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VALIDATED SPECTROPHOTOMETRIC DETERMINATION OF PANTOPRAZOLE SODIUM IN TABLETS USING 2, 4-DINITROFLUOROBENZENE THROUGH NUCLEOPHILIC SUBSTITUTION REACTION

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ABSTRACT

An accurate, simple and sensitive spectrophotometric method was developed for determination of pantoprazole sodium in bulk and pharmaceutical dosage form. The method was based on the reaction of the drug with 2, 4- dinitrofluorobenzene through nucleophilic substitution reaction, producing yellow colored product measured at λ_{max} 431 nm. Beer's law was obeyed in the concentration range from (10- 50 µg ml^{-1}) with molar absorpitivity 6.823 x 10^3 $Lmol^{-1}cm^{-1}$.

KEYWORDS

Pantoprazole sodium, 2, 4- Dinitrofluorobenzene and Nucleophilic substitution reaction.

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INTRODUCTION

Pantoprazole sodium is chemically known as 5-(Difluoromethoxy)-2-[[(3,4- dimethoxy- 2- pyridyl) methyl] sulfinyl] benzimidazole, sodium salt, sesquihydrate. Pantoprazole sodium is officially listed in B.P.2011 [1] and U.S.P.XXXII [2]. This drug is a proton pump inhibitor, used as an anti-ulcerative agent by inhibiting the gastric acid secretion. It is immensely used for the cure of erosion and ulceration of esophagus caused by a gastroesophagal reflux disease [3].

Literature survey revealed that there are methods based on electrochemical[4,5], UV spectrophotometric[6-8] and HPLC [9-13] methods were developed for the determination of pantoprazole sodium in bulk and in dosage forms.

This study reports simple, sensitive, economical and accurate spectrophotometric method for the quantitative estimation of pantoprazole sodium in its pure form and pharmaceutical formulation. The results of the analysis were validated by statistical analysis and recovery studies. Common additives used as excipients in pharmaceutical formulation do not interfere in the determination of the cited drug.

MATERIALS AND METHODS

Apparatus

Labomed Spectro UV-VIS Double Beam (UVD-2950) Spectrophotometer with matched 1 cm quartz cells connected to windows compatible computer using UV Win 5 Software v5.0.5.

Digital pH-meter (Cosort P400) for pH adjustment.

Thermostatically controlled (Wisebath) water bath.

Materials

Pantoprazole sodium (Sigma Pharmaceutical Industries, Quesna City, Egypt).

Pharmaceutical preparation (tablets)

The following pharmaceutical tablets were analyzed: Pantoloc tablets (MUP; Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt) labeled to contain 20 mg pantoprazole sodium sesquihydrate per tablet.

Reagents

2,4-Dinitrofluorobenzene (DNFB)

Sodium hydroxide, boric acid, hydrochloric acid and methanol

Preparation of standard drug solution

Standard solution of pantoprazole sodium (200 µg/ml) was prepared by dissolving 20 mg of the pure drug in 100 ml distilled water in a volumetric flask.

General Procedures for the Determination of Pure Drug through Nucleophilic Substitution Reaction with DNFB

Accurately measured aliquots of standard solution containing (100-500 µg/ml) of pantoprazole sodium were transferred into a series of 10-ml volumetric flasks. To each flask, 2 ml of borate buffer (pH 10.5) and 0.4 ml of DNFB were added and mixed well. The solutions were heated at 65^{o}C for 20 minutes in thermostatically controlled water bath. The solutions were cooled and acidified with 1 ml of 0.1 M HCl. The reaction products were diluted to 10 ml with methanol. The absorbance was measured at λ_{max} 431nm against the blank.

Pharmaceutical preparation (tablets)

Twenty tablets of Pantoloc were weighed and finely powdered. An accurately weighed amount of the powder equivalent to the concentration of the pure drug in the method was extracted with distilled water three times, the filtrate was collected and transferred to 100 ml volumetric flask and completed to the mark with distilled water. Aliquots from this solution equivalent to that in authentic sample were used for the application of the proposed method applying standard addition technique.

RESULTS AND DISCUSSION

2, 4-Dinitrofluorobenzene (DNFB; Sanger's reagent) is an active aryl halide reacts with primary and secondary amines, phenols, thiols and imidazoles forming stable condensation coloured products [14]. DNFB has been utilized as a chromogen and a fluorogen for the spectrophotometric[15-18] and spectrofluorometric [19, 20] determinations of many compounds of pharmaceutical interest. Also some drugs have been derivatized with DNFB before estimation with HPLC [19, 21].

This paper shows the possibility of the reaction of pantoprazole sodium with DNFB in alkaline medium through nucleophilic substitution reaction producing yellow colored product showing maximum absorption at λ_{max} 431 nm, Figure No. 2. The suggested mechanism is explained below.

Study of the Experimental Parameters

The different experimental parameters affecting the development of the reaction products were carefully studied and optimized.

Effects of pH and volume of buffer

Different buffers (borate, phosphate, hexamine) were studied. Borate buffer was found to be the most

suitable buffer for both methods, 2 ml of borate buffer (pH 10.5) gave best result. Figure No.3 and 4.

Pantoprazole sodium

Effect of the reagent concentration

The concentrations of the reagent were investigated; 0.4 ml of 0.3% v/v of DNFB solution was optimum reagent concentration. Figure No. 5.

Effects of temperature and heating time

It was found that the reaction proceeds very slowly at room temperature. A gradual increase in the temperature produced a significant increase in the absorbance. The reaction proceeded at 65^{o}C for 20 minutes. Figure No. 6 and 7.

Pantoprazole sodium

Effect of diluting solvent

Different organic solvents were tested as methanol, acetone, ethanol and isopropanol, methanol gives reasonable absorption intensity with maximum product stability.

Stoichiometry of the Reaction

The molar ratio of the reagent and the cited drug in the reaction mixture was studied according to Job's method of continuous variation [22]. The molar ratio was found to be 1:1 (drug: reagent). Figure No. 8

Validation of the Proposed Method

The validity of the proposed method was tested regarding linearity, range, limits of detection, limits of quantification, accuracy, precision, robustness and specificity according to ICH recommendation [23].

Linearity and Range

The calibration graphs obtained by plotting the values of the absorbance versus the final concentrations were found to be rectilinear over the concentration ranges cited in the table No. 1.

Limits of Detection and Limits of Quantification

Limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be detected according to the following equation: LOD = 3.3S/K

Limit of quantification (LOQ) was determined also by establishing the lowest concentration that can be detected according to the following equation:

$$LOQ = 10S/K$$

Where S is the standard deviation of the three replicate determination values under the same conditions as for the sample analysis in the absence of analyte and K is the sensitivity, namely, the slope of calibration graph. The results are summarized in Table No. 2.

Accuracy and Interference Liabilities

The accuracy of the proposed methods was checked by performing recovery experiments through standard addition technique. The results are shown in table No. 3. Before proceeding with the analysis of pantoprazole sodium in tablets, interference liabilities were carried out to explore the effect of common excipients that might be added during the tablet formulation. Samples were prepared by mixing known amount of pantoprazole sodium with 50 mg talc, 50 mg starch, 50 mg calcium stearate, and 50 mg sodium carbonate. These laboratory prepared samples were analyzed by the proposed methods. No interference from the excipients was observed.

Intraday precision was evaluated by calculating standard deviation (SD) of five replicate determinations using the same solution containing pure drug. The intraday SD values revealed the precision of the methods (values vary from 0.268 to 0.996). **For inter - day reproducibility**, a series was run, in which the standard drug solutions were analyzed each for five days. The inter-day SD values were in the range of 0.379 – 0.861.

Robustness

Robustness of the method was examined by small changes in the method variables such as change reagent concentration (±0.2 ml), pH of buffer solution (±0.2) and reaction time (±5 minutes). The minor changes that may take place during the experiment didn't affect the absorbance of the reaction products.

Analysis of Tablets

The proposed methods were applied to the analysis of the drug in tablets and the results were statistically compared with reference method [(24)] by calculating Student's *t*- and F-values. The evaluated *t*- and F-values were less than the tabulated values at the 95% confidence level. The results are listed in Table No. 4.

Table No.1: Analytical parameters for the reaction of pantoprazole sodium with DNFB reagent

S. No	Parameters		Pantoprazole sodium
1	λ_{max}, nm		431
2	Borate buffer ;pH		10.5
3	Borate buffer volume (ml)		2
4	2,4-DNFB Conc., % *v/v*		0.3
5	2,4-DNFB volume (ml)		0.4
6	Temperature (^oC)		65
7	Time (minutes)		20
8	Diluting solvent		Methanol
9	Beer's law limits ($\mu g\ ml^{-1}$)		10-50
10	Regression equation*	Slope (b)	0.016
11		Intercept (a)	0.005
12	Correlation coefficient		0.999

*A = a + bC where A is absorbance, C is the concentration of the drug in $\mu g\ ml^{-1}$

Table No.2: Statistical data for the reaction of pantoprazole sodium with DNFB reagent:

Parameters	Conc. taken, µg/ml	Conc. found, µg/ml	Recovery* %
	10	10.06	100.62
	20	20.06	100.31
	30	29.81	99.37
	40	39.75	99.37
	50	50.31	100.62
Mean recovery*			100.06

N		5
SD		0.640
RSD		0.640
SE		0.286
Variance		0.410
LOD, µg ml^{-1}		1.85
LOQ, µg ml^{-1}		5.53
Sandell's sensitivity (µg ml^{-1} per 0.001 A)		2.7 x 10^{-2}
Apparent Molar absorbitivity** L Mol^{-1} cm^{-1}		6.823 x 10^{3}

*Mean of three different experiments. **Calculated in the basis of molecular weight of the drug.

Table No. 3: Application of standard addition technique for the determination of pantoprazole sodium in tablets

Statistics	**Conc. added form pure drug (µg/ml)**	**Conc. taken from Pantoloc® (µg/ml)**	**Recovery*%**
	10	0	100
	10	10	99.06
	10	20	99.16
	10	30	99.37
	10	40	99.01
Mean recovery*			99.15
N			5
S.D.			0.403

R.S.D.			0.406
V			0.025
S.E.			0.201

*Mean of three different experiments.

Table No. 4: Statistical data for the determination of pharmaceutical tablets of pantoprazole sodium through the proposed method compared with the reference method

Statistics	**Reference method [24]**	**Proposed method**
Mean recovery*± SD	99.82 ± 0.605	99.15 ± 0.403
N	5	5
Variance	0.367	0.026
S.E.	0.272	0.201
*t-test***		2.06
F-test**		2.25

* Average of three experiments. **Theoretical *t* and F values are 2.306and 5.05, respectively at p=0.05.

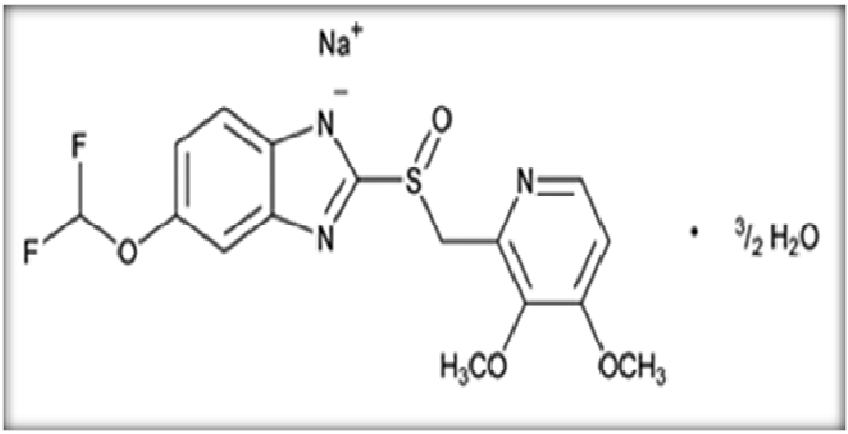
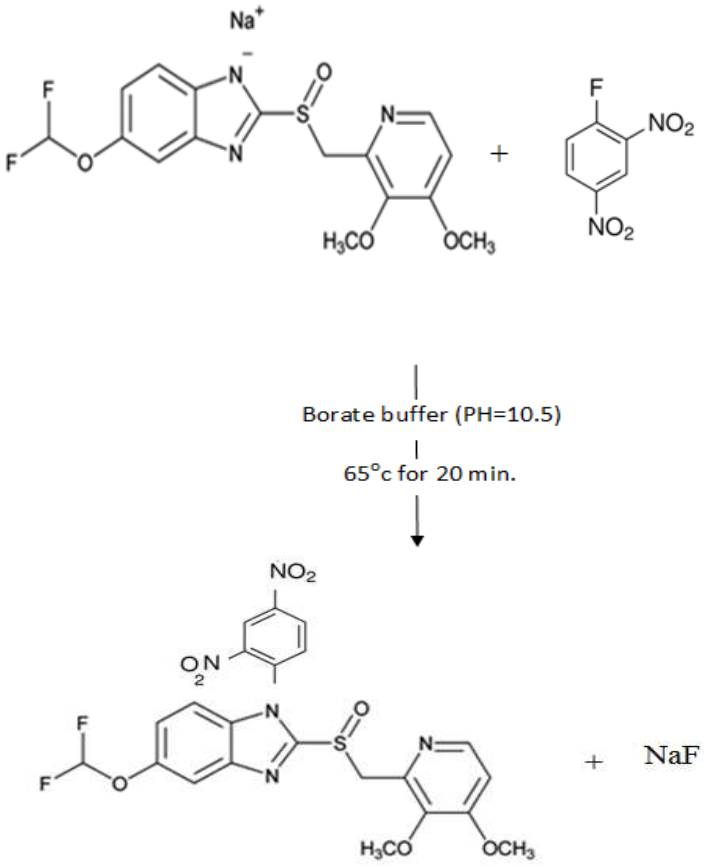


Figure No.1: Structure of Pantoprazole



Scheme: Proposed reaction mechanism between pantoprazole sodium and DNFB

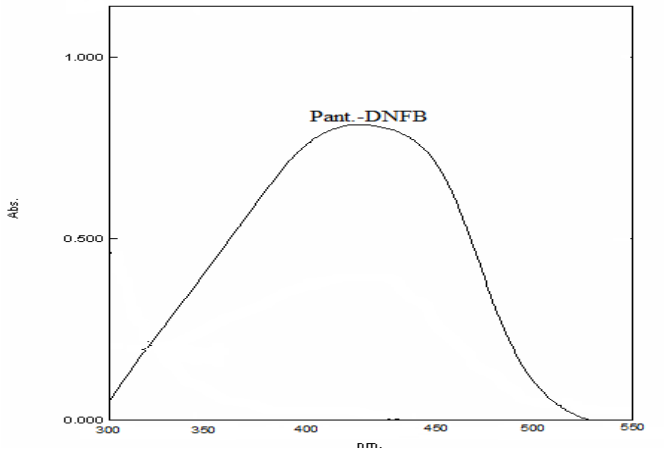


Figure No.2: Absorbance spectrum for the reaction between DNFB and 50 µg ml^{-1} pantoprazole sodium at λ_{max} 431 nm

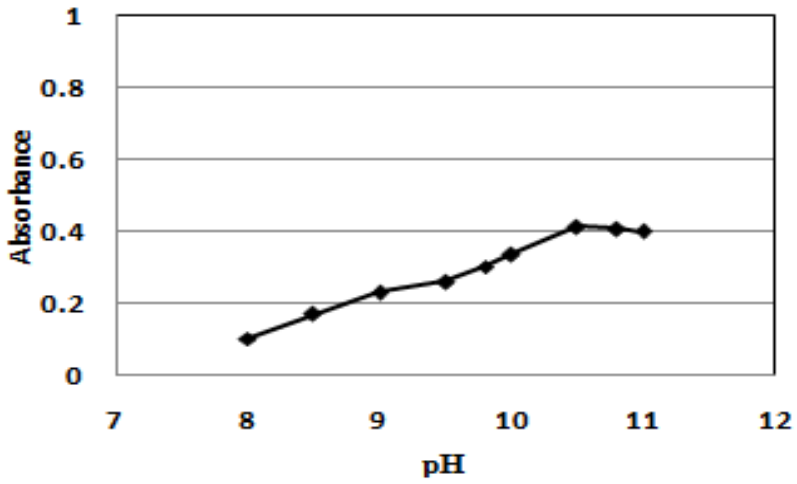


Figure No.3: Effect of buffer pH on the reaction of DNFB with 20 µg ml^{-1} pantoprazole sodium

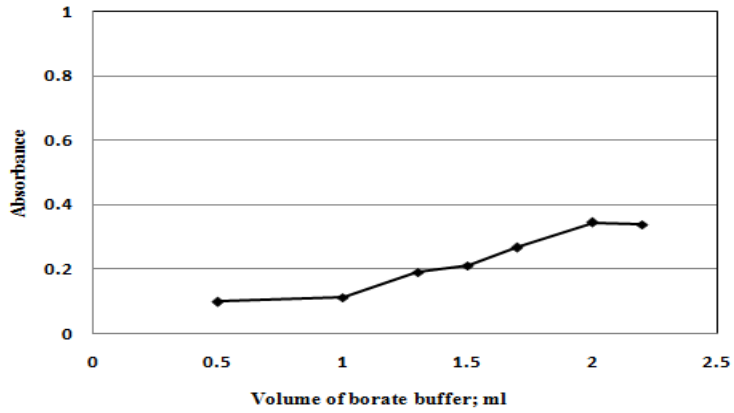


Figure No.4: Effect of buffer volume on the reaction of DNFB with 20 µg ml^{-1} pantoprazole sodium

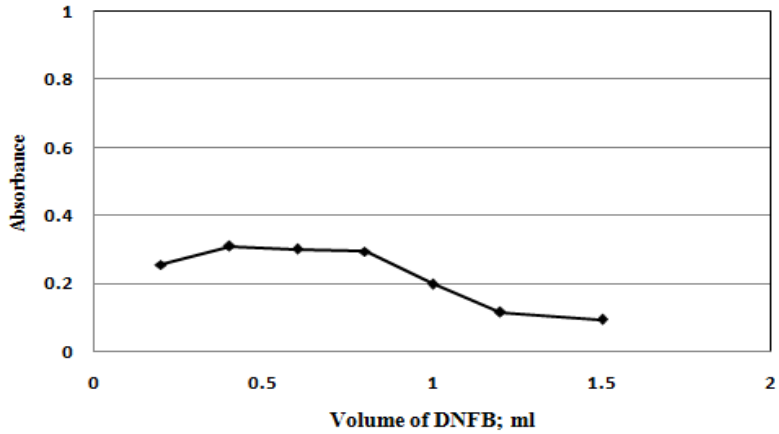


Figure No.5: Effect of volume of DNFB on the reaction with 20 µg ml^{-1} pantoprazole sodium

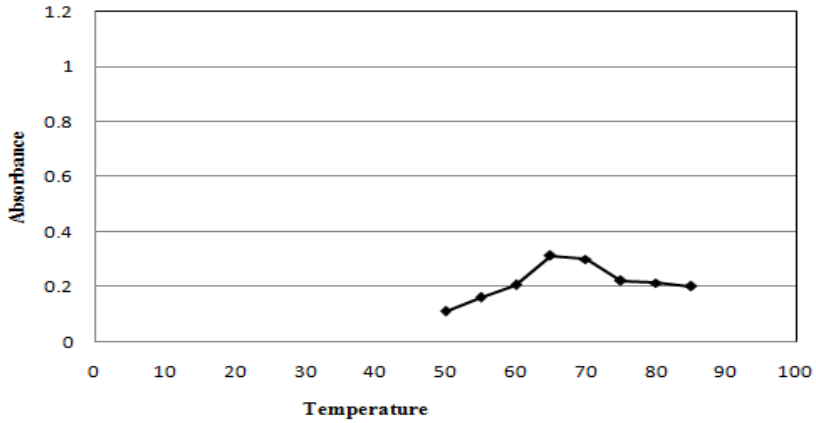


Figure No.6: Effect of temperature (^{o}C) on the reaction of DNFB with 20 µg ml^{-1} pantoprazole sodium

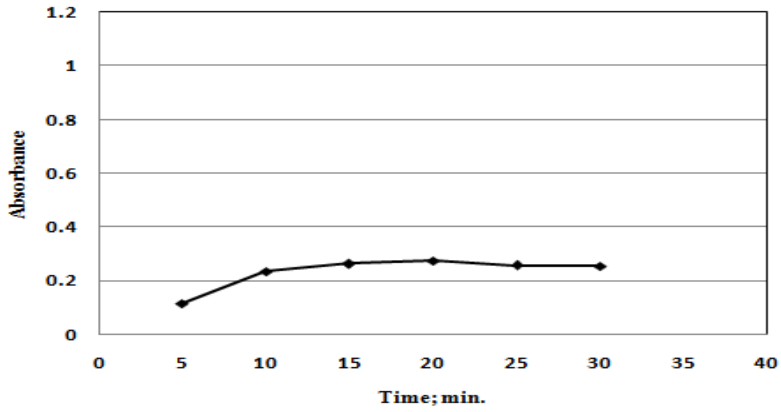


Figure No.7: Effect of heating time (min.) on the reaction of DNFB with 20 µg ml^{-1} pantoprazole sodium

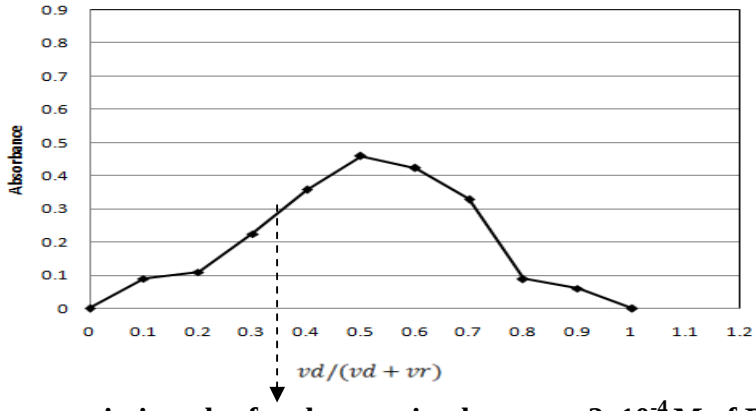


Figure No.8: Continuous variation plot for the reaction between: 2x10^{-4} M of DNFB and 2x10^{-4} M of pantoprazole sodium.

CONCLUSION

The proposed spectrophotometric method provided simple, sensitive, specific and inexpensive analytical procedures for determination of the cited drug either in pure form or in its pharmaceutical formulation without interference from common excipients. The satisfactory sensitivity and reproducibility as well as the convenience and simplicity, make the proposed method suitable for routine analysis in quality control laboratories.

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