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Development and validation of economically sensitive visible spectrophotometric assay methods for pramipexole dihydrochloride in pure and formulations

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ABSTRACT

Two new simple, sensitive and economical visible spectrophotometric methods were described for the estimation of pramipexole dihydrochloride in pure and dosage forms. This method is based on the diazocoupling reactions between the drug and pluroglucinol (Method –A) and resorcinol (Method –B). The colored products exhibited an absorption maximum at 520 nm (Method –A) and 600 nm (Method –B). Beer's law obeyed in the concentration range of 4.0-20 µg/ml, (Method –A) and 2.0-10 µg/ml (Method –B).The results obtained by the proposed method were in good agreement with the labeled amounts. The proposed methods offer the advantages of rapidity, simplicity, sensitivity and normal cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

Keywords: Pramipexole dihydrochloride, Spectrophotometer, 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 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 form using several analytical techniques. The present research paper describes the development and validation of economically sensitive two visible spectrophotometric method using diazotization reaction for assay of pramipexole dihydrochloride in pure and in formulations.

MATERIALS AND METHODS

Apparatus: All spectral and absorbance measurements were made on an Elico SL -159 digital spectrophotometer with 1cm matched quartz cells.

Chemicals and reagents: The reference sample of pramipexole dihydrochloride was provided as gift sample from Pharma Train, Hyderabad, India. The branded formulation PARPEX tablets containing 100 mg of pramipexole dihydrochloride was procured from the local pharmacy. All the other chemicals and reagents used were of analytical grade and solutions were prepared in distilled water.

Preparation of reagents:
Method- A: Phloroglucinol (Loba, 0.1%): Prepared by dissolving 100mg of Phloroglucinol in 100mL distilled water; $NaNO_2$ solution(Loba, 0.1%): Prepared by dissolving 100mg of $NaNO_2$ in 100mL distilled water;HCl solution (Qualigens,0.25M): Prepared by dissolving 100ml of HCl in 100mL distilled water;NaOH solution (Loba, 4%, 1.0M) : Prepared by dissolving 4g of NaOH to 100 mL distilled water and standardized.

Method-B: Resorcinol (Loba, 0.1%): Prepared by dissolving 100 mg of Resorcinol in 100 ml distilled water; $NaNO_2$ solution (Loba, 0.1%): Prepared by dissolving 100 mg of $NaNO_2$ in 100 mL distilled water; HCl solution (Qualigens,0.25M) : Prepared by dissolving 100 ml of HCl in 100 mL distilled water;NaOH solution (Loba, 4%, 1.0M) : Prepared by dissolving 4gms of NaOH to 100 mL distilled water and standardized.

Preparation of standard solutions: An accurately weighted sample of 10 mg of pramipexole dihydrochloride was dissolved in 10 ml of methanol to give standard stock solution of 100 µg/ml. A series of working standard solutions (4.0-20 µg/m for method-A and 2.0 – 10 µg/ml for method-B) were prepared by diluting the aliquots of stock solution with distilled water. All the above volumetric flasks containing standard solutions were wrapped with aluminium foil and stored in dark

Assay of pramipexole dihydrochloride in dosage form: Ten tablets of PARPEX[Label claim;100 mg of pramipexole dihydrochloride] were procured from local pharmacy and 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 a series of working sample solutions (4.0-20 µg/m for method-A and 2.0 – 10 µg/ml for method-B) were prepared by diluting the aliquots of stock solution with distilled water and the drug content in the tablet was quantified using the regression equation.

Fixation of optimum conditions in procedures: The optimum conditions for the color development of methods **[Method-A ($NaNO_2$ _ PLG), Method-B ($NaNO_2$ _ RES)]** were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in recommended procedures.

METHODS –A&B: Both the methods involve in the formation of diazo coupling product of diazotized pramipexole dihydrochloride with phloroglucinol/ Resorcinol. In order to establish optimum experimental conditions, the effect of various parameters such as volume of phloroglucinol/Resorcinol, HCl, $NaNO_2$ and NaOH solutions, waiting time for diazotization and stability of the colored species were studied. The results are shown in **Table-1**.

Proposed Procedures:
METHOD -A: Aliquots of (0.5 - 2.5 mL, 200 µg/mL) pramipexole dihydrochloride were transferred to a series of 25 ml calibrated flasks. To each of the above aliquots, 1.0 ml of Hydrochloric acid and 1.0 ml cold aqueous solution of sodium nitrite were added and set aside for 10 min. at 0 - 5^oC temperatures Later 1.0 ml of phloroglucinol and 1.5 ml of 1.0 M aqueous sodium hydroxide were added successively, and then the volume in each flask was made up to 25 ml with distilled water. The absorbance was measured at 520 nm against reagent blank. The amount of pramipexole dihydrochloride was computed from its calibration graph. **(Fig-3.A)**

METHOD-B: To each of 25 ml volumetric flasks (0.5 - 2.5 ml, 100 µg/ml) pramipexole dihydrochloride was transferred. Then 1.0 ml of hydrochloric acid and 1.0 ml cold aqueous solution of sodium nitrite were added and set aside for 10 min. at 0 - 5^oC temperature. Later 1.0 ml of resorcinol and 1.5 ml of 1.0M aqueous sodium hydroxide were added successively, and then the volume in each flask was made up to 25 ml with distilled water. The absorbance was measured at 600 nm against reagent blank. The amount of pramipexole dihydrochloride was calculated from its calibration graph. **(Fig-3.B)**

RESULTS AND DISCUSSION

a.Optical and Regression analysis: The absorption curves of the colored species in each method show characteristic absorption maxima graphically represented in **Figs-2.A&2.B.**The Beer's law plots of these systems were recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded graphically **(Figs-3.A&3.B)**. Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range for pramipexole dihydrochloride with each of the mentioned reagents were calculated and recorded in **Table-2**. Least square regression analysis was carried out for getting the slope, intercept and correlation coefficient and the values are shown **Table-2.**.

b. Precision: The precision of each proposed method was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of pramipexole dihydrochloride in total solution. The percentage relative standard deviation and percent age of range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods **Table-2.**

c. Accuracy: To determine the accuracy of each proposed method, different amounts of bulk samples of pramipexole dihydrochloride within the Beer's law limits were taken and analyzed by the proposed methods. The results (percentage error) are recorded in **Table-2.**.

d. Interference studies: The effect of wide range of excipients and other active ingredients usually present in the formulations for the assay of pramipexole dihydrochloride in methods **(A&B)** under the above studied optimum conditions were investigated. The commonly used excipients and other active ingredients usually present in formulations did not interfere even if they were present in more amounts than they usually exist.

e. Analysis of formulations: Commercial formulations (tablets) containing pramipexole dihydrochloride were successfully analysed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically and found that they donot differ significantly in precision and accuracy from reference method. The results are summarized in **Table-3.**.

Table-1 Optimum conditions established in methods-A&B

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	510-530 590-610	520(Method-A) 600(Method-B)	
Effect of Volume of $NaNO_2$ solution.	0.5-1.5mL	1.0mL(both A& B_5)	The absorbance increases over the range of 0.5-1.0mL and remains constant even after further addition.
Volume of (0.25M) HCl solution.	0.5-1.5mL	1.0mL(both M_4 & M_5)	>1.5mL results in high blank valves. When no acid is present diazotization does not occur.
Volume of Phloroglucinol/Resorcinol solution.	0.5-1.5mL	1.0mL(both A B)	Variation of volume beyond this range gave erratic results.
Effect of volume of 1.0M NaOH solution.	1.0-2.0mL	1.5mL(both A&B)	Minimum amount of 1.0mL of NaOH solution was necessary to maintain alkaline conditions necessary for coupling.
Time required for diazotization.	5-10min.	10min.(both A&B)	The same absorbance was noticed over the time interval 2-15min.Beyond that interval, the results were erratic.
Effect of temperature on daizotisation.	0-5^oC	0-5^oC(both A&B)	The same results were obtained over the temperature range 0-5^oC, so it is necessary to cool the solution in ice. Low absorbance was obtained if the temperature is beyond10^oC.
Solvent for final dilution.	Distilled water	Distilled water	
Stability of the colored species after final dilution.	8hrs.	-----	----

Table-2 Results of Optical and regression characteristics of the proposed methods

Parameter	METHOD-A	METHOD-B
λ_{max} (nm)	520	600
Beer's law limits (µg/mL)	4 -20	2-10
Detection limit (µg/mL)	0.3574	0.0502
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	7.70×10^3	2.59×10^4
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.1456	6.43×10^{-2}
Optimum photometric range (µg/mL)	6.5-18.5	3.5-9.5
Regression equation (Y=a+bc) ;slope (b)	0.0178	0.0606
Standard deviation on slope (S_b)	1.60×10^{-4}	1.53×10^{-4}
Intercept (a)	1.7×10^{-3}	1.8×10^{-3}
Standard deviation on intercept (S_a)	2.12×10^{-3}	1.01×10^{-3}
Standard error on estimation (S_e)	2.02×10^{-3}	9.66×10^{-4}
Correlation coefficient (r)	0.9998	0.9999
Relative standard deviation (%)*	0.9159	0.3845
% Range of error (confidence limits)	----	-----
0.05 level	0.9616	0.4037
0.01 level	1.5079	0.6331

** Average of six determinations considered*

f. Chemistry of the colored species: The presence of amino group in pramipexole dihydrochloride enabled the use of diazotization of the drug with nitrous acid and coupling the resulting diazonium salt with phloroglucinol, to

form purple colored chromogen in method-A(**Fig-4**) exhibiting λ_{max} at 520 nm. In method-B (**Fig-4**) diazotisation reaction was followed by coupling with resorcinol in presence of sodium hydroxide solution resulting in the formation of violet chromogen exhibiting λ_{max} at 600 nm.

Table-3 Results of analysis of tablet containing pramipexole dihydrochloride

PHARMACEUTICAL FORMULATION	AMOUNT OF PRAMIPEXOLE DIHYDROCHLORIDE*			% RECOVERY	
	LABELLED	FOUND			
		METHOD -A	METHOD -B	METHOD -A	METHOD -B
PARPEX	100 mg	99.95	99.97	99. 95	99.97

** Average of three determinations*

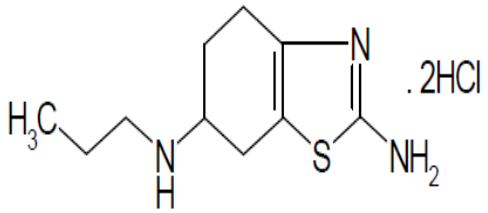

Fig.1:Molecular structure of pramipexole dihydrochloride

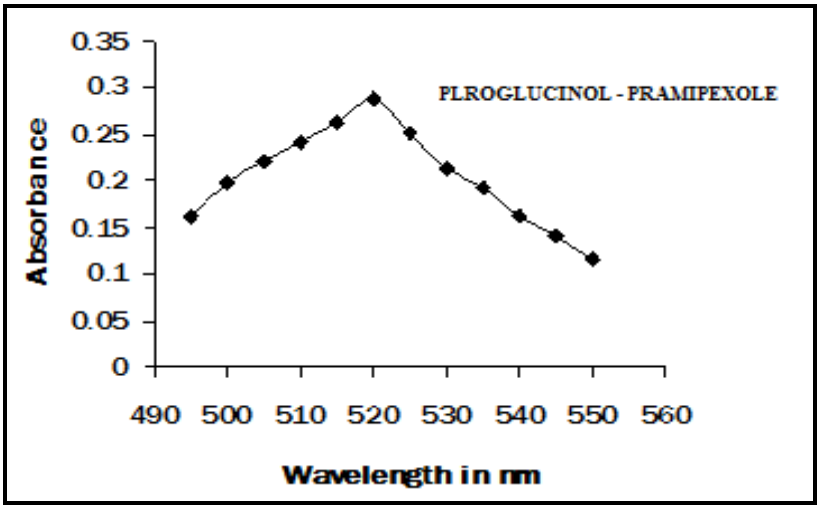

Fig.2.A: Absorption spectrum of PMP with $NaNO_2$ - Phloroglucinol (Method-A)

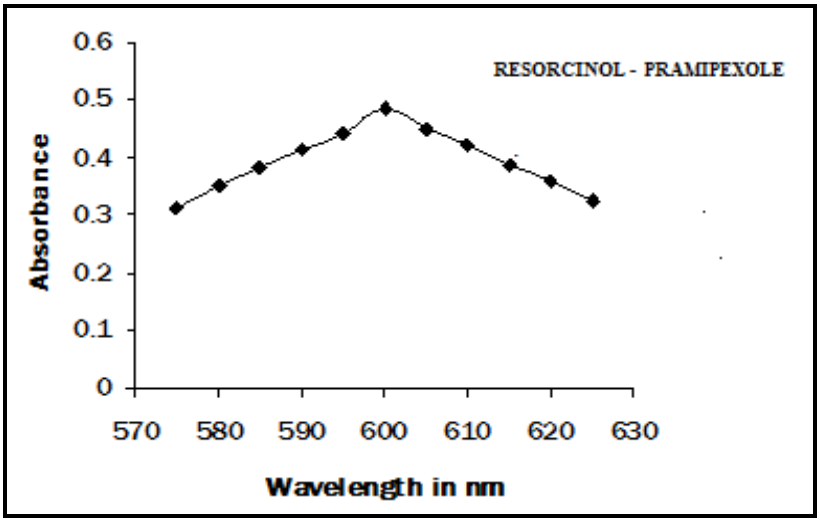

Fig.2.B: Absorption spectrum of PMP with $NaNO_2$ - Resorcinol (Method-B)

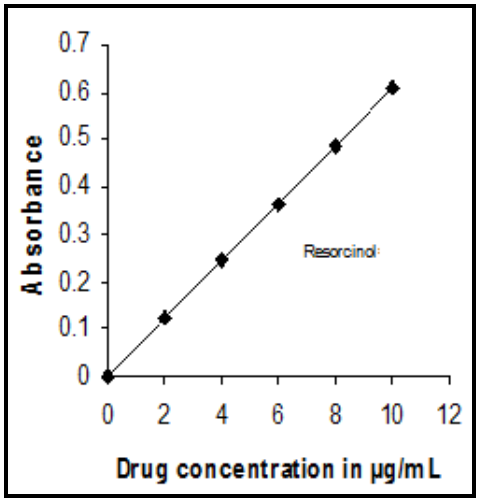

Fig. 3.A: Beer's Law plot of PMP with $NaNO_2$ - Phloroglucinol (A)

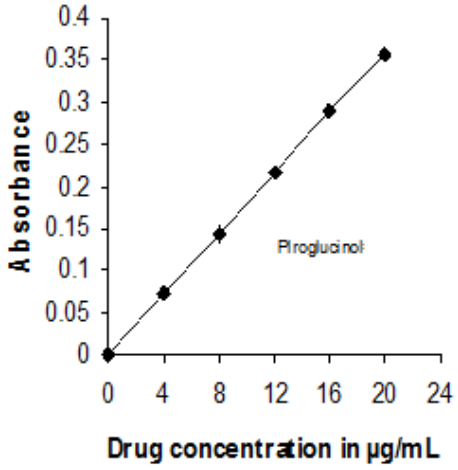

Fig. 3.B: Beer's Law plot of PMP with $NaNO_2$ - Resorcinol (B)

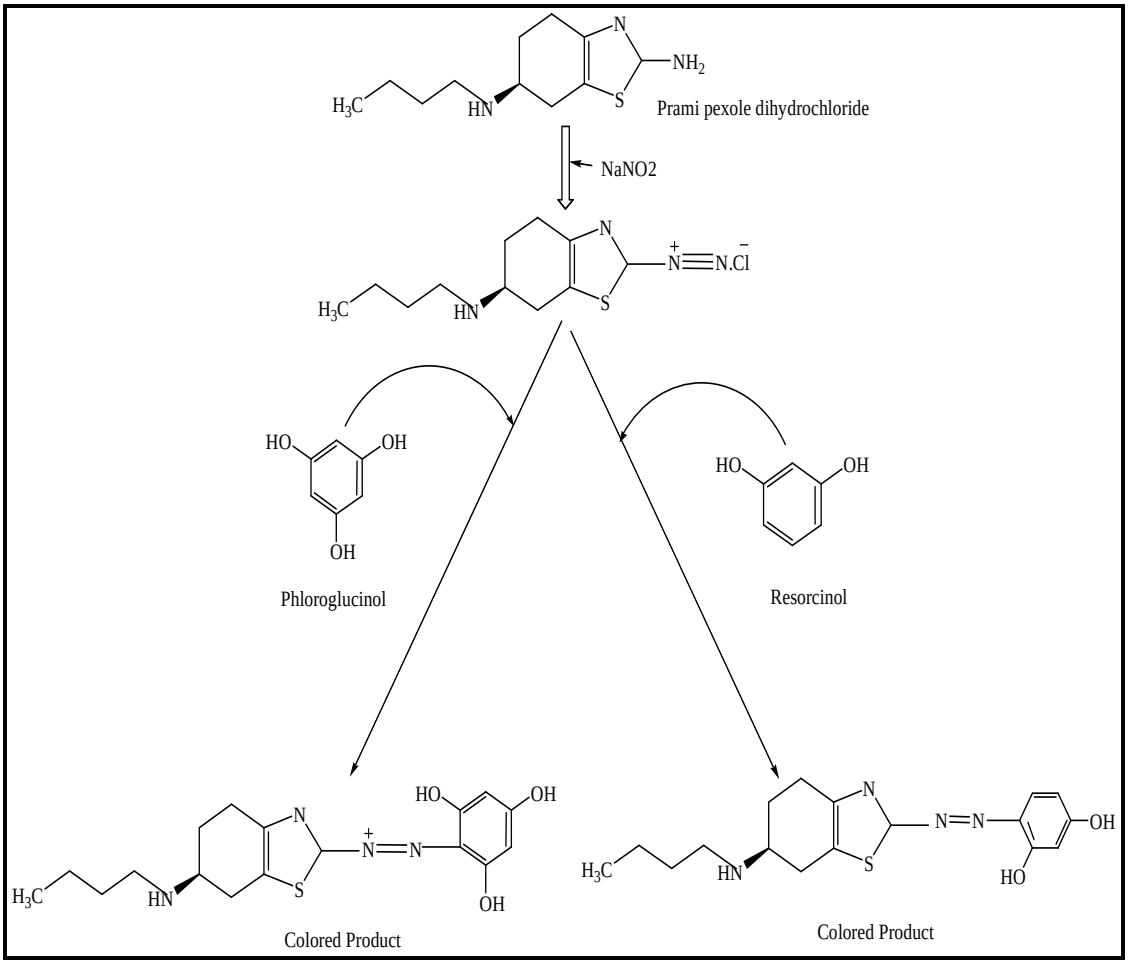

Fig.4.Reaction scheme of pramipexole with phloroglucinol and resorcinol

CONCLUSION

The reagents utilized in the proposed methods are of normal cost, readily available and the procedure does not involve any critical reaction conditions. The proposed visible spectrophotometric method exhibited reasonable precision, accuracy and is simple, sensitive and can be used as an alternative method to the other methods published

in papers for the routine determination of pramipexole dihydrochloride in quality control departments depending on the need and situation

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