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Article

Spectrophotometric Method for the Estimation of Pantoprazole Sodium Using Reagents – 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone, Chloranilic Acid, and 1-Chloro-2,4-dinitrobenzene with Charge – Transfer Complex Reaction

N. Subrahmanyeswara Rao, S. Arul Antony*, T. Thirupathi Rao, S. Siva Kumar

Department of Chemistry, Meenakshi Academy of Higher Education and Research, Meenakshi University, Chennai, Tamil Nadu, India

ABSTRACT

Three simple, sensitive, and rapid spectrophotometric methods were developed for the estimation of pantoprazole sodium (PNT) in pure and its commercial dosage forms. These methods were based on the formation of charge–transfer reaction between the drug, an n-electron donor and π-acceptors, 2, 3-dichloro-5, 6-dicyano-p-benzoquinone, chloranilic acid, and 1-chloro-2, 4-dinitrobenzene. The absorbance of the formed charge–transfer complexes was measured and utilized for the determination of PNT in its pure and commercial dosage forms. The developed methods were evaluated in terms of standard deviation, relative standard deviation, correlation coefficient, limit of detection, and limit of quantitation. Molar absorptivity and Sandell's sensitivity were calculated at the optimum experimental conditions. The validity of the proposed methods was ascertained by recovery studies which indicated that the present methods can be successfully applied for the determination of PNT in pure and commercial dosage forms.

Key words: Spectrophotometric methods, Pantoprazole sodium, Charge–transfer reaction, 2,3-Dichloro-5, 6-dicyano-p-benzoquinone, Chloranilic acid, 1-Chloro-2, 4-dinitrobenzene.

1. INTRODUCTION

Pantoprazole sodium (PNT), 6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2- yl)methylsulfinyl]-1H-benzimidazole (Figure 1), is an irreversible proton-pump inhibitor which is used for the treatment of acid-related gastrointestinal diseases. PNT inhibits proton pump on the gastric parietal cell and reduces gastric acid secretion. It is used for short-term treatment of erosion and ulceration of the esophagus caused by gastroesophageal reflux disease.

The drug has lower affinity than omeprazole or lansoprazole for hepatic cytochrome P450 and shows no clinically relevant pharmacokinetic or pharmacodynamic interactions with diazepam [1-3], carbamazepine [4], phenytoin [5], and warfarin [6]. In literature, few high-performance liquid chromatography [7-9] methods were reported for the determination of PNT in biological fluids and pharmaceutical formulations. A voltammetric method [10] was reported for its determination by differential pulse adsorptive stripping voltammetry using a carbon paste electrode. Some spectrophotometric procedures were reported for the determination of [11-13] PNT. Visible spectrophotometry, because of its simplicity, cost-effectiveness, sensitivity, selectivity, fair accuracy, and precision, has remained competitive in an era chromatographic techniques for pharmaceutical analysis.

In view of the importance of PNT in the treatment of diseases, there is a need to develop simple, cost effective, accurate, and sensitive analytical methods for its assay in pure and dosage forms.

Hence, in the present work, three methods were developed which were based on the formation of charge–transfer complex between PNT and 2,3-dichloro-5, 6-dicyano-p-benzoquinone (DDQ), chloranilic acid (CAA), and 1-chloro-2,4-dinitrobenzene (CDNB) (Figure 1).

2. EXPERIMENTAL PROCEDURE

2.1. Instrumentation

All measurements were carried out using a Shimadzu ultraviolet (UV)–visible spectrophotometer (UV-160A) with a matched pair of 10 mm quartz cells. Mettler Toledo analytical balance (accuracy 0.1 mg) was used for weighing all the samples.

2.1.1. Materials and reagents

PNT from M/s HETERO Drugs Limited, India, as a gift sample, (Pantoprazole tablets IP) Pantolup, Pantosec, and Pantop were purchased in the local market, Tirupati. All the chemicals used were of analytical reagent grade. Double-distilled water is used throughout the experiment.

2.1.2. Preparation of standard solutions

A stock solution of PNT was prepared by dissolving accurately weighed 100 mg of pure drug in 100 ml of water and sonicated to get required concentration of 1 mg/ml. Further, it was diluted with double-distilled water as required for the present investigation.

2.1.3. Preparation of reagents

About 2.5% (w/v) of DDQ solution was prepared by dissolving 2.5 g of compound in 100 ml of acetonitrile for method A, 1.0% (w/v) of CAA

***Corresponding author:**
E-mail: aruantsam@gmail.com

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solution was prepared by dissolving 1 g of compound in 100 ml of ethanol for method B, and 3.0% (w/v) of CDNB solution was prepared by dissolving 3 g of compound in 100 ml of DMSO for method C.

2.2. Method Development

2.2.1. Method A (DDQ method)

The standard drug solution was transferred into a series of clean and dry volumetric flasks. To each flask, 3.2 ml of 0.2% DDQ solution was added and concentrations range of 5–25 µg/ml. A wine red color was developed, and absorbance was measured at 465 nm against the reagent blank.

2.2.2. Method B (CAA method)

Different volumes of standard drug solutions were prepared from stock solution in the concentrations range of 6–30 µg/ml in clean and dry volumetric flasks and 2.0 ml of 1.0% CAA solution was added to each flask. The entire contents were kept aside for 30 min; maximum absorbance of produced pale greenish-brown color solution was measured at 432 nm against the reagent blank.

2.2.3. Method C (CDNB method)

Into a series of volumetric flasks, various volumes of standard drug solution were transferred. To each flask, 3.2 ml of 3% CDNB solution was added and made up the solution in the concentration range of 6–30 µg/ml. Total contents were heated up to 98±2°C and cooled to room temperature. Maximum absorbance of each yellow-colored solution was measured at 415 nm against the reagent blank.

2.2.4. Procedure for analysis of pure drug

Accurately weighed amount of PNT was transferred into clean and dry volumetric flask, subsequently diluted with water to get the required concentration and analyzed by above-mentioned procedure.

2.2.5. Procedure for commercial dosage forms

The dosages of Pantolup, Pantosec, and Pantop containing PNT were purchased from the local market and analyzed by the developed methods. Twenty tablets of each formulation were weighed and grinded to make a fine powder. A quantity of grinded powder equivalent to 100 mg was taken into volumetric flask and analyzed as described above.

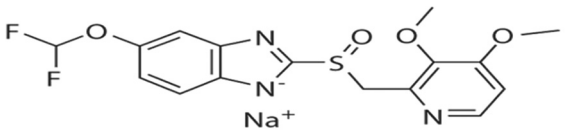

Figure 1: Structure of pantoprazole sodium.

3. RESULTS AND DISCUSSION

In the present work, DDQ, CAA, and CDNB were π-acceptors and PNT as n-donor. Charge–transfer complex was formed by electron that is transfer from the donor to acceptor, which produces high intense color in the visible region of the electromagnetic spectrum.

3.1. Spectral Characteristics

3.1.1. Absorption spectrum

The reaction of DDQ, CAA, and CDNB with PNT results in the formation of wine red, yellowish-green and yellow complexes, respectively, which exhibit maximum absorbance at 465 nm, 432 nm, and 415 nm, respectively (Figures 2-4).

3.1.2. The effect of reagent concentration

To measure the effect of concentration of the reagent on the formation of colored product at the chosen wavelength, various volumes of reagent were added to a fixed concentration of drug solution and absorbance was measured. It was found that 3.2 ml of 0.2% DDQ (method A), 2 ml of 1.0% CAA (method B), and 3.2 ml of 3% CDNB solutions (method C) were optimum for the production of high-intensity color and no change was observed after addition of few more ml of respective reagents.

3.1.3. Effect of the concentration drug

To study the effect of concentration of drug solution on the absorbance maximum, fixed volume of reagent, i.e.,, DDQ, CAA, and CDNB was added to each volumetric flask containing different aliquots of drug solution which was measured the absorbance at 465 nm, 432 nm, and 415 nm, respectively, against reagent blank. It was found that PNT obeyed Beer's law in the range of 5–25 µg/ml, 6–30 µg/ml, and 6–30 µg/ml with DDQ, CAA, and CDNB, respectively.

3.2. Analytical Method Validation

Validation is one of the important steps in analytical method evaluation [14]. The validation parameters, i.e., linearity, accuracy, precision, recovery, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness were evaluated to assess the method suitability.

3.2.1. Linearity

Linearity of the concentration drug solution for the developed methods was studied, and calibration plots were constructed (Figure 5). From the calibration plots, a linear correlation was calculated between the absorbance and the concentration. Beer's law limit, Sandell's sensitivity, and molar absorptivity are reported in Table 1.

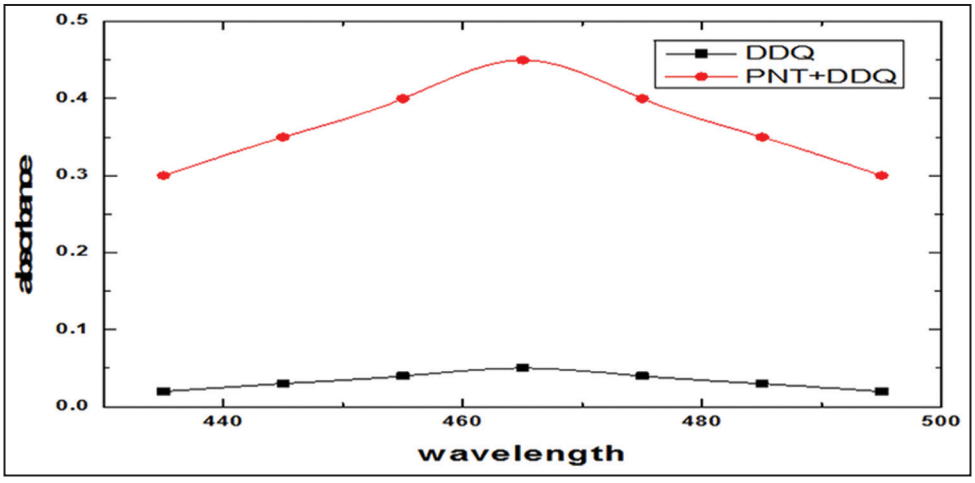

Figure 2: Absorption spectrum of pantoprazole sodiumwith 2,3-dichloro-5, 6-dicyano-p-benzoquinone (Method A).

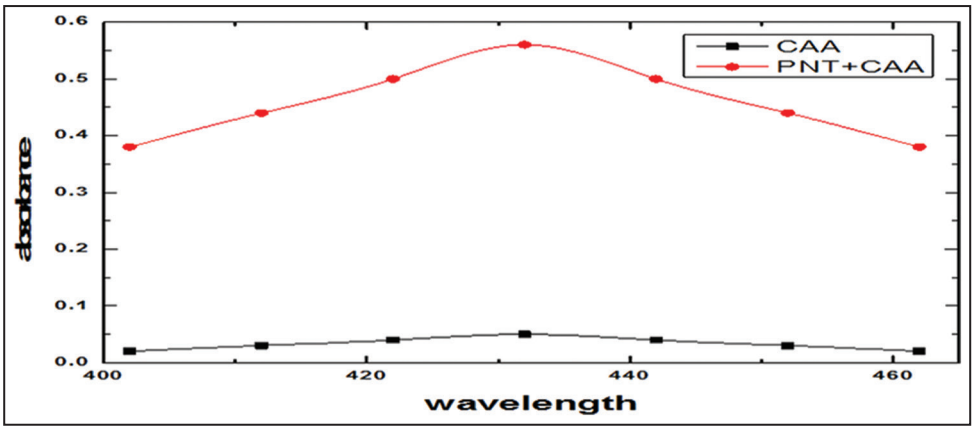

Figure 3: Absorption spectrum of pantoprazole sodiumwith chloranilic acid (Method B).

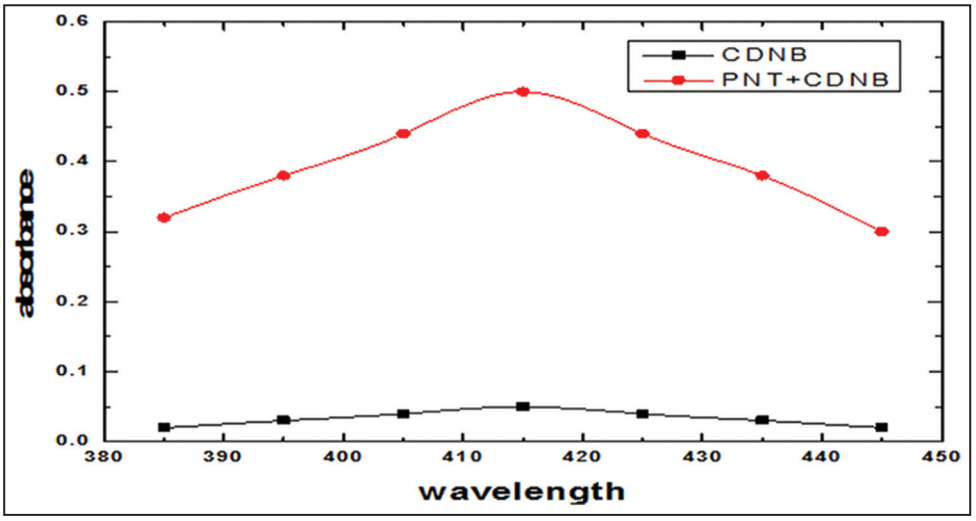

Figure 4: Absorption spectrum of pantoprazole sodiumwith 1-chloro-2,4-dinitrobenzene (Method C).

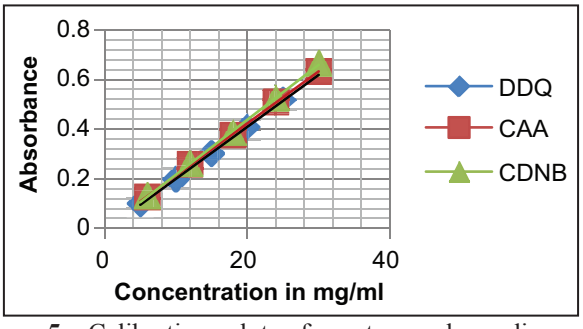

Figure 5: Calibration plot of pantoprazole sodium with analytical reagents.

3.2.2. Robustness and ruggedness

For the evaluation of robustness, some parameters such as concentration of drug and reagent, wavelength range, and shaking time were interchanged. The capacity remained unaffected by small changes in these parameters. Method ruggedness was expressed as relative standard deviation (RSD%) of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical difference between different analysts and instruments, suggesting that the developed methods were robust and rugged.

3.2.3. Accuracy, precision, and recovery

Accuracy of the proposed methods was proved by recovery studies (Tables 2 and 3). The recovery studies were carried out using the developed methods by adding known quantity of pure drug. The obtained results proved that the recovery values in drug and in dosages were within the acceptance limit.

Repeatability is determined using different concentrations and studied the variances in intraday and interday using proposed analytical methods and found the % RSD <1.0, which indicated that the developed methods were precise.

Table 1: Spectral characteristics of the drug with reagent.

Parameter	Method A	Method B	Method C
λ_{max} (nm)	465	432	415
Beer's law limit (µg/ml)	5–25	6–30	6–30
Molar absorbance ($L.mol^{-1}$ cm^{-1})	8574	7070	8137
Sandell's sensitivity ($\mu g.cm^{-2}$/0.001 AU)	0.0022	0.0021	0.0024
Correlation coefficient (r^2)	0.9989	0.9999	0.9997
Slope (m)	0.0213	0.0183	0.0215
Intercept (c)	0.0131	0.0012	0.0073
% RSD	0.2272	0.2010	0.2222
Color	Wine red	Yellowish green	Yellow
LOD	0.1403	0.1631	0.1394
LOQ	0.4674	0.5430	0.4641

LOD: Limit of detection, LOQ: Limit of quantification

Table 2: Evaluation of accuracy and precision of the proposed method in bulk form.

Method	Taken (mg/ml)	Intraday				Interday			
		*Found	Recovery %	±SD	% RSD	*Found	Recovery %	±SD	% RSD
A	2	1.99	99.25	0.010	0.53	1.99	99.25	0.010	0.53
	4	3.98	99.50	0.014	0.36	3.97	99.17	0.018	0.44
	6	5.97	99.47	0.017	0.29	5.97	99.42	0.019	0.31
B	2	1.97	98.58	0.019	0.98	1.98	99.08	0.012	0.59
	4	3.97	99.33	0.015	0.38	3.96	98.88	0.035	0.89
	6	5.96	99.33	0.021	0.35	5.92	98.67	0.055	0.94
C	2	1.98	99.17	0.010	0.52	1.98	99.17	0.014	0.69
	4	3.97	99.13	0.019	0.47	3.97	99.17	0.018	0.44
	6	5.96	99.33	0.021	0.35	5.97	99.47	0.028	0.47

*Average of six determinations, RSD: Relative standard deviation, SD: Standard deviation

Table 3: Evaluation of accuracy and precision of the proposed method in pharmaceutical dosage forms.

Method	Pharmaceutical formulation	Taken (mg/ml)	Intraday				Interday			
			*Found	Recovery %	±SD	% RSD	*Found	Recovery %	±SD	% RSD
A	Pantolup	4	3.94	98.58	0.033	0.83	3.97	99.13	0.024	0.61
	Pantosec	6	5.98	99.61	0.014	0.23	5.97	99.42	0.021	0.35
	Pantop	8	7.92	99.00	0.048	0.60	7.98	99.73	0.012	0.15
B	Pantolup	4	3.96	99.08	0.026	0.65	3.97	99.33	0.015	0.38
	Pantosec	6	5.97	99.56	0.018	0.29	5.96	99.28	0.023	0.38
	Pantop	8	7.95	99.38	0.027	0.34	7.88	98.54	0.096	1.22
C	Pantolup	4	3.97	99.13	0.021	0.52	3.94	98.38	0.035	0.89
	Pantosec	6	5.97	99.50	0.018	0.30	5.95	99.22	0.025	0.42
	Pantop	8	7.96	99.50	0.024	0.31	7.94	99.23	0.042	0.52

*Average of six determinations, RSD: Relative standard deviation, SD: Standard deviation

Table 4: Determination of pantoprazole in the presence of excipients.

Excipients	Amount taken (mg/ml)	*Found (mg/ml)	Recovery (%)	±SD	RSD%
Glucose	5	4.96	99.17	0.023	0.47
Sucrose	10	9.96	99.60	0.021	0.21
Lactose	15	14.90	99.30	0.057	0.38
Dextrose	10	9.97	99.65	0.019	0.19
Talc	15	14.91	99.38	0.046	0.31
Starch	20	19.78	98.89	0.185	0.94

*Average of six determinations, RSD: Relative standard deviation, SD: Standard deviation

3.2.4. Specificity and selectivity

To assess the specificity and selectivity of developed method, the effect excipients such as starch, lactose, glucose, sugar, and talc were studied. The results indicated (Table 4) that there was no effect of interference from the excipients on the developed methods.

3.2.5.LOD and LOQ

LOD and LOQ were calculated for the proposed methods using the formula.

$$LOD=3.3\ s/S \text{ and } LOQ=10\ s/S$$

Where, S = standard deviation of the response and S = slope of the calibration curve.

4. CONCLUSIONS

PNT was analyzed using the current developed methods in both bulk and pharmaceutical preparations. The linearity of the calibration standards of the drug by spectrophotometric method was good from the result of correlation coefficient. The overall recovery of the drug by the proposed method was good. Hence, the proposed analytical method is free from interface due to the excipients and other impurities present in the tablet forms. LOD, LOQ, molar absorptivity, and Sandal's sensitivity values indicated that the proposed analytical method, i.e.,, spectrophotometric method was more accurate, precise, and cost effective for the determination of PNT drug in bulk and pharmaceutical formulations.

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****Bibliographical Sketch***

Dr. S. Arul Antony currently working as Associate professor PG & Research Department of Chemistry, Presidency College, Chennai. He obtained his M.Sc degree in Chemistry from Madurai Kamaraj University, Tamil Nadu, in the year 1987. He was awarded M. Phil. in Chemistry from Madurai Kamaraj University, Tamil Nadu, in 1988 and Ph. D. in Chemistry from University of Madras, Tamil Nadu, in February 2002. He is a resource person for TRB and TNPSC. He is author for +1, +2 TN Text book Society chemistry books and also Ph.D. Viva voce Examiner for various universities. He published 96 research articles in reputed national and international journals.