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Der Pharma Chemica, 2011, 3 (6):1-9
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Validated RP- HPLC and specrophotometric determination of Ropinirole hydrochloride in bulk and in pharmaceutical dosage form

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ABSTRACT

Ropinirole hydrochloride is a nonergot dopamine agonist, indicated for the treatment of the signs and symptoms of Parkinson's disease. The objective of this study is to develop validated HPLC (Method-I) and first order derivative spectrophotometric method (Method -II) for the estimation of ropinirole hydrochloride. A sharp peak of the drug was obtained at 2.66 min, on a Thermo C 18 column (4.6 mm i.d × 250 mm) utilizing Acetonitrile: 0.02 M KH_2PO_4 solution in the ratio 72:28 v/v as mobile phase. The flow rate was 1.1 mL/ min with UV detection at 250.0 nm. The methods are validated for specificity, precision, accuracy and robustness. Method –II is a simple, precise and economical first order derivative method for the estimation of ropinirole hydrochloride from its formulation. Ropinirole hydrochloride shows absorption maxima at 249.0 nm and in the first order derivative spectra, zero crossing at 249.0 nm was obtained with a sharp peak at 238.0 nm when n=1. Results of the analysis were validated statistically and were found to be satisfactory.

Keywords: Ropinirole hydrochloride; First order derivative spectrophotometric method; RP-HPLC; Validation.

INTRODUCTION

Ropinirole hydrochloride (RPN) is a novel orally administered non-ergot dopamine agonist. Chemically it is hydrochloride salt of 4-[2-(dipropyl amino) ethyl]-1, 3-dihydro-2H-indol-2-one. It is used in the treatment of early and advanced Parkinsons disease. RPN is listed in Merck Index[16], Martindale, The complete drug reference[17].

Literature survey reveals that very few analytical methods were developed for the RPN. RP-HPLC method for RPN[1], UV-spectroscopy and HPTLC method[2], Stability-Indicating HPLC Method[3], spectrophotometric and spectrofluorimetric methods[4], Chemometrical evaluation of ropinirole and its impurity's chromatographic behavior[5], effect of madopar on the pharmacokinetics of Ropinirole[6], LC-MS for determination of RPN in human plasma[7], Separation of ropinirole impurities using capillary liquid chromatography[8], Isolation and

characterization of impurities[9], Determination of impurities using capillary zone electrophoresis[10].

An attempt has been made to develop simple, rapid and accurate HPLC (Method-I) and first order derivative spectrophotometric method (Method -II) for the estimation of ropinirole hydrochloride.

MATERIALS AND METHODS

Reagents and chemicals

The gift sample of RPN was obtained from USV Ltd. (Mumbai, India). All chemicals used throughout this work were of analytical grade and the solvents were of HPLC grade, purchased from Hexon Laboratories, Pune. Borosilicate glassware used throughout analysis.

Instrument

Method -I

The system (Merck Hitachi) consisting of quaternary gradient pump, and UV detector (L-7400) was employed for analysis. Chromatographic data was acquired using Winchrom software. Thermo BDS-Hypersil C18 column (4.6 mm i.d × 250 mm) was used as stationary phase. RPN was eluted with a flow rate 1.1 mL/min using a mobile phase consisting of acetonitrile: 0.02M potassium dihydrogen ortho phosphate solution (KH_2PO_4) (72:28 v/v). The wavelength of UV detector was set to 250.0 nm. The mobile phase was freshly prepared, filtered through 0.45 µm membrane filter (Millipore) and sonicated before use. The HPLC system was operated under ambient conditions.

Method –II

Shimadzu UV/Visible spectrophotometer, model 1700 (Japan) with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction employing a pair of quartz cells was used for analysis. Shimadzu electronic analytical balance (AX-200) was used for weighing the samples.

Preparation of standard stock solution

Method-I

About 10 mg of RPN was weighed and transferred to 100 mL volumetric flask. It was dissolved in mobile phase and the solution was made up to volume with mobile phase to obtain 100µg/mL of RPN. Working solutions were prepared by dilution of aliquots of the stock solution with the mobile phase.

Method-II

Accurately about 10 mg of the RPN was weighed and dissolved in 25 mL distilled water and volume was made up to 100 mL with distilled water to give standard stock solution of concentration 100 µg/mL. Aliquots of standard stock solution were pipetted out and suitably diluted with distilled water to get the final concentration of standard solutions.

Analysis of bulk substance

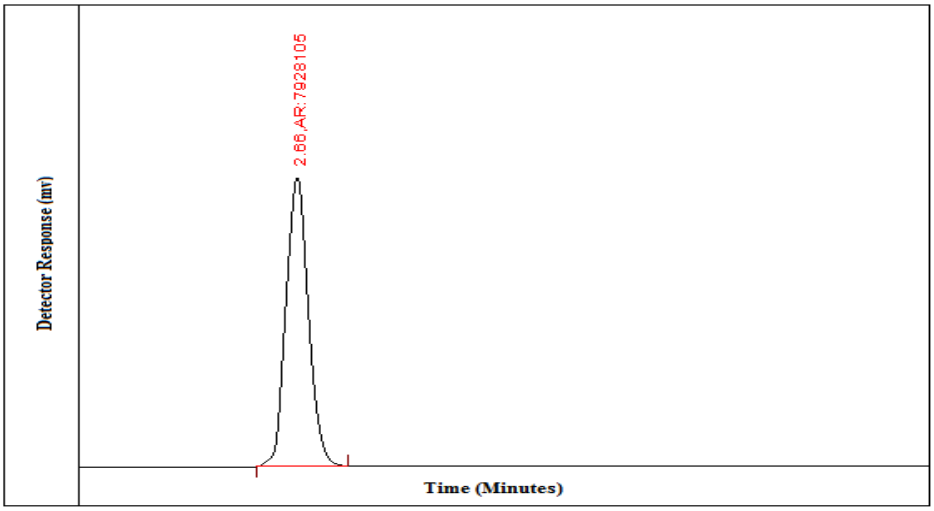
Method-I

Preparation of the calibration graph

working solutions containing 0.5–50 µg/mL of RPN were prepared by dilution of aliquots of the stock solution and injections were made of each solution and the peak areas of RPN were plotted against the corresponding concentration in µg/mL to obtain the calibration graph and the

corresponding regression equation was derived. Typical chromatogram of standard RPN is shown in Fig. No. 1.

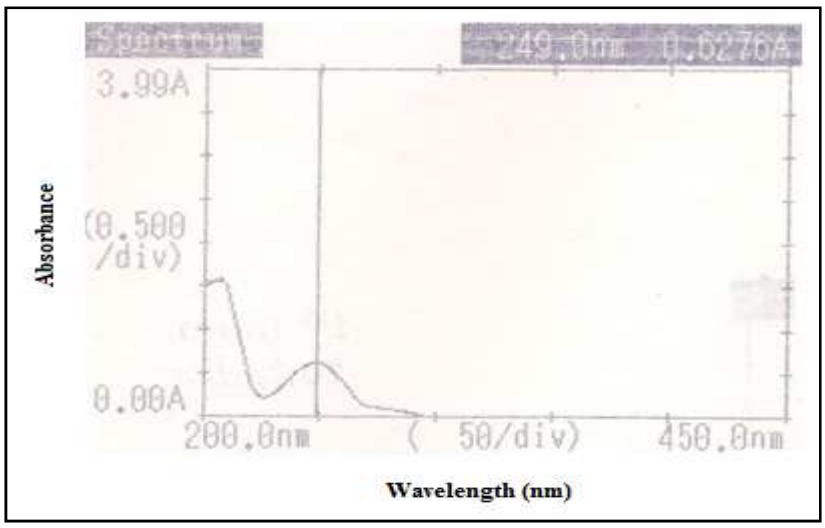
Fig. No. 1: Typical chromatogram of standard RPN



Method-II

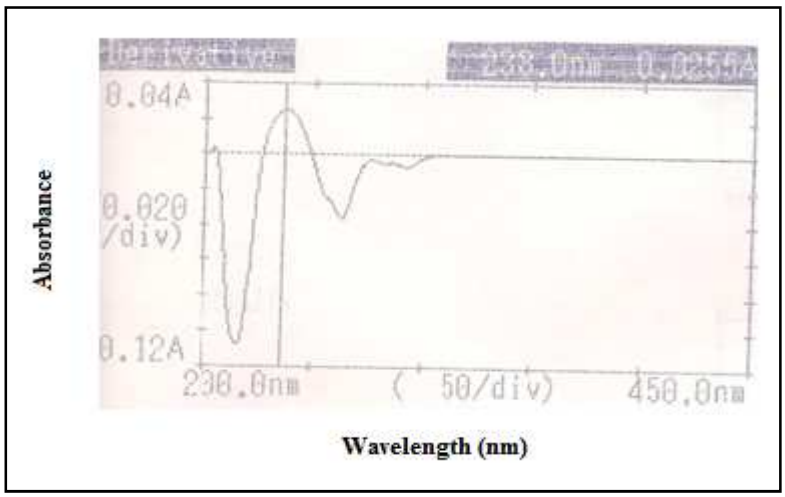
The solutions were scanned in the range of 400 nm to 200 nm and the absorption maxima was observed at 249.0 nm (fig. No. 2). The concentration range selected was 5-30 µg/mL for the calibration curve.

Fig. No. 2: UV Spectrum Of RPN



The first order derivative spectra at n=1, showed a sharp peak at 238.0 nm (Fig. No. 2). The absorption difference at n=1 (dA/dλ) is calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solution. The standard drug solution was diluted so as to get the final concentration in the range if 5-30 µg/mL and scanned in the first order derivative spectra. The calibration curve of dA/dλ against concentration of the drug showed linearity.

Fig. No. 3: First order derivative spectum of RPN



Analysis of dosage forms

Method-I

The marketed formulation (ROPARK mfg by Sun Pharma, Sikkim) containing RPN is available. Twenty tablets were accurately weighed. Average weight of one tablet was calculated. Tablets were finely triturated and thoroughly mixed. Accurately weighed quantity of tablet powder equivalent to about 5 mg RPN was transferred into a 50.0 mL volumetric flask; 25 mL of mobile phase was added and the contents of flask were sonicated for 30 min. The volume was then made up to the mark with mobile phase and solution was filtered through Whatman filter paper No. 41. From the filtrate, 0.2 mL portion was diluted to 10.0 mL with mobile phase to get final concentration of 20 µg/mL of RPN. The solution was mixed and filtered through 0.2 µ membrane filter and 20 µL was injected and analyzed for six times. The amount of drug present in the sample was determined by comparing mean peak areas with that of standard. (Table No.1)

Table No. 1: **Analysis of marketed formulation**

Drugs	Mean content (%)*	S.D.*	% R.S.D.*	S.E.*
RPN	99.26	0.3086	0.3109	0.1260

**mean of three determinations*

Method-II

The marketed formulation (ROPARK mfg by Sun Pharma, Sikkim) containing RPN is available. Powder equivalent to 5 mg RPN was weighed and dissolved in quantity sufficient with distilled water to get stock solution of concentration of 100 µg/mL. The solution was kept for sonication for about 30 min. then filtered through Whatman filter paper No.41. 20 µg/mL of the solution was prepared and analyzed for six times and concentration was confirmed by using the results of standard drugs by three methods. (Table No. 2)

Table No. 2: **Analysis of marketed formulation**

Component	*Mean	*S.D.	*%R.S.D.	*S.E.
RPN	99.55	1.5100	1.5168	0.6168

** mean of six determinations*

Optimization of chromatographic conditions

Of several solvents and solvent mixtures investigated, the mobile phase consisting of Acetonitrile: 0.02 M KH_2PO_4 solution in the ratio 72:28 v/v was found to provide better peak shape with less tailing and reasonable time of 2.66 min A HPLC Quaternary gradient system (Lachrom HPLC) consisting of L-7100 Merck Hitachi Pump, UV visible detector (L-7400), Rhenodyne injection syringe with 20µL was used for analysis. The optimum flow rate was found to be 1.1 mL/min. The performance of any chromatographic system may continuously change during their regular use, which can affect the reliability of the analytical results. System suitability tests were performed to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. Study of system suitability revealed following: tailing factor was found to be 1.33; Number of theoretical plates was found to be 2750. Retention time is 2.66 (fig.1).

Content uniformity testing

Content uniformity testing was carried out as per USP 2007 guidelines. Ten tablets were taken for analysis. Each tablet was weighed crushed and taken into a 10 mL volumetric flask. 5 mL of mobile phase was added and the contents of flask were sonicated for 30 minutes. The volume was then made up to the mark with mobile phase and solution was filtered through Whatmann filter paper No. 41. From the filtrate, 8 mL portion was diluted in to 10.0 mL with mobile phase to get final concentration of 20 µg/mL of RPN. The solutions were analysed as per the assay procedure mentioned earlier. The results are shown in Table No. 3.

Table No. 3: Results of content uniformity testing of RPN tablets

Parameters	Label claim(%) (µg/mL)	Total amount recovered (µg/mL)	Label claim (%) Pramipex tablets
	0.25	0.2690	107.60
	0.25	0.2558	102.32
	0.25	0.2636	105.45
	0.25	0.2354	94.14
	0.25	0.2476	99.04
	0.25	0.2565	102.61
	0.25	0.2214	88.57
	0.25	0.2586	103.44
	0.25	0.2298	91.91
	0.25	0.2367	94.66
Acceptance Value(AV)[b]			15.20
Max allowed Acceptance Value(LI)[b]			15
Mean			98.97
%RSD			6.40

a Each result is the average of six separate determinations
b USP (2007)

Method validation

Linearity

Method-I

The linearity test solutions for RPN were prepared in the concentration range of 5-50 µg/mL. All linearity test solutions, soon after preparation, were injected in the HPLC instrument. Each concentration level was analyzed in triplicate. Averaged peak-area values were directly correlated to the corresponding substance concentration. The peak area versus concentration data was performed by least-squares linear regression analysis. The correlation coefficients obtained were 0.9997 calibration curves. Results of regression analysis (Validation and System Suitability

Studies) are shown in Table No. 4. The results demonstrate that an excellent correlation existed between the peak area and concentration of RPN.

Table No. 4: Validation and System Suitability Studies

Parameter	Result
Linearity range (µg/mL.)	0.5-50
Detection Wavelength	250 nm
Retention time (min)	2.66
LOD (µg/mL)*	0.0011
LOQ (µg/mL)*	0.0036
Slope ± S.D*	396121.5±3164.3
Correlation coefficient ± S.D*	0.9998±0.0007
Theoretical plates	2750

**Mean of three determinations*

For method-II

Aliquots of standard stock solution of RPN were taken in 10 mL volumetric flasks and diluted up to the mark with Water to get final concentration of RPN in the range 5-30 µg/mL.

and scanned in the first order derivative spectra. The calibration curve of dA/dλ against concentration of the drug showed linearity. The correlation coefficient (r^2) value was found to be 0.999. Optical parameters for the calibration curve are shown in Table No. 5

Table No. 5: Optical parameters for the calibration curve

Parameters	RPN
Linearity range (µg/mL)	5-30
***Slope ± S.D.**	0.031±0.004
***Intercept ± S.D.**	0.0043± 0.00153
***Regression coefficient (r^2) ± S.D**	0.999±0.001

** mean of three determinations*

Limit of Detection (LOD) and limit of Quantitation (LOQ)

For method-I

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ.

LOD = 3.3 * standard deviation / slope LOQ = 10 * standard deviation / slope

The value of LOD was and the value of LOQ was 0.0011 and 0.0036 respectively.

For Method-II

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ.

LOD = 3.3 * standard deviation / slope LOQ = 10 * standard deviation / slope

The value of LOD was and the value of LOQ was 1.65 and 5.00 respectively.

Precision

For method-I

Precision of the method was determined with the pure drug samples. Suitable aliquots of the standard stock solutions (100µg/mL) of RPN were pipette out and transferred to 10 mL volumetric flasks. The volume was made up to the mark. Triplicate dilutions of each concentration of standard were prepared separately and analyzed in duplicate by the proposed methods to access the repeatability of sample application and measurement of sample concentrations. Intraday and interday precision was determined by repeating the assay three times on the same day for intraday precision and on different days for inter day precision studies (Table No. 6)

Table No. 6: Statistical validation data of precision

Precision parameter	% Mean*	S.D.*	% R.S.D*	S.E.*
Repeatability	99.48	0.0666	0.0669	0.0272
Intra-day	99.44	0.0942	0.0947	0.0384
Inter-day	99.50	0.0589	0.0598	0.0241

**mean of three determinations*

For method-II

Precision of the method was done by intraday and interlay study. Suitable aliquots of the tablets solutions (100µg/mL) of RPN were pipette out and transferred to 10 mL volumetric flasks. Intraday and interday precision was determined by repeating the assay three times on the same day for intraday precision and on different days for inter day precision studies (Table No. 7)

Table No. 7: Statistical validation data of precision

Precision Parameter	% Mean*	*S.D.	*%R.S.D.	*S.E.
Intra-day	98.50	1.738	1.7563	0.7095
Inter-day	99.11	0.6719	0.6779	0.2743

** mean of six determinations*

Accuracy (recovery studies)

Method-I

The accuracy of the assay method, performed using standard addition method, was evaluated in triplicate at three concentration levels, i.e. 80 %, 100 % and 120 % of final assay concentration. The percentage recoveries and respective standard deviations were calculated.

The percentage recovery of RPN Tablet powder ranged from 99.39% to 100%. (Table No. 8). The recovery values meet the acceptance criteria of 100 ±2% indicating the accuracy of proposed method. In addition, these results provide the working range for the method.

Table No. 8: Statistical validation data of recovery studies

Level of % Recovery	% *Mean Recovery	*S.D.	*% R.S.D.	*S.E.
80	99.31	0.6481	0.6526	0.3742
100	99.07	0.9673	0.9763	0.5585
120	99.35	0.9662	0.9725	0.5579

**mean of three determinations*

Robustness

Method-I

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered. The effect on retention time and peak asymmetry was evaluated. To study the effect of change in flow rate, the flow rate was altered by 0.1 units, i.e. 1.1 and 1.3 mL/min from the actual flow 1.2 mL/min. The effect of change in mobile phase ratio was studied by changing the acetonitrile: Potassium dihydrogen phosphate solution ratio by ± 1 mL. The effect of change in pH was studied by changing the pH of KH_2PO_4 solution by ± 0.1 mL. (Table No. 9)

Table No. 9: Results of robustness studies

Parameters	Retention time	Tailing factor	Peak areas*
Flow rate (mL/min)			
1.0	2.69	1.29	7919917
1.1	2.66	1.33	7959092
1.2	2.59	1.35	8037805
*Mean ± S.D	2.65±0.51	1.32±0.03	7972271.33±60039
% of ACN in the mobile phase (v/v)			
71	2.60	1.36	7897935
72	2.66	1.33	7959092
73	2.70	1.30	7900012
*Mean ± S.D	2.65± 0.50	1.33±0.03	7919013±34725
pH of KH_2PO_4 solution			
5.9	2.71	1.34	7924738
6	2.66	1.33	7959092
6.1	2.62	1.31	8096754
*Mean ± S.D	2.66 ±0.045	1.32±0.0153	7993528±91032

**mean of three determinations*

Method-II

To evaluate the robustness of the developed method, the conditions were deliberately altered. The change in results were studied by using different model of UV Spectrophotometers, by changing the operators.(Table No. 10)

Table No. 10: Results of robustness studies

Parameter	Abs	Derivative
UV-1	0.627	0.254
UV-2	0.633	0.259
UV-3	0.599	0.248
Operator-1	0.615	0.260
Operator-2	0.629	0.264

RESULTS AND DISCUSSION

Ropinirole hydrochloride (RPN), is a nonergot dopamine agonist, selective for dopamine D2 and D3 receptors, indicated for the treatment of the signs and symptoms of Parkinson's disease. Here two simple HPLC and UV methods are reported. Methods can be applied for the analysis of formulation. The methods were validated as per ICH guidelines. Standared deviation, % relative standerd deviation , standered error calculated for both the methods are within the limits indicating high degree of precision and accuracy of the methods. The correlation coefficient (r^2) values for RPN were 0.9998 and 0.999 for method-I and method-I respectvely. The value of

LOD and LOQ were 0.0011 and 0.0036 respectively for method-I. The value of LOD and LOQ were 1.65 and 5.00 respectively for method-II.

CONCLUSION

RPN is available dosage form for the treatment of Parkinson's disease. In the present work, a RP-HPLC and specrophotometric for the determination of RPN was developed and validated, in accordance with the ICH parameters (linearity, accuracy, precision, and limit of detection, limit of quantification, robustness and system suitability). Both the Methods are suitable for routine analysis and quality control of pharmaceutical preparations containing RPN including content uniformity testing of tablets by method-I. Results of the analysis were validated statistically and were found to be satisfactory.

Acknowledgement
We are highly thankful to our Director and Principal, Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune for providing excellent research facilities and to USV Ltd., Mumbai, for providing us the gift sample of the pure drug.

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