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SHORT COMMUNICATION

SENSITIVE AND VALIDATED SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF PANTOPRAZOLE SODIUM IN PHARMACEUTICALS USING N-BROMOSUCCINIMIDE BASED ON REDOX AND COMPLEXATION REACTIONS

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ABSTRACT. Two simple, sensitive and rapid methods are described for the determination pantoprazole sodium sesqui hydrate (PNT) in bulk drug and in formulations using N-bromosuccinimide (NBS) as the oxidimetric reagent. The methods involve the addition of a known excess of NBS to PNT in HCl medium followed by estimation of the unreacted oxidant by two reaction schemes involving the use of iron(II) and thiocyanate (method A) or tiron (method B). In both methods, the absorbance is found to decrease linearly with PNT concentration. Beer's law is obeyed over the ranges 0.25-3.5 and 1-15 μg mL^{-1} for method A and method B, respectively. The calculated molar absorptivity values are 1.4×10^5 and 2.5×10^4 L $mol^{-1}cm^{-1}$ for method A and method B, respectively. The limit of detection (LOD) and quantification (LOQ) are also reported for both methods. The RSD values for intra-day and inter-day precision studies were less than 2.5 and 3.0 %, respectively. Both the methods were applied to the determination of PNT in dosage forms and the results were satisfactory, and were comparable with those obtained by the reference method. The accuracy and reliability of the proposed methods were further ascertained by recoveries studies, and the recoveries of the spiked drug ranged between 98.5 and 102.5 %.

KEY WORDS: Pantoprazole sodium, Determination, N-bromosuccinimide, Complexation reactions, Pharmaceuticals

INTRODUCTION

Pantoprazole sodium sesqui hydrate(PNT) is chemically known as sodium 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-p-methyl]sulfinyl]-1H-benzimidazole sesqui hydrate [1]. Pantoprazole inhibits H^+ K^+ AT Pase pump function thereby healing the acid related conditions. PNT is chemically more stable than omeprazole and lansoprazole in neutral to mildly acidic conditions, but under strongly acidic medium, active species is formed. PNT like omeprazole and lansoprazole also has a role in the eradication of Helicobacter Pylori [2].

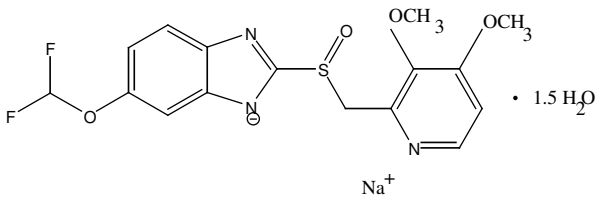

Figure 1. Structure of Pantoprazole sodium sesquihydrate.

The literature survey reveals that only few methods are available for the determination of PNT in dosage forms and include HPLC [3-5], HPTLC [6], UV spectrophotometry [7] and chemometry [8].

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Visible spectrophotometry, because of its simplicity, cost-effectiveness, sensitivity, selectivity, fair accuracy and precision, has remained competitive in an era of chromatographic techniques for pharmaceutical analysis. However, only three reports are found in the literature for the assay of PNT. In a method reported by Salama *et al*. [9] PNT was quantified by stability-indicating procedure through chelation with iron(III) in aqueous-ethanol medium to form an orange chelate peaking at 455 nm. The method is applicable over 30-300 μg mL^{-1} range. In a report by Moustafa *et al*. [10], two methods based on charge transfer complexation reaction using 2,3-dichloro-5,6-dicyano-1,4 benzo quinone(DDQ), a π acceptor and iodine as σ-acceptor with linearity ranges of 10-60 and 17.7-141.6 μg mL^{-1}, respectively, are described. The same article describes one more procedure based on ternary complex formation of PNT with eosin and copper(II) with a linear range of 4-26 μg mL^{-1}. Rahman *et al*. [11] have published determination of PNT using Fe(III) and potassium ferricyanide as reagents. But all the methods involve the use of organic solvents and the last method involves liquid-liquid extraction step. In addition, the methods are poorly sensitive.

The present investigation aims to develop sensitive and cost-effective methods for the determination of PNT in pure form and in dosage forms using the visible spectrophotometric technique.

The methods utilize N-bromosuccinimide, ammonium thiocyanate and Tiron as reagents. The methods have the advantages of speed and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for industrial quality control.

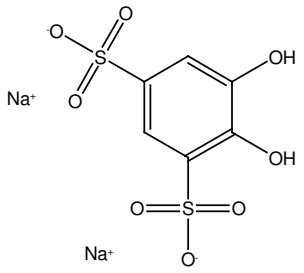

Figure 2. Structure of Tiron.

EXPERIMENTAL

Apparatus. A Systronics (Ahmedabad, India) model 106 digital spectrophotometer provided with 1-cm matched quartz cells was used for all absorbance measurements.

Reagents and standards. All chemicals were of analytical reagent grade and distilled water used to prepare solutions.

N-Bromosuccinimide (NBS, CAS No. 128-08-5). An approximately 0.01 M NBS solution was prepared by dissolving about 1.8 g of chemical (SRL Research Chemicals, India) in water with the aid of heat and diluted to one litre with water and standardized [12]. The solution was stored in an amber coloured bottle and was diluted appropriately to get 180, and 650 μg mL^{-1} NBS for use in method A and method B, respectively. The NBS solution was kept in a refrigerator when not in use.

Hydrochloric acid. Concentrated hydrochloric acid (S.D. Fine Chem, Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 5 M for method A and 1 M for use in method B.

Ferrous ammonium sulfate, FAS (400 and 1400 μg mL^{-1}). A stock solution equivalent to 0.01 M FAS was prepared by dissolving about 400 mg of the salt (S.D. Fine Chem, Mumbai, India) in 50 mL of water containing 1 mL of 5 M H_2SO_4, and diluted to 100 mL with water, and standardized [13] using pure potassium dichromate. The stock solution was then diluted appropriately with water to get 400 and 1400 μg mL^{-1} FAS for method A and method B, respectively.

Tiron (1.0 %) (sodium 4,5-dihydroxy benzene-1,3-disulfonate; CAS No: 149-45-1). About 1.0 g of Tiron (Loba Chemie, Mumbai, India) was dissolved in 100 mL of water.

Ammonium thiocyanate (3 M). Prepared by dissolving 23 g of the chemical (S.D. Fine Chem, Mumbai, India) in 100 mL water.

Sodium acetate trihydrate (1.5 M). Prepared by dissolving 24.5 g of the chemical (S.D. Fine Chem, Mumbai, India) in 100 mL water.

Buffer of pH 1.09. Prepared by mixing of 50 mL of 1 M sodium acetate and 70 mL of 1 M HCl and diluting to 250 mL with water.

Standard solution of Pantoprazole sodium. Pharmaceutical grade PNT, certified to be 99.8 % pure was procured from Cipla India Ltd, Mumbai, India, and was used as received. A stock standard containing 500 μg mL^{-1} PNT solution was prepared by dissolving accurately weighed 50 mg of pure drug in water and diluting to 100 mL in a calibrated flask with water. The solution was diluted stepwise to get working concentrations of 10 and 50 μg mL^{-1} PNT for method A and method B, respectively.

Procedures

Method A. Different aliquots (0.25-3.5 mL) of standard 10 μg mL^{-1} PNT solution were accurately measured and transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 4.0 mL by adding water. To each flask was added 1 mL each of 5 M HCl and NBS (180 μg mL^{-1}), the last being added using 10 mL microburette. The content was mixed and the flasks were let stand for 5 min. Then, 1 mL of 400 μg mL^{-1} FAS was added to each flask (micro burette), and again the flasks were let stand for 5 min followed by 1 mL of 3 M thiocyanate. The volume was diluted to the mark with water, mixed well and absorbance of each solution was measured at 470 nm against water blank.

Method B. Varying aliquots (0.2-3.0 mL) of standard PNT solution (50 μg mL^{-1}) were accurately measured into a series of 10 mL calibrated flasks by means of a 10 mL micro burette and the total volume was brought to 3 mL by adding water. The solution in each flask was acidified by adding 1 mL of 1 M HCl before adding 1 mL of NBS (650 μg mL^{-1}) by means of micro burette. The content was mixed well and allowed to stand for 15 min with occasional shaking. To each flask was then added 1 mL of 1400 μg mL^{-1} FAS, and after 5 min, 1 mL each of 1.5 M sodium acetate, buffer of pH 1.09 and 1 % Tiron were added and diluted to the mark with water. The absorbance of each solution was measured at 670 nm against water blank.

In either spectrophotometric method, a standard graph was prepared by plotting the decreasing absorbance values versus concentration of PNT. The concentration of the unknown was read from the standard graph or computed from the respective regression equation derived using the Beer's law data.

Procedure for tablets. A quantity of the finely ground tablet powder equivalent to 50 mg of PNT was accurately weighed into a 100 mL calibrated flask, 60 mL of water was added and shaken

for 20 min; the volume was finally diluted to the mark with water, mixed well and filtered using a Whatman No. 42 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (500 µg mL^{-1} PNT) was diluted appropriately to get 10 and 50 µg mL^{-1} concentrations for analysis by method A and method B, respectively.

RESULTS AND DISCUSSION

Method development. The methods are based on the oxidation of PNT by a known excess of NBS in hydrochloric acid medium, reducing the unreacted oxidant by iron(II) and subsequent determination of iron(III) by thiocyanate method [14] or by Tiron method of Vector Potter and Armstrong [15] and modified by Keshavayya *et al.* [16]. When a fixed concentration of NBS is made to react with increasing concentration of PNT, there occurs a concomitant fall in the former's concentration. When the unreacted NBS is reduced by a fixed concentration of iron(II), there will be a proportional decrease in the concentration of iron(III). This is observed as a proportional decrease in the absorbance of iron(III)-thiocyanate complex and iron(III)-Tiron complex on increasing the concentration of PNT, which formed the basis for the determination of drug.

Various parameters associated with the oxidation of PNT by NBS and subsequent reduction of the residual oxidant by iron(II) were optimized. Considering 5.5 µg mL^{-1} as the upper limit of iron(III) that could be determined by thiocyanate method, 18 µg mL^{-1} NBS was found to produce it from 38.7 µg mL^{-1} FAS. However, slightly higher concentration (40 µg mL^{-1}) FAS was used to ensure a quantitative reaction in method A. Similarly in method B, fixing 18 µg mL^{-1} as the upper limit of iron(III) that could be determined by tiron method, 140 µg mL^{-1} FAS and 65 µg mL^{-1} NBS were used. One mL of 5 M HCl in a total volume of 6 mL was used for the oxidation step and the same quantity of acid was used for the reduction of NBS and complexation of iron(III) with thiocyanate. However, the formation of iron(III)-Tiron complex(1:1) is pH dependent and 1 mL of 1 M HCl in a total volume of ~5 mL was used to cause oxidation of drug by NBS and the latter's reduction by iron(II), and later the pH was raised to ~1.0 by adding 1.0 mL of 1.5 M sodium acetate solution. To ensure an optimum pH for the complex formation reaction, 1 mL of buffer of pH 1.09 was also added. The oxidation of PNT was complete in 5-15 min but the reduction of NBS by iron(II) and subsequent complexation of iron(III) with thiocyanate or tiron was instantaneous.

Analytical parameters. A linear relation is found between absorbance and concentration in the ranges given in Table 1. In both methods, Beer's law is obeyed in the inverse manner. Correlation coefficients for the calibration data, sensitivity parameters such as molar absorptivity and Sandell sensitivity values, and the limits of detection and quantification calculated according to ICH guidelines [17] are also compiled in Table 1, and demonstrate the high sensitivity of the methods.

Table 1. Analytical parameters of the proposed methods.

Parameter	Method A	Method B
λ_{max}, nm	470	670
Beer's law limits, µg mL^{-1}	0.25 – 3.5	1 – 15
Molar absorptivity, L mol^{-1} cm^{-1}	1.4×10^5	2.5×10^4
Sandell sensitivity, µg cm^{-2}	0.0030	0.0171
Limit of detection, µg mL^{-1}	0.05	0.17
Limit of quantification, µg mL^{-1}	0.15	0.52
Correlation coefficient, (r)	-0.9974	-0.9968

Method validation

Evaluation of accuracy and precision. Intra-day and inter-day precisions were assessed from the results of seven replicate analyses on pure drug solution. The mean values and relative standard deviation (RSD) values for replicate analyses at three different concentration levels were calculated. To calculate the inter-day precision, analysis was performed over a period of five days preparing all solutions afresh each day. The accuracy of the methods was determined by calculating the percentage deviation observed in the analysis of pure drug solution and expressed as the relative error (RE). Table 2 summarizes the intra-day precision and accuracy data for the determination PNT by the proposed methods.

Table 2. Evaluation of accuracy and precision.

Method	PNT taken, μg mL^{-1}	PNT Found* μg mL^{-1}	Range, μg mL^{-1}	RE %	SD, μg mL^{-1}	SEM, μg mL^{-1}	RSD, %
Method A	0.50	0.49	0.06	2.00	0.008	0.003	1.63
	1.50	1.49	0.08	0.67	0.011	0.004	0.74
	2.50	2.48	0.10	0.80	0.021	0.008	0.85
Method B	4.0	3.95	0.07	1.25	0.066	0.025	1.67
	8.0	7.95	0.05	0.62	0.085	0.032	1.07
	12.0	11.92	0.05	0.67	0.102	0.039	0.86

RE: relative error; SD: standard deviation; SEM: standard error of mean; RSD: relative standard deviation; *Mean value of seven determinations.

Application to tablet analysis. Table 3 gives the results of assay and reveals that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically with those obtained by a literature method [7] by applying student's t-test for accuracy and F-test for precision. At the 95 % confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.77 and F = 6.39) suggesting that the proposed methods are as accurate and precise as the reference method.

Table 3. Results of determination of PNT in tablets and statistical comparison with the reference method.

Tablet brand name^{x}	Nominal amount, mg	% found^{y} ± SD		
		Literature method	Method A	Method B
PAN^{a}	20	99.8±0.56	99.2±1.01 t = 1.21 F = 3.25	100.2±1.02 t = 0.80 F = 3.32
PANTOCIP^{b}	40	102.3±0.95	101.8±1.02 t = 0.80 F = 1.15	101.2±1.05 t = 1.74 F = 1.22
PANTOP^{c}	40	101.2±0.62	100.5±1.32 t = 1.14 F = 4.53	101.9±1.33 t = 1.13 F = 4.60

^{x}Marketed by: a. Alkem Ltd.; b. Cipla Ltd.; c. Aristo Ltd. ^{y}Mean value of five determinations. Tabulated t-value at 95 % confidence level is 2.77. Tabulated F-value at 95 % confidence level is 6.39.

Accuracy and validity of the methods were further ascertained by performing recovery experiments via standard addition technique. To a fixed and known amount of PNT in tablet powder (pre analysed), pure drug was added at three levels and the total was found by the proposed methods. Each test was repeated three times. The recovery of pure PNT added to tablet powder ranged from 97.2 to 102.5 % (Table 4) indicating that commonly encountered tablet

excipients and additives such as talc, starch, lactose, sodium alginate, magnesium stearate, calcium gluconate and calcium dihydrogenorthophosphate did not interfere in the assay procedures.

Table 4. Results of recovery experiments by standard addition method.

Formul-ation studied	Method A				Method B			
	Amount of drug in tablet, μg mL^{-1}	Amount of pure drug added, μg mL^{-1}	Total found, μg mL^{-1}	Pure drug recover-ed[a], %	Amount of drug in tablet, μg mL^{-1}	Amount of pure drug added, μg mL^{-1}	Total found, μg mL^{-1}	Pure drug recovered[a] %
PAN 20	0.50	0.50	0.99	98.5	5.01	2.0	7.03	100.8
	0.50	1.50	2.02	101.6	5.01	4.0	9.06	101.3
	0.50	2.00	2.51	100.3	5.01	6.0	10.94	98.8
PANTO P 40	0.50	0.50	1.00	99.3	5.10	2.0	7.09	99.6
	0.50	1.50	1.96	97.2	5.10	4.0	9.17	101.8
	0.50	2.00	2.55	102.5	5.10	6.0	11.23	102.2

[a]Mean value of three determinations.

Table 5. Comparison of the proposed method with the reported vis-spectrophotometric method

S.No.	Reagent[a]	λ_{max} nm	Linear range, μg mL^{-1}	∈ Lmol^{-1}cm^{-1}	Remarks	Ref
1.	Iron (III)	455	30-300	-	Wide linear range but less sensitive	9
2.	DDQ	455	10-60	-	Less sensitive	10
	Iodine	359	17.7 - 141.6	-	Measured at shorter wavelength; less sensitive	
	Eosin and copper(II)	549	4 - 26	-		
3.	Fe(III)-potassium ferricyanide	725	5 - 90	-	Reaction product is stable only for 8 min. Less sensitive.	11
4.	NBS/thiocyanate	470	0.25 - 3.5	1.4×10^5	Highly sensitive; stable coloured species	Present methods
	NBS/Tiron	670	1-15	2.5×10^4	Wide linear dynamic range	

[a] DDQ: 2,3-dichloro-5,6-dicyano-1,4 benzo quinone; NBS: N-bromosuccinimide.

CONCLUSION

Two new methods have been developed and appropriately validated for the assay of PNT. Both spectrophotometric methods are based on well-characterised complexation reactions and the thiocyanate method is the most sensitive in terms of wide linear dynamic concentration range and molar absorptivity. An additional advantage of the methods is that the absorbance is measured at longer wavelengths where the interference from excipients is far less than at shorter wavelengths. The stability of the coloured species and sensitivity of the reactions used are not

critically dependent on any experimental variable unlike many reported methods. These advantages coupled with a fairly degree of accuracy and precision qualify the methods for use in quality control laboratories.

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