Spectrophotometric Determination of Pantoprazole Sodium in Pharmaceuticals Using N-Bromosuccinimide, Methyl Orange and Indigo Carmine as Reagents

Basavaiah, K.*+, Anil kumar, U.R., Kalsang tharpa
Department of Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, INDIA

Vinay, K.B.
Process Analytical Laboratory, Advinus Therapeutics, Peenya, II Phase, Bangalore-58, INDIA

ABSTRACT: *Two sensitive spectrophotometric methods are presented for the assay of pantoprazole sodium sesqui hydrate (PNT) in bulk drug and in formulations using N-bromosuccinimide (NBS) and two dyes, methyl orange and indigo carmine, as reagents. The methods involve the addition of a known excess of NBS to PNT in acid medium, followed by determination of unreacted oxidant by reacting with a fixed amount of either methyl orange and measuring the absorbance at 520 nm (method A) or indigo carmine and measuring the absorbance at 610 nm (method B). In both methods, the amount of NBS reacted corresponds to the amount of PNT and the measured absorbance is found to increase linearly with the concentration of PNT which is corroborated by the correlation coefficients of 0.9959 and 0.9985 for method A and method B, respectively. The systems obey Beer's law for 0.1 - 2.0 $\mu g\ mL^{-1}$ and 0.5 - 6.0 $\mu g\ mL^{-1}$ for method A and method B, respectively. The limits of detection and quantification are also reported for both methods. Intra-day and inter-day precision, and accuracy of the methods have been evaluated. The methods were successfully applied to the assay of PNT in tablet preparations and the results were statistically compared with those of the reference method by applying Student's t-test and F-test. No interference was observed from the common tablet excipients. The accuracy of the methods was further ascertained by performing recovery studies via standard-addition method.*

KEY WORDS: *Pantoprazole sodium, Assay, Spectrophotometry, N-bromosuccinimide, 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

** To whom correspondence should be addressed.*
+ E-mail: basavaiahk@yahoo.co.in
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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].

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].

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. However, only two visible spectrophotometric methods 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 *Monstafa et al*. [10] two methods based on charge transfer complexation reaction using 2,3-dichloro-5,6-dicyano-1,4 benzo quinine (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}. But all the methods involve the use of organic solvents and the last method involves liquid-liquid extraction step.

The present investigation aims to develop simple, 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 NBS, methyl orange and indigo carmine as reagents. The proposed 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.

EXPERIMENTAL

Apparatus

A Systronics model 106 digital spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements.

Reagents and Standards

All chemicals used were of analytical purity grade and all solutions were prepared in distilled water.

N-bromosuccinimide (NBS): 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 [11]. The solution was kept in an amber coloured bottle and was diluted appropriately to get 80 and 340 μg mL^{-1} NBS for use in method A and method B, respectively. The NBS solution was stored in a refrigerator when not in use.

Hydrochloric acid (5 M): Concentrated hydrochloric acid (S.D. Fine Chem., Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 5 M acid.

Methyl orange (50 μg mL^{-1}): A 500 μg mL^{-1} dye solution was first prepared by dissolving accurately weighed 58.8 mg of dye (S.D. Fine Chem., Mumbai, India, 85 % dye content) in water and diluting to 100 mL in a calibrated flask and filtered using glass wool. It was further diluted to obtain a working concentration of 50 μg mL^{-1}.

Indigo carmine (200 μg mL^{-1}): A 1000 μg mL^{-1} stock standard solution was first prepared by dissolving accurately weighed 112 mg of dye (S.D. Fine Chem., Mumbai, India, 90 % dye content) in water and diluting to volume in a 100 mL calibrated flask. The solution was then diluted 5-fold to get the working concentration of 200 μg mL^{-1}.

Methanol-ammonia 4.0 % v/v: Methanol (S.D. Fine Chem., Mumbai) and ammonia (S.D. Fine Chem., Mumbai) were prepared as per the reference method [7].

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 solution 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 5 and 20 μg mL^{-1} PNT for method A and method B, respectively.

Procedures

Spectrophotometry using methyl orange (method A). Different aliquots (0.2 - 4.0 mL) of a standard 5 μg mL^{-1} PNT solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 4 mL by adding adequate quantity of water. To each flask were added 1 mL each of 5 M

HCl and 1 mL of NBS solution (80 μg mL^{-1}), the last being measured accurately. The flasks were stoppered, content mixed and let stand for 15 min with occasional shaking. Finally, 1 mL of 50 μg mL^{-1} methyl orange solution was added (accurately measured) and the volume was diluted to the mark with water and mixed well. The absorbance of each solution was measured at 520 nm against a reagent blank after 10 min.

Spectrophotometry using indigo carmine (method B). Varying aliquots (0.25-3.0 mL) of a standard 20 μg mL^{-1} PNT solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 3 mL by adding water. To each flask were added 1 mL each of 5 M hydrochloric acid and 340 μg mL^{-1} of NBS solution (by means of a micro burette). The content was mixed well and the flasks were kept aside for 10 min with intermittent shaking. Finally, 1 mL of 200 μg mL^{-1} indigo carmine solution was added to each flask, the volume was diluted to the mark with water, mixed well and absorbance measured against a reagent blank at 610 nm after 10 min. In either method, a standard graph was prepared by plotting the absorbance versus the concentration of PNT . The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using Beers' 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 5 and 20 μg mL^{-1} concentrations for analysis by method A and method B, respectively. For comparison [8] the same tablet powder was extracted with 0.1 M NaOH, and after appropriate dilution to 10 μg mL^{-1} with 0.1 M NaOH, the absorbance was measured at 295 nm.

RESULTS AND DISCUSSION

Method development

The proposed spectrophotometric methods are indirect and are based on the determination of the residual NBS after allowing the reaction between PNT and a measured amount of NBS to be complete. The residual NBS was determined by reacting it with a fixed amount of either methyl orange or indigo carmine dye. The methods make use of bleaching action of NBS on the dyes, the decolouration being caused by the oxidative destruction of the dyes.

PNT when added in increasing concentrations to a fixed concentration of NBS, consumes the latter proportionally and there occurs a concomitant fall in the concentration of NBS. When a fixed concentration of dye is added to decreasing concentrations of NBS, a concomitant increase in the concentration of dye results. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentration of PNT

Preliminary experiments were performed to fix the upper concentrations of the dyes that could be determined spectrophotometrically, and these were found to be 5 and 20 μg mL^{-1} for methyl orange and indigo carmine respectively. A NBS concentration of 8.0 μg mL^{-1} was found to bleach the red colour due to 5 μg mL^{-1} methyl orange whereas 34.0 μg mL^{-1} NBS was required to destroy the blue colour due to 20 μg mL^{-1} indigocarmine. For both steps, i.e., the reaction between PNT and NBS, and the determination of the latter by reacting with the dye, HCl medium was found to be ideally suited. The absorbance of the dye was not affected in 0.5 to 1.5 M HCl concentrations. However, 1 mL of 5 M HCl was selected for oxidation of drug in both methods and the same quantity of acid was maintained for bleaching step. The oxidation reaction was found to be complete in 15 min for method A and 10 min for method B and contact times upto 30 min had no effect on the absorbance of dyes. The absorbance of either dyes solution even in the presence of reaction product was found to be stable for several days.

Analytical data

A linear correlation was found between absorbance at λ_{max} and concentration of PNT in the ranges given in table 1. Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in table 1.

Table 1: Analytical and regression parameters of spectrophotometric methods.

Parameter	Method A	Method B
λ_{max}, nm	520	610
Beer's law limits, μg mL^{-1}	0.1 - 2.0	0.5 - 6.0
Sandell sensitivity, μg cm^{-2}	0.003	0.01
Limit of detection, μg mL^{-1}	0.02	0.06
Limit of quantification, μg mL^{-1}	0.07	0.19
Regression equation, Y* Intercept (a) Slope (b)	 0.015 0.3460	 -0.003 0.1046
Correlation coefficient, (r)	0.9959	0.9985
S_a	0.02	0.03
S_b	0.02	0.02

**Y = a+bX, where Y is the absorbance and X concentration in μg mL^{-1}.*
S_a=Standard deviation of intercept, S_b=Standard deviation of slope.

Table 2: Evaluation of accuracy and precision.

Method	PNT taken, μg mL^{-1}	PNT Found*, μg mL^{-1}	Range, μg mL^{-1}	RE, %	RSD, %
Method A	0.5 1.0 1.5	0.49 0.99 1.48	0.08 0.06 0.09	2.00 1.00 1.33	1.63 0.71 0.68
Method B	1.0 3.0 5.0	0.99 2.95 4.94	0.07 0.10 0.05	1.00 1.67 1.20	1.52 0.54 0.91

RE: Relative error; RSD: Relative standard deviation
** Mean value of seven determinations.*

The optical characteristics such as Beer's law limits and Sandell sensitivity values for both methods are given in table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [12] are also presented in table 1 and reveal the very high sensitivity of the methods.

Method Validation

To evaluate the accuracy and intra-day precision of the methods, pure drug solution at three different concentration levels was analysed, each determination being repeated seven times. The relative error (%) and relative standard deviation (%) were = 2 and indicate high accuracy and precision of the methods (table 2). For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed in which standard drug solution at three different levels was determined each day for five days with all solutions being prepared afresh each day. The day-to-day relative standard deviation values were less than 3.0 % and represent the best appraisal of repeatability of the proposed methods.

Application

In order to check the validity of the proposed methods, PNT was determined in some commercial tablets. Table 3 gives the results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically by a Student's t- test for accuracy and variance ratio F- test for precision with those of the literature method [7] at 95 % confidence level. The calculated t- and F-values (table 3) did not exceed the tabulated values (t=2.77, F=6.39) and indicate that there was no significant difference between

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

Tablet brand name[#]	Nominal amount, mg	% found* ± SD		
		Literature method[7]	Method A	Method B
PAN[a]	20	101.5±0.62	100.8±1.29 t=1.16 F=4.33	100.2±1.18 t=2.28 F=3.62
PANTOCIP[b]	40	98.2±1.06	99.4±1.48 t=1.49 F=1.95	99.5±1.92 t=1.38 F=3.28
PANTOP[c]	40	99.2±0.62	99.8±1.32 t=0.98 F=4.53	98.7±1.33 t=0.81 F=4.60

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

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

Formulation studied	Method A				Method B			
	PNT in tablet, μg mL^{-1}	PNTadded, μg mL^{-1}	Total found, μg mL^{-1}	PNT recovered*, %	PNT in tablet, μg mL^{-1}	PNT added, μg mL^{-1}	Total found, μg mL^{-1}	PNT recovered* %
PAN 20	0.50	0.25	0.74	97.8	2.00	1	3.01	101.3
	0.50	0.75	1.27	102.3	2.00	2	4.04	102.2
	0.50	1.25	1.75	100.3	2.00	3	4.96	98.8
PANTOP 40	0.50	0.25	0.75	99.3	1.97	1	2.97	99.6
	0.50	0.75	1.23	97.8	1.97	2	4.03	103.2
	0.50	1.25	1.79	103.2	1.97	3	5.01	101.3

**Mean value of three determinations.*

the proposed methods and the literature method in respect to accuracy and precision.

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analysed tablet powder was spiked with pure PNT at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that co-formulated substances such as talc, starch, gelatin, gum acacia, calcium carbonate, calcium gluconate, calcium dihydrogen orthophosphate, sodium alginate and magnesium stearate did not interfere in the determination. However, ascorbic acid was found to interfere strongly in the assay. Drugs such as omeprazole and lansoprazole were also found to interfere. The results of recovery study are compiled in table 4.

CONCLUSIONS

Two useful micro methods for the determination of PNT have been developed and validated. The methods are simple and rapid taking not more than 20 min for analysis. Besides, they are more sensitive than the reported visible spectrophotometric methods [9,10] and also the chemometric methods reported by *Wahbi et al.* [8]. Whereas the already reported methods [8] have a working range of 0.5 -3.5 μg mL^{-1} with detection limit of 0.035 μg mL^{-1}, the present methods (method A) has a detection limit of 0.02 μg mL^{-1} and has a wide linear dynamic range of 0.1-2.0 μg mL^{-1}. Precision wise the present methods (RSD, 0.54 -1.63 %) is comparable to that of the reported methods [8] (RSD, 0.5 %). The proposed methods rely on the use of simple and cheap chemicals and techniques but provide a sensitivity

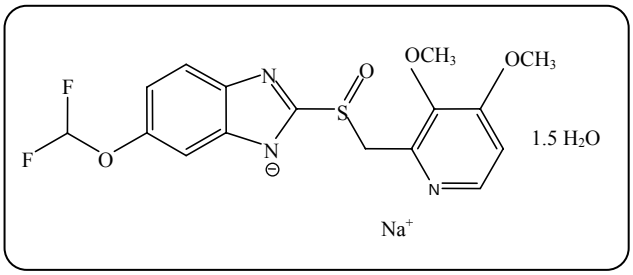

Fig. 1: Structure of pantoprazole sodium sesqui hydrate.

comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets as a part of industrial quality control.

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