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Research Article

RP–HPLC Method for the Determination Pramipexole Dihydrochloride in Tablet Dosage Form

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ABSTRACT

A simple, sensitive, rapid, selective, precise and accurate high performance liquid chromatographic method was developed and validated for the determination of Pramipexole dihydrochloride in bulk and tablet dosage forms. HPLC separation was carried out by reversed phase chromatography on a Thermo Scientific C18 column (4.6 ×150mm, 85µm), held at ambient temperature. The mobile phase consisted of methanol: ammonium acetate buffer (75:25 v/v), run at a flow rate of 0.7 ml/min and with UV detection at 262 nm. The method was found to be linear over an analytical range of 1-100 µg/ml with LOD limit for pramipexole is 3.17 (S/N ratio) and LOQ limit for the drug is 10.3 (S/N ratio) respectively. The proposed method was validated successfully and applied to the quantification of the drug in tablet dosage forms.

INTRODUCTION

Pramipexole dihydrochloride (PPD) [1-6], a no ergot dopamine agonist approved in the US (1997), is used as an antidyskinetic for treatment of Parkinson's disease. Its chemical name is **((S)-N6- propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine dihydrochloride) .** 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 UVspectrophotometry , visible spectrophotometry , HPTLC have been reported by several workers. High-performance liquid chromatography with mass spectrometer (HPLC-MS) capillary electrophoresis with laser-induced fluorescence detection [14], gas chromatography with mass spectrometer (GC-MS) and Ultra-performance liquid chromatography with mass spectrometer (UPLCMS) 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 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 Jančić was carried out using a C18 column with mobile phases containing different ratios of acetonitrile and water phase (aqueoustriethylamine/orthophosporic acid). Yau 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 method for PPD in pure and in its pharmaceutical dosage forms has been reported by RAO 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 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 rigid pH control, narrow linear concentration range and less sensitivity. Here 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.

MATERAILS AND METHODS:

Apparatus : All HPLC experiments were carried out on a isocratic high pressure liquid chromatographic system (water 2695 separations module).with UV detector ,the analytical column used for this separation is 4.6 ×150mm,85µm particle size thermoscintific c18 column .

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,

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India. Methanol and acetonitrile of HPLC grade were from Rankem laboratories, Mumbai, India.

Preparation of Mobile phase: Mobile phase 'A' consisted of methanol. Mobile phase 'B' was acetate buffer. The mobile phase used for analysis was prepared by mixing mobile phase 'A' and mobile phase 'B' in the ratio, 72:25 v/v. The same mobile phase was also used as a diluent for the sample.

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.

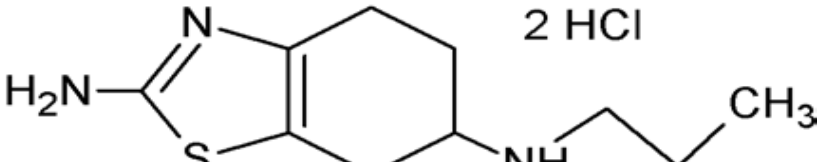
Parpex (1 mg, Zydus cadila, Ahmedabad, India)

Pramipex (0.5 mg and 1 mg, Sun pharma, Mumbai, India)

Chromatographic conditions: The mobile phase was a mixture of methanol and acetate buffer (75:25 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 262 nm.

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 262 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.

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.



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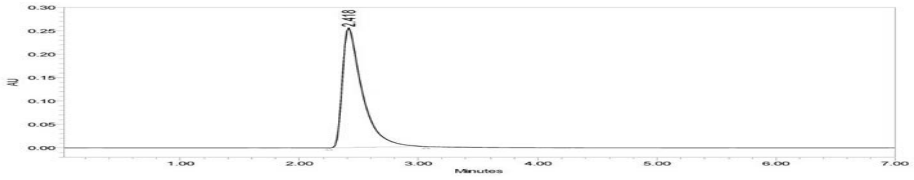


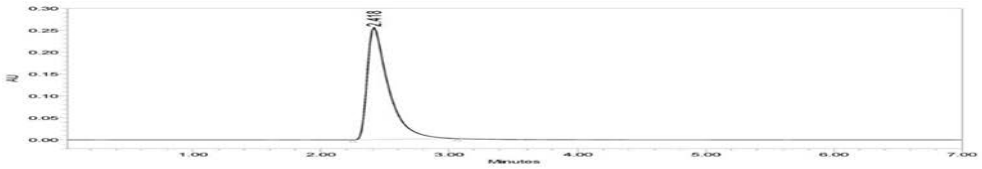
Chromatograph of Standard PPD (100 micro gram/ml)

RESULTS AND DISCUSSION

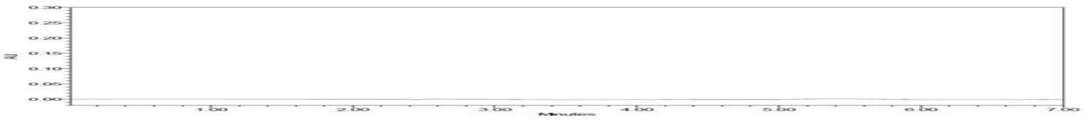
Method development: In order to develop an efficient and simple RPHPLC method for the analysis of the drug in bulk and in its tablet dosage forms, preliminary tests were conducted to select satisfactory and optimum conditions. HPLC parameters, such as detection wavelength, ideal mobile phase & their proportions and flow rate were carefully studied. Preliminary experiments indicated that the Thermo Scientific C18 (4.6 × 150mm, 8.5 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 262 nm. After trying different ratios of mixtures of methanol and acetate buffer, the best results were achieved by using a mixture of methanol : acetate buffer (75:25 v/v) as mobile phase. At a flow rate of 0.7 ml/min, the retention time for PPD was 2.415 min. The analyte peak area was well defined and free from tailing under the described experimental conditions.

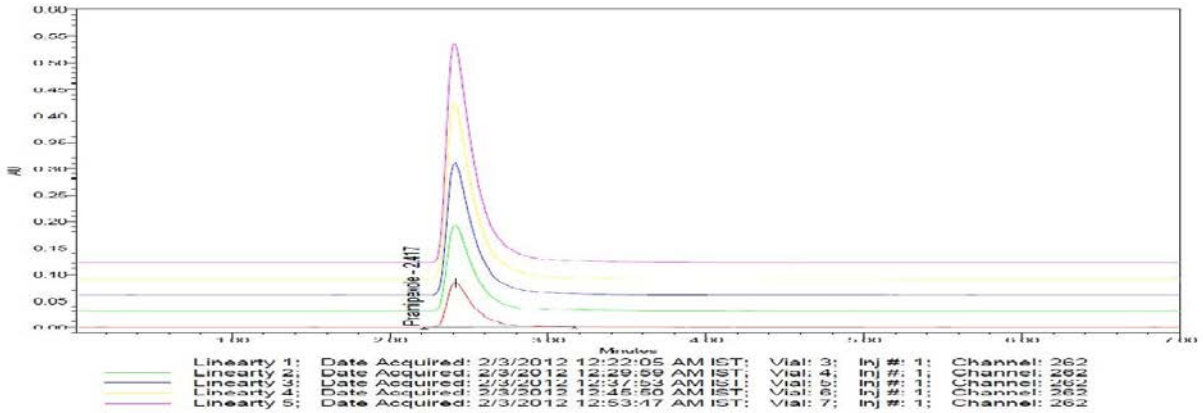
System suitability: System suitability test was carried out on freshly prepared solution of PPD (50 µg/ml) to ensure the validity of the analytical procedure. Data from five injections were used to confirm system suitability parameters like retention time, peak area, peak



Chromatograph of Standard PPD (100 micro gram/ml)



Chromatogram of diluent blank(methanol and acetate buffer 75:25 v/v)



Overlay Report of Linearity

S.no	Linearity Level	Concentration	Area
1	I	20 ppm	999097
2	II	40 ppm	1977688
3	III	60 ppm	3002020
4	IV	80 ppm	4008727
5	V	100 ppm	5084461
Correlation Coefficient			*0.999*

asymmetry, theoretical plates, and plates per meter and height equivalent to theoretical plate.

Tailing factor for the peaks due to pramipexole peaks in standards solutions should not be more than **2.0**

Theoretical plates for the pramipexole peaks in standard solutions should not be less than **2000.**

Tailing factor : obtained from the standard injection is 1.54

Theoretical plates **:** obtained from the standard injection is **2110.4.**

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 did not show any peak other than that of PPD, which confirmed the selectivity of the method. The selectivity of the method

Table 1: Linearity Results:

%Concentration (At Specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1010124	5.0	4.95	99.05%	
100%	3003753	10.0	9.97	99.76%	
150%	5112926	15.0	14.98	99.91%	99.58%

Table 2: The Accuracy Results:

Injections	Area
Injection-1	2984864
Injection-2	2996289
Injection-3	2998848
Injection-4	2994098
Injection-5	3011143
Average	2997048
Standard Deviation	9482.1
%RSD	0.32

Table 3: Precision Results

S.NO	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	UPS Tailing
1	0.6	2050.1	1.54
2	0.7	2110.4	1.54
3	0.8	2008.1	1.54

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 diluents blank with PPD peak was observed.

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 for PPD show good linearity with excellent regression coefficient (0.9993) in the concentration range of 1-100 µg/ml.

The linearity results are presented in table 1.

LOD and LOQ : The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the S/N ratio .S/N ratio value for LOD solution obtained is 3.17 (acceptance value =3)

S/N ratio value for LOQ solution obtained is 10.3 (acceptance value =10)

Accuracy: The Accuracy of the Method was performed by the known amount of standard drug was spiked in triplicate to the prea-nalysed samples and the recovery of the drug was calculated.

Accuracy was performed at 3 levels: 50%, 100%, and 150% of sample concentrations, in Triplicate at each level, using the drug. Samples were prepared by adding corresponding weight of Working Standard.

Inject the standard solutions of Accuracy-50%, Accuracy-100% and Accuracy-150% solutions.

Calculate the Amount Food and Amount Added for Pramipexole and calculate the Individual Recovery and Mean Recovery values.

The **% Recovery** for each level should be between **98.0** to **102.0%.** The results of

Accuracy showed in table no 2:

Precision: The Standard Solution (60µg/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 limit. The **%RSD** for the area of five standard injections results should not be more than **2%.**

The results of Precision showed in table no 3:

Robustness: Robustness of an Analytical Method is measure of its Capacity to remain unaffected, but small deliberate variations in method parameters and provides an indication of its Reliability during Normal usage.

As part of the Robustness, deliberate change in the Flow Rate, Mobile Phase Composition was made to evaluate the impact on the Method.

Flow Rate: The Flow Rate was varied at 0.6 ml/min to 0.8ml/min.

Standard solution 60ppm of Pramipexole was prepared and analyzed using the varied flow rates along with method Flow Rate.From the results it can be concluded that the variation in Flow Rate does not affect the method significantly. Hence it indicates that the method is robust even by change in the Flow Rate of **±0.1%.**

The results are shown in table no 4:

Change in Mobile Phase Composition:

The Organic Composition in the Mobile Phase was varied from **70% to 80%.**

Standard solution 60ppm of Pramipexole was prepared and analyzed using the varied Mobile Phase composition along with the Actual Mobile Phase composition in the method. From the results it can be concluded that the variation in 5 % Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile Phase **±5%.**

Table 4: Flow Rate System Suitability Results

S.NO	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	UPS Tailing
1	5% less	2027.9	1.54
2	*Actual	2110.4	1.54
3	5% more	2193.1	1.54

CONCLUSION

A Sensitive and Specific RP-HPLC method was developed for the quantitative estimation of Pramipexole Bulk & Tablet Formulation. The developed method consisting the mobile phase Methanol : Buffer (2gm of Ammonium Acetate was added in 1000 ml of water and pH adjusted to 4.5 with Glacial Acetic Acid in the ratio 75 : 25 with Isocratic programming, Kromacil C18 column (150mm × 4.6mm, 8.5µm) as Stationary Phase with a Flow Rate of 0.7 mL/min.

The proposed method was proven to be Specific, Linear, Accurate and Robust and is suitable for its application purpose for Pramipexole Bulk and Tablet Dosage form. So the above work performed gives documented evidence for the Assay and Validation of Pramipexole in Bulk and Tablet Dosage form.

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