added (mg)	Amount Present (mg)	Mean amount found(n=5)*	Mean % recovery
RPR	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

Acceptance Criteria: Recovery should be within 98.0% to 102.0%

7.4.4.4. Assay of RPL

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 99.50 and 100.50% 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 7.4.6.

Table 7.4.6: Assay of RPR

Brand name of the tablet	Labeled claim(mg)	Amount estimated*(mg)	Mean ±S.d	%Purity ±S.d
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

7.4.4.5. 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 7.4.7.

Table 7.4.7: System Suitability Parameters

Parameter	RPR
Retention time (min)	4.867
Theoretical Plates	4803
Tailing factor	0.90
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

Acceptance Criteria:

1. RSD should not be more than 2.0% for five replicate injections of standard.
2. Tailing for RPR peak is not more than 2.0

3. The column efficiency as determined for RPR peak, Plate Count should be not less than 2000.

7.4.4.6. Ruggedness

Ruggedness of the method (intermediate precision) was estimated by preparing six dilutions of the RPR 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 7.4.8.

Table.7.4.8: Ruggedness of RPR

S.NO	Labeled claim(mg)	Amount estimated*(mg)	Mean ±S.d	%RSD
Set-1	2	2.03	2.03±0.03	1.50
Set-2	2	1.97	1.97±0.02	1.02

Mean of six values

Acceptance Criteria: Over all RSD should not be more than 2.0%

7.4.4.7. Robustness

Robustness of the proposed method was evaluated by changing mobile phase composition from buffer: acetonitrile 50:50 v/v to buffer: acetonitrile 55:45 v/v, changing the flow rate from 0.5 mL to 0.7mL/min, changing the pH (±0.2), changing the temperature (±5^{0}c) and changing the wave length (±5.0 nm) and System suitability parameters were found to be within acceptable limits. Results were shown in table 7.4.9 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 7.4.9: Robustness of RPR

Parameter	Variation	System suitability		
		Theoretical plates	**Tailing factor**	**%RSD**
Standard	-	4803	0.90	0.30
Flow	0.5 to 0.7	3959	0.85	0.10
Wave length	-5.0 nm	5965	0.73	0.25
	+5.0 nm	6239	0.72	0.32
Mobile Phase	50: 50 to 55: 45	3583	0.99	0.21
Temperature	-5^{0}C	3446	0.90	0.13
	+5^{0}C	3739	1.10	0.15
pH	-0.2	5823	0.70	0.48
	+0.2	6232	0.50	0.16

Acceptance Criteria:

1. RSD should not be more than 2.0% for five replicate injections of standard.
2. Tailing for RPR peak is not more than 2.0

3. The column efficiency as determined for RPR peak, Plate Count should be not less than 2000.

7.4.4.8. 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 = $10\sigma/S$.

7.4.4.9. 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.

7.4.5. Results and discussion

7.4.5.1. Method development

The development of HPLC methods for the determination of drugs has received greater attention because of their importance in the quality control. The main objective of method development was to determine the drug content present in the formulation and its % purity. The goal of this study was to develop and validate a RP-HPLC method for the estimation of RPR in bulk and pharmaceutical commercial preparations. Initial stages of method development we optimized various chromatographic conditions such as solvent, column, mobile phase composition, flow rate and detection wavelength was optimized and the method was developed, validated success fully. Our experiments and data reported in the literature showed that both the methanol and acetonitrile could be used an organic modifier in the mobile phase. The use of acetonitrile as a mobile phase organic modifier resulted in better sensitivity compared to methanol. During method development tests involving the use of mixtures of acetonitrile and different buffer solutions (e.g., Potassium dihydrogen

phosphate, potassium phosphate, ammonium acetate) were made to optimize the mobile phase with different pH values, finally Potassium dihydrogen phosphate buffer and acetonitrile in the ratio of 50:50 v/v, pH was adjusted to 6.0 with phosphoric acid has been selected whose mobile phase combination given good peak symmetry, sensitivity, and shorter retention time without interfering peaks. RPR was eluted successfully in the given mobile phase within significant shorter retention time of 4.867 min and gave good single sharp symmetrical peak (tailing factor < 2) without interfering peaks. The typical chromatograms of RPR were shown fig 7.4.1 and fig 7.4.2. The use of hydrophobic stationary phases usually provides adequate retention of organic non polar molecules.

The chromatographic separation was achieved using an RP C_{18} column for RPR with symmetrical peak shape. The absorption spectrum of RPR was recorded in methanol and determined detection wavelength as 245 nm. Different flow rates were tested and finally the flow rate of 0.5 mL/min was optimized. The mode of elution also optimized and isocratic mode was selected than gradient where the elution was achieved using complicated mobile phases. The developed method more advantageous with respect to simplicity, isocratic conditions, shorter run time, low injection volume, less flow rate and inexpensive mobile phases. For the quantification the linearity range was optimized by preparing the solution of RPL in different concentration ranges and finally the range 5-50µg/mL was optimized which follows the beers law. The optimized chromatographic conditions were shown in the table 7.4.1. The proposed method was found to be simple, rapid, economic and accurate and the method was applicable to routine laboratory analysis. The developed was successfully validated as per ICH guidelines.

4.4.4.2. Method validation

The method was validated for various parameters as per ICH guidelines. The linearity of the method was evaluated by plotting calibration curve using least squares linear regression analysis of concentration and mean peak area. The method was showing good linearity in the concentration range of 5-50 µg/mL With (R^2 = 0.9972, y = 156.31x + 70.591) the 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 table 7.4.2 and statistical data of calibration curves were shown in table 7.4.3. The calibration curve of RPR was shown in fig 7.4.3. The intraday and inter day precision studies were performed result revealed the precision with %RSD (0.27% and 0.26%) respectively for intraday and inter day. Results were shown in table 7.4.4. Accuracy studies were performed to know the reliability of the method and the mean % recoveries were in between 99.3-100.4% and were given in table 7.4.5. The method was applied for the estimation of RPR in two different commercial tablet dosage form having label claim of 2mg and 1mg. The average drug content was found to be 99.5% and 100.5% of the labelled claim of two different marketed tablet formulations namely PARKIROP and ROPITOR having labelled claim of 2mg and 1mg, respectively. The results of assay were shown in table 7.4.6. 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 7.4.7. The method was found be specific, rugged and robust and suitable for routine laboratory analysis.

7.4.5. Summary

A simple and accurate RP-HPLC method has been developed for the estimation of RPL in bulk and pharmaceutical dosage forms using C_{18} 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 RPR was found to be 4.867 min. The proposed method has permitted the quantification of RPR over linearity in the range of 5-50µg/mL and its percentage recovery was found to be 99.3-100.4%. The intraday and inter day precision were found 0.27% and 0.26% respectively.

7.6. Conclusion

The results of the study reveal that the proposed RP-HPLC method for the estimation of RPR is simple, rapid and accurate in bulk and pharmaceutical dosage forms and suitable for routine laboratory analysis.

7.7. REFERENCES

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