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Original Article

Stability indicating RP-HPLC assay method for pramipexole dihydrochloride in pure and formulations

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ABSTRACT

A simple, rapid, accurate and precise RP-HPLC method is developed for the determination of pramipexole dihydrochloride in pure and dosage forms. Separation of the pramipexole dihydrochloride was achieved on a reverse phase Hypersil BDS C18 Column (250 mm x 4.6 mm, 5µm) using the mobile phase [phosphate buffer [pH-3.0]: Acetonitrile in the ratio of 40:60, v/v] with a flow rate of 1.0mL/min and detection at 264 nm. The method showed a linear response in the range of 10-30 µg/mL and retention time was 6.791 min. The method was statistically validated for linearity, accuracy, precision and selectivity as per ICH guidelines. The drug was subjected to stress conditions of hydrolysis (acid and base), oxidation, photolysis and thermal degradation to show the stability-indicating power of the developed RP-HPLC method. The present method can be successfully used for routine quality control analysis of pramipexole dihydrochloride and stability studies.

Keywords: Pramipexole dihydrochloride, RP-HPLC, Validation.

INTRODUCTION

Pramipexole dihydrochloride [1] is chemically (s)-2-amino-4,5,6,7-tetrahydro-6-(propylamino) benzothiazole dihydrochloride (**Fig. 1**) which is a non-ergot dopamine agonist recently approved for the treatment of early and advanced Parkinson's disease (PD). It is used along with levodopa. Various analytical methods [2-16] were reported for the analysis of pramipexole dihydrochloride in bulk and pharmaceutical dosage forms using several analytical techniques. Extensive literature survey revealed that there is no rapid stability-indicating HPLC method for the determination of pramipexole dihydrochloride in pure and pharmaceutical dosage forms and this made the authors in the present research work to develop a suitable, single and rapid stability-indicating HPLC method for the determination of pramipexole dihydrochloride and its related substances.

The present research paper describes the development and validation of a stability-indicating liquid chromatographic analytical method for assay of pramipexole dihydrochloride in pure as well as in formulations.

EXPERIMENTAL

Instrumentation: The present analysis was performed on HPLC system (Waters Alliance 2695 separations module) equipped with 600e controller pump, 776 auto sampler, 2487 dual variable wavelength UV detector equipped with Empower software on Dell computer. A stainless steel Hypersil BDS, C_{18} RP-Column (4.6 mmx250 mm) purchased from Waters Corporation (Bedford, MA, USA) was used in the present assay. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. Dona analytical balance was used for weighing the reagents in the present assay.

Chemicals and Reagents: The reference sample of pramipexole dihydrochloride was provided as gift sample from Pharma Train, Hyderabad, India and its market formulation PARPEX tablets containing 100 mg of Pramipexole dihydrochloride was procured from the local pharmacy.

HPLC grade acetonitrile was purchased from E. Merck (India) Ltd., Mumbai, India. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai, India. Milli-Q water was used throughout the experiment

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Prepartion OF Buffer Solution: 2.72 grams of potassium dihydrogen phosphate was weighed and transferred into a 1000 mL beaker, dissolved and diluted to 1000 mL with HPLC water. The pH of the buffer was adjusted to 3.0 with orthophosporic acid. This buffer solution was filtered and degassed prior to the assay.

Preparation of Mobile Phase: The mobile phase in the present assay was prepared by dissolving 0.02 M ammonium dihydrogen phosphate buffer (pH-3.0) and Acetonitrile in the ratio of 40:60v/v. This mobile phase is filtered and degassed prior to the assay.

Preparation of Diluent: Mobile phase is used as diluent in the present assay.

Standard Stock Solution: Standard stock solution (pramipexole dihydrochloride-100 µg/ml) was prepared by dissolving 10 mg of pramipexole dihydrochloride pure reference standard in 30 ml of methanol and then diluted to 100 ml with the diluent. A series of working standard solutions of pramipexole hydrochloride in the concentration range of 10.0 µg/ml to 30 µg/ml were obtained by diluting the aliquots of the above prepared stock solution with the same diluent. All the above volumetric flasks of working standard solutions were wrapped with aluminium foil and stored in the dark.

RESULTS AND DISCUSSIONS

In developing the new RP-HPLC method a systematic study of the effect of various factors (i.e, the influence of column, aqueous and organic phase for mobile phase, mobile phase proportion, wavelength, diluent, concentration of analyte and other chromatographic parameters) was carried out by varying one parameter at a time and keeping all other conditions constant. From these studies it is revealed that Hypersil BDS, C_{18} RP-Column (4.6 mmx250 mm) having 5 µm particle size was used as stationary phase for pramipexole dihydrochloride among the other columns because of its advantages of high degree of retention, high resolution capacity, better reproducibility, ability to produce lower back pressure and low degree of tailing. A good symmetrical peak for pramipexole dihydrochloride was obtained, when water was replaced by phosphate buffer (adjusted to acidic pH by ortho-phosphoric acid) as aqueous phase in mobile phase. Preliminary trials on mobile phase proportion were carried out to provide good resolution for pramipexole dihydrochloride using different compositions of mobile phase. From these trails, the proportion of phosphate buffer (pH-3.0) and Acetonitrile in the ratio of 40:60v/v was finalized as it gave good symmetrical peak for pramipexole dihydrochloride. The appropriate wavelength for determination of pramipexole dihydrochloride was scanned by UV-visible spectrophotometer and it was observed that the maximum absorbance (λ_{max}) was obtained at 264 nm. At this wavelength pramipexole dihydrochloride offered high response with good linearity. The best separation with adequate resolution and symmetric peak of pramipexole dihydrochloride was obtained with the injection volume of 20 µL at a flow rate of 1.0 ml/min for the mobile phase respectively. On this finalized chromatographic conditions, chromatogram of pramipexole dihydrochloride exhibited good peak symmetry with higher theoretical plates. The representative chromatogram of pramipexole dihydrochloride is shown in **Fig.2**.

Method Validation: After fixing the optimization studies the developed method was validated as per ICH guidelines which include system suitability, specificity, linearity, accuracy test, precision, robustness, ruggedness, sensitivity, limit of detection and quantification. The column efficiency, resolution and peak asymmetry were calculated for the standard solutions of pramipexole dihydrochloride. The values demonstrated the suitability of the system for the analysis of pramipexole dihydrochloride in dosage forms and the results of these studies were summarized in **Table.1**. The specificity of the proposed method for pramipexole dihydrochloride was studied and calculated basing on the resolution factor of the peak and was found to be free of interference from the excipients used in pharmaceutical formulation and it indicates the specificity of the system. In the present study, the drug was subjected to various stress degradation studies as per the ICH recommended guidelines. As pramipexole dihydrochloride is soluble in methanol all solutions of pramipexole dihydrochloride for use in forced degradation studies were prepared in methanol. This is done by subjecting pramipexole dihydrochloride standard reference powder to acidic (0.1N HCl), basic (0.1N NaOH), oxidizing (3% H_2O_2), and photo stability stress conditions. The chromatograms obtained under acidic stress, basic stress and photostability stress conditions revealed that pramipexole dihydrochloride is more stable, did not show any degradation and is eluted from the column respectively. The oxidative stress studies revealed that pramipexole dihydrochloride is not fully degraded and its degradation products were eluted separately at different retention times respectively. From the respective chromatographs**,** it was observed that the degradation products did not interfere in the detection analysis of pramipexole dihydrochloride establishing the high stability of the developed method. For linearity

studies concentration levels corresponding to 50, 75, 100,125 and 150% of test solution[10 µg/ml – 30 µg/ml] of pramipexole dihydrochloride were prepared separately and 20 µl of each concentration was injected into the prescribed HPLC system and the response was read at 264 nm and the corresponding chromatograms were recorded. From these chromatograms, a calibration curve was constructed by plotting the peak areas of the drug versus concentration of pramipexole dihydrochloride **(Figs.3)**. The linear regression equation for the calibration curve of pramipexole dihydrochloride was found to be **Y= 119269.74x+4304334.40** with a coefficient of regression, r^2=0.9998 respectively. The calibrated results of pramipexole dihydrochloride were tabulated in **Table.2**. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by calculating the signal to noise (S/N) ratio. The LOD and LOQ values of pramipexole dihydrochloride were found to be 0.0193 µg/ml and 0.0645 µg/ml respectively.

Precision of the proposed method was determined by repeatability (intra-day precision). It was expressed as % relative standard deviation (%RSD).The percent relative standard deviation (% RSD) was calculated and it was found to be 0.022, which is within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in **Table.3**. The accuracy of the proposed method was assessed by determination of recovery for three concentrations in triplicate (corresponding to 50, 100 and 150 % level of test solution concentration) of pramipexole dihydrochloride covering the within the linearity range of the proposed method. The percentage recovery was calculated and results are compiled in **Table.4.** These results indicate a high degree of accuracy of the proposed method for determination of pramipexole dihydrochloride. The ruggedness of the present RP-HPLC method was determined by carrying out the experiment by different analysts using different columns of similar types. The percentage of assay (%RSD) of different preparations assay values with two different analysts and columns were 0.22 and 0.156% respectively (**Table.5)**. Robustness of the method was determined by small deliberate changes in flow rate, and temperature. The robustness limit for flow rate variation and temperature variation were well within the limit, revealing that the proposed method is robust under given set of defined experimental conditions **(Table.6)**.

The proposed RP-HPLC method has been validated for the assay of pramipexole dihydrochloride in tablet as per guidelines of ICH. Ten tablets of PARPEX [Label claim; 100mg of pramipexole dihydrochloride] were procured from local pharmacy and were powdered. An accurately weighed portion of powder equivalent to 25 mg of pramipexole dihydrochloride was dissolved in 25 ml of methanol and filtered through 0.45 µm membrane filter. From this filtrate, 0.1 ml was pipetted in to 10 ml graduated test tube and made up to volume with the mobile phase. 20 µL of this sample was injected into the column and the drug content in the tablet was quantified using the regression equation and the chromatogram and the results are shown in **Table.7**.

CONCLUSIONS

Based on all the results, it can be concluded that a simple, accurate and stability indicating RP-HPLC method has been developed and validated for the analysis of pramipexole dihydrochloride in tablet dosage forms. Based on peak purity results, obtained from the analysis of force degradation studies, it can be inferred that the absence of coeluting peak along with the main peak of pramipexole dihydrochloride shows that the developed method is specific for the estimation of dexamethasone in presence of degradation products. Statistical analysis proved that the method is suitable for the analysis of pramipexole dihydrochloride in pure and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of pramipexole dihydrochloride and can be conveniently used for the routine assay of pramipexole dihydrochloride by the pharmaceutical manufacturing units.

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TABLE.1: SYSTEM SUITABILITY PARAMETERS

PARAMETERS	PRAMIPEXOLE DIHYDROCHLORIDE
Retention time	6.791
Peak area	7301167
USP Tailing	1.6

TABLE.2: CALIBRATION OF THE RP HPLC FOR THE ESTIMATION OF PRAMIPEXOLE DIHYDROCHLORIDE

Concentration (µg.mL)	Area (mAU)
10	5477091
15	6104459
20	6707825
25	7296414
30	7862857
Regression equation; Intercept (a)	4304334.40
Slope (b)	119269.74
Correlation coefficient	0.9998
Standard deviation on intercept (S_a)	770.442869
Standard deviation on slope (S_b)	1335.48036
LOD	0.0193
LOQ	0.0645

TABLE.3:RESULTS OF INTRA-DAY PRECISION

S No	Name	Area
1	**Injection-1**	6645719
2	**Injection-2**	6645447
3	**Injection-3**	6644604
4	**Injection-4**	6641291
5	**Injection-5**	6669204
6	**Injection-6**	6675402
Avg		6653611
Std Dev		14695.8
% RSD		0.220

TABLE.4: RECOVERY STUDIES OF THE PROPOSED RP-HPLC METHOD

*All the values are the averages of three determinations

Proposed methods	PPD in tablet µg.mL^{-1}	Pure PPD added µg.mL^{-1}	Total found µg.mL^{-1}	Pure PPD recovered %±SD*
50%	10.0	5.0	14.99	99.93
100%	20.0	5.0	24.94	99.76
150%	30.0	5.0	34.98	99.94

TABLE.5: EVALUATION DATA OF ROBUSTNESS STUDY

ROBUST CONDITIONS		PRAMIPEXOLE DIHYDROCHLORIDE	
		RT	**PEAK AREA**
Flow rate	**0.8 ml/min**	6.855	6660097
	1.2 ml/min	6.787	6679389
TEMPERATURE	**30^{O}C**	6.790	6101647
	45^{O}C	6.854	6683871

TABLE.6: EVALUATION DATA OF RUGGEDNESS STUDY

No of injections	Ruggedness	
	Analyst -1	Analyst-2
	Area	Area
Injection-1	6645719	6645014
Injection-2	6645447	6655432
Injection-3	6644604	6676544
Injection-4	6641291	6660981
Injection-5	6669204	6655321
Injection-6	6675402	6654876
AVG*	6653611	6658028
STDEV*	14695.8	10439.62
%RSD*	0.220	0.156

*All the values are the averages of three determinations

TABLE.7:RESULTS OF ANALYSIS OF TABLET CONTAINING PRAMIPEXOLE DIHYDROCHLORIDE

PHARMACEUTICAL FORMULATION	AMOUNT OF PRAMIPEXOLE DIHYDROCHLORIDE*		% RECOVERY
	LABELLED	FOUND	
PARPEX	100 mg	99.96	99. 96

* Average of three determinations

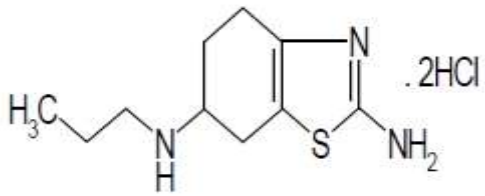

Fig.1:MOLECULAR STRUCTURE OF PRAMIPEXOLE DIHYDROCHLORIDE

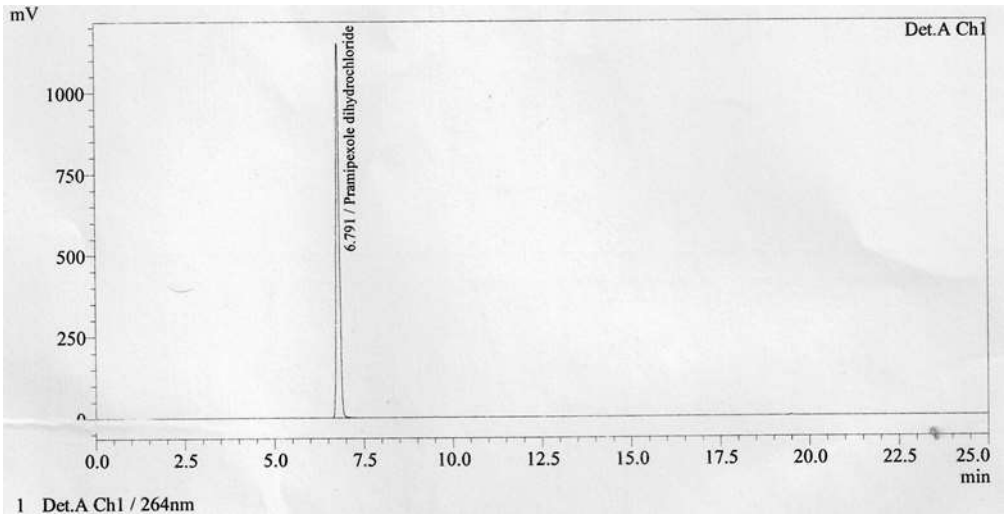

Fig.2.: VALIDATIVE CHROMATOGRAM OF PRAMIPEXOLE DIHYDROCHLORIDE

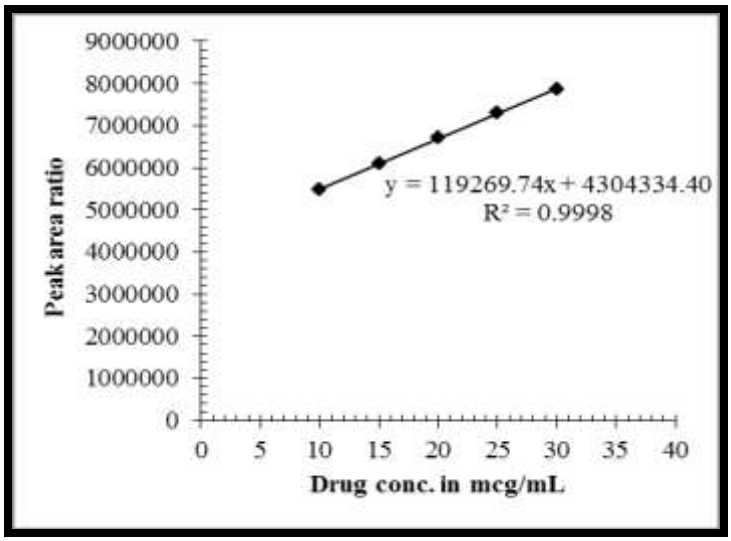

Fig.3: LINEARITY CURVE OF PRAMIPEXOLE DIHYDROCHLORIDE

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