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Rp-Hplc Method for the Determination of Pramipexole Dihydrochloride in Tablet Dosage Form

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I. Introduction

Pramipexole dihydrochloride (PPD) [1-6], a nonergot dopamine agonist approved in the US (1997), is used as an antidyskinetic for treatment of Parkinson's disease. Its chemical name is (S)-N^6-propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine dihydrochloride (Fig. 1). The ability of PPD to alleviate the signs and symptoms of Parkinson's disease is supposed to be linked to its ability to stimulate dopamine receptors in the striatum.

Various analytical methods have been reported in the literature for the assay of PPD in pure and in its pharmaceuticals preparations. Procedures using UV-spectrophotometry [7], visible spectrophotometry [8,9], HPTLC [10] have been reported by several workers. High-performance liquid chromatography with mass spectrometer (HPLC-MS) [11-13], capillary electrophoresis with laser-induced fluorescence detection [14], gas chromatography with mass spectrometer (GC-MS) [15] and Ultra-performance liquid chromatography with mass spectrometer (UPLC-MS) [16] have been used for the analysis of PPD in biological samples.

Only few HPLC methods with UV detection have been described in the literature for determination of PPD. Pathare et al [17] developed a chiral liquid chromatographic method for the enantiomeric resolution of Pramipexole dihydrochloride monohydrate on a Chiralpak AD (250 mm × 4.6 mm, 10 µm) column using a mobile phase system containing n-hexane:ethanol: diethylamine (70:30:0.1 *v/v/v*). A method developed for determination of PPD and its impurities by Jačić et al [18] was carried out using a C18 column with mobile phases containing different ratios of acetonitrile and water phase (aqueous triethylamine/orthophosporic acid). Yau et al [19] reported a HPLC method for the determination of pramipexole in human plasma and urine. Separation is achieved by ion-pair chromatography on a Zorbax Rx C8 column (250 mm × 4.6 mm, 5 µm) and a Brown lee RP-8 pre-column (15 mm x 3.2 mm, 7 µm) with electrochemical detection at 0.6 V for plasma and ultraviolet detection at 286 nm for urine. A RP-HPLC [20] method for PPD in pure and in its pharmaceutical dosage forms has been reported by RAO et al and was carried out on an hypersil ODS-C18 (250 mm × 4.6 mm, 5µm) column with acetonitrile and acetate buffer (90:10 *v/v*) as the mobile phase and a detection wavelength of 260 nm. Srinubabu et al [21] have reported an RP-HPLC method for the assay of PPD in tablet formulations on an ODS-C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase of acetonitrile and phosphate buffer (60:40 *v/v*) and detection at 260 nm. The reported HPLC methods for the determination of PPD in pharmaceutical dosage forms suffer from one or more disadvantages like preparation of buffer, rigid pH control, narrow linear concentration range and less sensitivity.

In this paper, an attempt is made to develop and validate a simple, efficient and reliable method, without incorporating the use of an internal standard, for the determination of PPD in tablet dosage forms by HPLC using UV detection.

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II. Materials and Methods

a) Apparatus

All HPLC experiments were carried out on a isocratic High Pressure Liquid Chromatography system (Shimadzu HPLC class VP series, Shimadzu Corporation, Kyoto, Japan) with two LC-10 AT, VP pumps, variable wavelength programmable UV/Visible detector SPD-10A, VP, CTO-10AS VP column oven, SCL-10A, VP system controller. The HPLC system was equipped with the software "class VP series version 5.03" (Shimadzu). The analytical column used for the separation was 250 mm × 4.6 mm I.D., 5 µm particle size, Thermo Scientific C18 (Phenomenex, Torrance, CA, USA).

b) Chemicals and reagents

All chemicals and reagents were of HPLC grade quality. Milli-Q-water was used throughout the process and it was obtained from Merck Specialties Private Ltd, Hyderabad, and Andhra Pradesh, India. Methanol and acetonitrile of HPLC grade were from Rankem laboratories, Mumbai, India.

c) Preparation of Mobile phase

Mobile phase 'A' consisted of methanol. Mobile phase 'B' was acetonitrile. The mobile phase used for analysis was prepared by mixing mobile phase 'A' and mobile phase 'B' in the ratio, 40:60 v/v. The same mobile phase was also used as a diluent for the sample preparations.

d) Standard solutions and tablet dosage forms

Pharmaceutical grade PPD was kindly gifted by Matrix laboratories, Hyderabad, India, and was used as received. The following available pharmaceutical dosage forms containing 0.5 mg and 1 mg of active ingredient were purchased from the local pharmacy and used in the present investigation:

- Parpex (1 mg, Zydus cadila, Ahmedabad, India)
- Pramipex (0.5 mg and 1 mg, Sun pharma, Mumbai, India)

Stock solution of PPD (1 mg/ml) was prepared by dissolving 100 mg of PPD in 50 ml of diluent in a 100 ml volumetric flask and then made up to the mark with diluent.

e) Chromatographic conditions

The mobile phase was a mixture of methanol and acetonitrile (40:60 v/v). The contents of the mobile phase were filtered before use through 0.45 µm membrane filter, degassed with a helium sparge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1 ml/min. The column temperature was maintained at 25±10C. The injection volume of samples was 20 µl. The analyte was monitored at a wavelength of 263 nm.

f) Recommended procedure

Working standard solutions equivalent to 1 to 100 µg/ml PPD were prepared by appropriate dilution of the stock standard solution (1 mg/ml) with the diluent. Prior to injection of the drug, the mobile phase was pumped for about 30 minutes to saturate the column thereby to get the base line corrected. 20 µl of each solution was injected automatically onto the column in triplicate and the peaks were determined at 263 nm. The peak areas of PPD were plotted against the corresponding nominal concentration to obtain calibration graph. The concentration of the drug was obtained from the calibration graph or the regression equation.

g) Procedure for tablet dosage forms

Fifty tablets containing PPD were exactly weighed and ground into a fine powder. From this powder, an amount of the tablet powder equivalent to 25 mg PPD was transferred to a 25 ml standard flask containing 10 ml of diluent and shaken for 10 minutes. The volume was made up to the mark with diluent and mixed well. The solution was filtered through a 0.45 µm membrane filter. The filtered solution was appropriately diluted with diluent to obtain a concentration of 100 µg/ml. From this solution, 20 µL was injected into the HPLC system. The area under the peak was noted and the drug content in the tablets was quantified using the calibration graph or regression equation.

III. Results and Discussion

a) Method development

In order to develop an efficient and simple RP-HPLC method for the analysis of the drug in bulk and in its tablet dosage forms, preliminary tests were conducted to select satisfactory and optimum conditions. HPLC parameters, such as detection wavelength, ideal mobile phase & their proportions and flow rate were carefully studied.

Preliminary experiments indicated that the Thermo Scientific C18 (250 mm × 4.6 mm, 5 µm) column provides efficient and reproducible separation of PPD at ambient temperature. Hence Thermo Scientific C18 column was selected for method development and validation. PPD was determined by injecting the drug solution on to Thermo Scientific C18 column with UV detector set at 263 nm. After trying different ratios of mixtures of methanol and acetonitrile, the best results were achieved by using a mixture of methanol-acetonitrile (40:60 *v/v*) as mobile phase. At a flow rate of 1.0 ml/min, the retention time for PPD was 4.458 min. The analyte peak area was well defined and free from tailing under the described experimental conditions.

b) System suitability

System suitability test was carried out on freshly prepared solution of PPD (50 µg/ml) to ensure the

validity of the analytical procedure. Data from five injections were used to confirm system suitability parameters like retention time, peak area, peak asymmetry, theoretical plates, plates per meter and height equivalent to theoretical plate. The results are presented in Table 1. The values obtained demonstrated the suitability of the system for the analysis of the PPD.

c) Selectivity

Selectivity is the ability of an analytical method to distinguish between the analyte of interest and other components present in the sample. To identify the interference by the excipients in the tablet dosage form, the tablet extract was prepared according to procedure described under "Procedure for tablet dosage forms" and injected. The resulting chromatogram (Fig. 2) did not show any peak other than that of PPD, which confirmed the selectivity of the method. The selectivity of the method was also demonstrated by interference check by injecting the diluent blank to determine whether any peaks in the diluent are co-eluting with PPD peak. No interference of peaks eluted in the diluent blank with PPD peak was observed (Fig.3).

d) Linearity

The linearity was determined by constructing calibration curve. A calibration curve was constructed using least squares method by plotting the peak area vs concentration of PPD. The calibration curves (Fig.5) for PPD show good linearity with excellent regression coefficient (0.9993) in the concentration range of 1-100 µg/ml. The linear regression equation and regression coefficient of the calibration curve is presented in Table 2.

e) LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of y-intercepts of regression lines or standard deviation of blank readings and the slope of the calibration curve by using three calibration curves. Results of LOD and LOQ for PPD are shown in Table 2.

f) Accuracy and precision

The precision and accuracy of the method was determined by performing five repeated analysis of three different standard solutions containing 5, 50, 90 µg/ml PPD, on the same day, under the optimized experimental conditions. The precision and accuracy are expressed as RSD and relative error, respectively. The results of this study are presented in Table 3. The values of the relative standard deviation and relative error were found satisfactory. Hence the proposed method is precise and accurate.

g) Recovery studies

The accuracy of the proposed method was also further assessed by performing recovery experiments using the standard addition method. Known amount of the pure PPD was added to pre-analyzed formulation and the total concentration was once again determined by the proposed method. The obtained mean recoveries and relative standard deviations were in the range 99.66–100.33 and 0.378–0.614 %, respectively (Table 4). The results revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed method. The closeness of the recoveries suggests lack of interference from tablet excipients and thereby establishes some degree of selectivity.

h) Application to tablet dosage forms

To find out the suitability of the proposed method for the assay of tablet dosage forms containing PPD was analyzed by the proposed method. The results obtained from the proposed method were compared statistically with reference method7 by applying Student's t-test for accuracy and F-test for precision. From the results (Table 5) it was found that the proposed method does not differ significantly in precision and accuracy from the reference method.

IV. Conclusion

A simple, sensitive, selective, accurate and precise RP-HPLC method was developed for the determination of PPD in bulk and in tablet dosage forms. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The short chromatographic time makes this method appropriate for the processing of numerous samples in a limited time. The method has wider linear range with good accuracy and precision. The method shows no interference from tablet excipients. Hence, the proposed method could be useful and fit for the quantification of PPD in bulk and tablet dosage forms.

V. Acknowledgements

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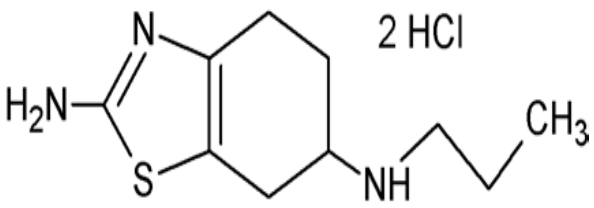

Figure 1 : Structure of pramipexole dihydrochloride.

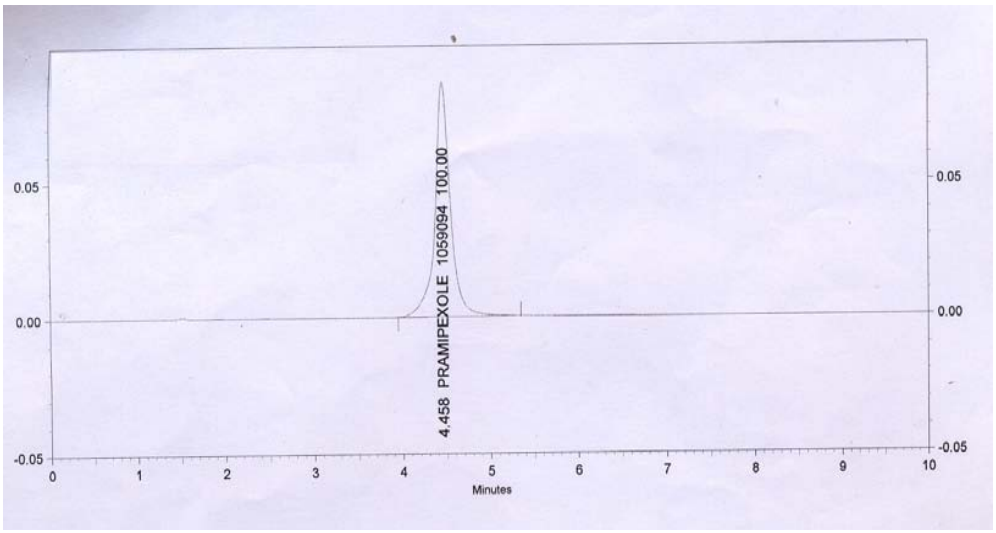

Figure 2 : Chromatogram of standard PPD (100 µg/ml).

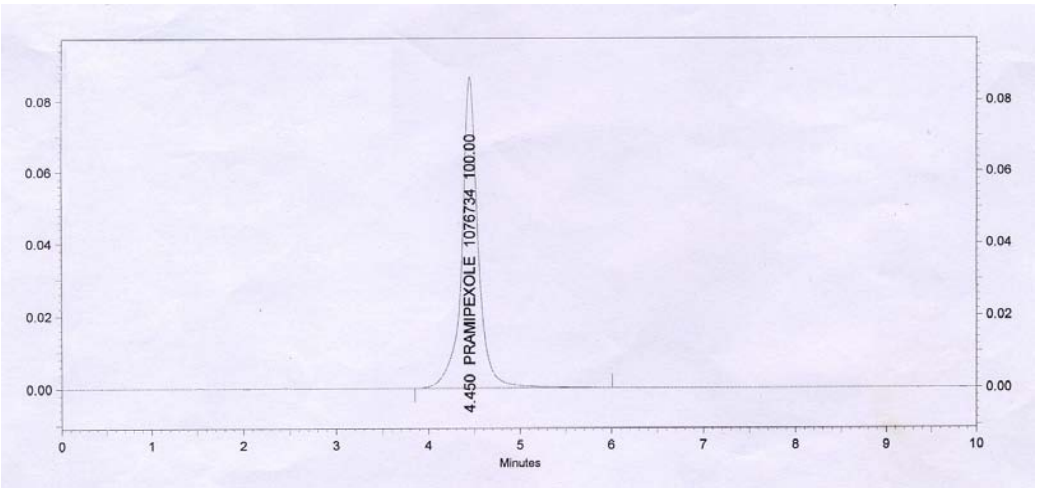

Figure 3 : Chromatogram of PPD tablet dosage form (100 µg/ml).

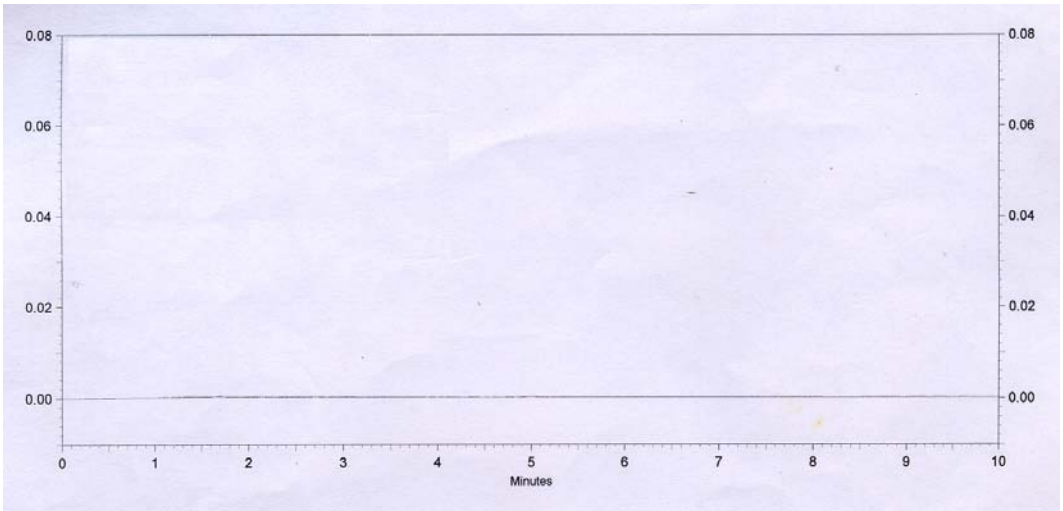

Figure 4 : Chromatogram of diluent blank (methanol and acetonitrile in the ratio of 40:60 v/v).

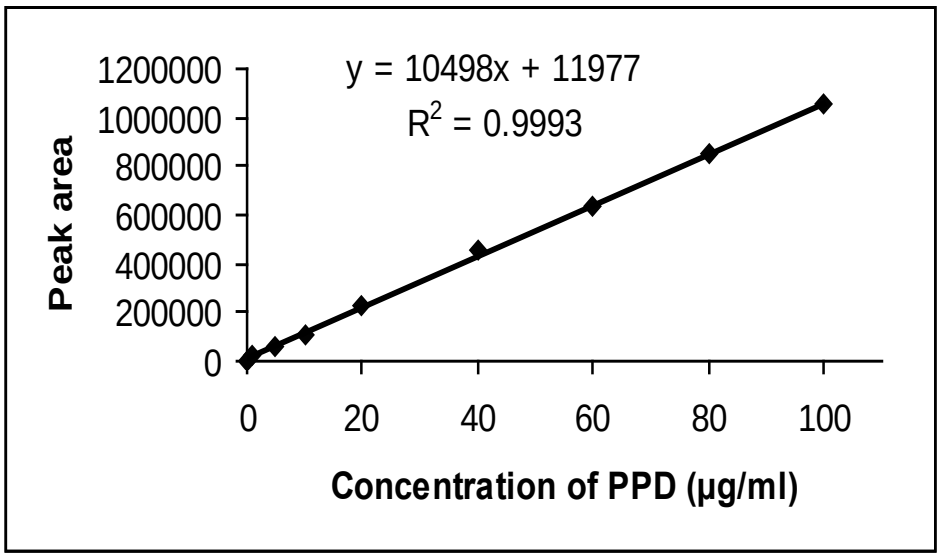

Figure 5 : Calibration curve for assay of PPD.

Table 1 : System suitability studies

Parameter	Value	RSD (%)
Retention Time (t) (Min)	4.458	1.052
Peak area	541238	0.957
Theoretical Plates (n)	8464	0.834
Plates per Meter (N)	17500	1.127
Height equivalent to theoretical plate (HETP) (mm)	2.958×10^{-5}	1.096
Peak asymmetry	1.95	0.946

Table 2 : Linearity and regression characteristics.

Parameter	Value
Linearity range (µg/ml)	1-100
Regression equation (Y = a + bc):	
Slope (b)	10498
Intercept (a)	11977
Regression Coefficient (r^2)	0.9993
Limit of Detection (µg/ml)	0.075
Limit of Quantification (µg/ml)	0.227

Table 3 : Precision and accuracy studies.

Concentration of PPD (µg/ml)		RSD (%)	Recovery (%)	Error (%)
Taken	Found ± SD(n=5)			
5	4.92 ± 0.067	1.361	98.40	1.60
50	50.09 ± 0.459	0.916	100.18	0.18
90	91.02 ± 0.729	0.800	101.13	1.13

Table 4 : Recovery studies.

Formulation	Labelled claim (mg)	Concentration of PPD (mg)		RSD (%)	Recovery (%)
		Added	Found ± SD (n=5)		
Parpex	1.0	0.50	1.495 ± 0.0083	0.555	99.66
Pramipex	1.0	0.50	1.505 ± 0.0057	0.378	100.33
Pramipex	0.5	0.25	0.748 ± 0.0046	0.614	99.73

Table 5 : Assay of PPD in tablet dosage forms.

Formulation	Labelled claim (mg)	Found (mg) ± SD (n=5)		t Value*	F value*
		Proposed method	Reference method		
Parpex	1.0	0.992 ± 0.0056 %R=99.20 %RSD=0.564	0.995 ± 0.0036 %R=99.50 %RSD=0.361	1.56	3.61
Pramipex	1.0	1.051 ± 0.0037 %R=105.10 %RSD=0.352	0.997 ± 0.0028 %R=99.70 %RSD=0.280	1.20	3.08
Pramipex	0.5	0.509 ± 0.0067 %R=101.80 %RSD=1.316	0.502 ± 0.0038 %R=100.40 %RSD=0.756	0.96	2.51

R – Recovery

**Tabulated t value at 95 % confidence level = 2.77 and Tabulated F value at 95% confidence level = 6.39.*

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