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RESEARCH ARTICLES

Determination of Pantoprazole in Tablet Dosage Forms by Two Different Spectrophotometric Methods

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Determination of pantoprazole in tablet dosage forms by two different spectrophotometric methods

Summary : *Pantoprazole is a gastric hydrogen-potassium adenosine triphosphatase (H^+/K^+ATPase) inhibitor. In this study spectrophotometric methods have been developed for the determination of pantoprazole in its tablet dosage forms. Pantoprazole in methanol - water (1:9, v/v) solution was determined at the wavelength ranges of 200-350 nm by the two spectrophotometric methods. Analysis was performed at 295 nm and 303 nm for UV and first derivative UV spectrophotometric methods, respectively. Linearity ranges were found as 2.50 - 80.00 µg mL^{-1} for UV spectrophotometric method and 0.5-70 µg mL^{-1} for first derivative UV spectrophotometric method. Limits of quantitation were determined as 2.31 µg mL^{-1} and 0.5 µg mL^{-1} and limits of detection as 0.69 µg mL^{-1} and 0.15 µg mL^{-1} for UV and first derivative UV spectrophotometric methods, respectively. Developed methods were validated and showed good precision and accuracy. The proposed methods were successfully applied to the assay of pantoprazole in pure and tablet dosage form. No interference was found from tablet excipients at the selected wavelengths and assay conditions. The data were compared with those obtained from the spectrophotometric method given in the literature and no difference was found statistically.*

Keywords: *Pantoprazole, UV Spectrophotometry, First Derivative UV Spectrophotometry, Tablet Dosage Form.*

Tablet Dozaj Formlarındaki Pantoprazol'ün İki Farklı Spektrofotometrik Yöntem ile Tayini

Özet: *Pantoprazol bir gastrik hidrojen-potasyum adenozin trifosfat (H^+/ K^+ATPaz) inhibitörüdür. Bu çalışmada tablet dozaj formlarındaki pantoprazol'ün tayini için spektrofotometrik yöntemler geliştirilmiştir. Pantoprazol'ün metanol-su (1:9 h/h) içindeki çözeltisi 200-350 nm dalga boyu aralığında iki spektrofotometrik yöntem ile tayin edildi. UV ve birinci türev UV spektrofotometrik yöntemler için analizler sırasıyla 295 nm ve 303 nm'de yapıldı. Doğrusallık aralıkları UV spektrofotometrik yöntem için 2.50 - 80.00 µg mL^{-1} ve birinci türev UV spektrofotometrik yöntem için 0.5 - 70 µg mL^{-1} olarak bulundu. UV ve birinci türev UV spektrofotometrik yöntemler için tayin alt sınırı sırasıyla 2.31 µg mL^{-1} ve 0.5 µg mL^{-1} , teşhis sınırı sırasıyla 0.69 µg mL^{-1} ve 0.15 µg mL^{-1} olarak tayin edildi. Geliştirilen yöntemler valide edildi ve iyi kesinlik ve doğruluk gösterdi. Önerilen yöntemler saf madde ve tablet dozaj formlarındaki pantoprazol'ün analizine başarıyla uygulandı. Seçilen dalga boylarında ve analiz şartlarında tablet yardımcı maddelerinden dolayı herhangi bir girişim bulunmadı. Sonuçlar literatürde verilen spektrofotometrik yöntem ile elde edilenler ile karşılaştırıldı ve istatistiksel farklılık bulunmadı.*

Anahtar kelimeler: *Pantoprazol, UV Spektrofotometri, Birinci Türev UV Spektrofotometri, Tablet Dozaj Form.*

INTRODUCTION

Pantoprazole sodium sesquihydrate (P) is widely used as anti-ulcer drugs (proton pump inhibitors) through inhibition of hydrogen-potassium adenosine triphosphatase (H^+/ K^+ - ATPase) in gastric perietal cells[1-7]. P reduces the gastric acid secretion regardless of the nature of stimulation.

P, the active ingredient of Pantpas® tablets, is described chemically as: sodium 5- (difluoromethoxy) - 2- [3,4 - dimethoxy - 2 - pyridyl) methylsulfinyl] - 1H benzimidazole sesquihydrate (Fig. 1).

Methods for the determination of P in pharmaceutical formulations and biological materials which have been reported previously included high performance liquid chromatography (HPLC)[8-13], capillary electrophoresis[14,15] and spectrophotometric determination[16-18].

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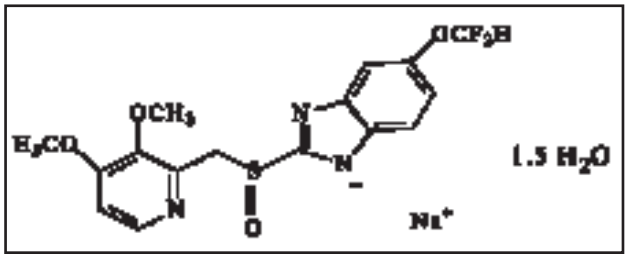

Figure 1. Chemical structure of P.

Derivative spectrophotometry is an analytical technique for the enhancement of sensitivity and specificity in qualitative and quantitative analysis of various compounds including pharmaceuticals.

The main purpose of the present study was to establish a relatively simple, single - step, sensitive, validated and inexpensive spectrophotometric method for the determination of P in pure form and in pharmaceutical dosage form, since most of the previous methods have been found to be relatively complicated and expensive, such as HPLC and CE.

The developed methods were relatively more sensitive and the limit of detection (LOD) and limit of quantitation (LOQ) values for proposed methods were lower than the UV spectrophotometric method in the literature[16-18].

EXPERIMENTAL

Instrument

Spectrophotometric determinations were performed by using an Agilent 8453 UV - Visible spectrophotometric system. UV and first derivative UV spectra of reference and sample solutions were recorded in 1 cm quartz cells at a scan speed of 50 nm min^{-1} with a fixed slit width of 3 nm, using a diode-array detector. The concentrations of P in methanol - water solutions (1:9, v/v) were determined at the wavelength ranges of 200 - 350 nm for UV and first derivative UV spectrophotometric measurements.

Reagent and Solutions

The P standard was obtained from the Central Institute of Hygiene of Turkey. Purity of P was tested by checking its melting point, UV and IR spectra. No impurities were found. Pantpas® tablets (Bayer A.Ş.) are available in tablet forms of 40 mg pantoprazole which is equivalent to 45.1 mg P, red ferric oxide (E172), black ferric oxide (E 172), yellow ferric oxide (E 172) and titanium dioxide (E 171). All analytical grade chemicals were purchased from Merck. Stock solution of P (1000 µg mL^{-1}) was prepared in methanol - water (1:9, v/v). Working standard solutions were prepared by diluting the stock solution in the concentration range of 2.50 - 80.00 µg mL^{-1} with water daily.

Procedure

A total of 10 tablets of P were powdered and weighed. The average content of one tablet was calculated. An accurately weighed quantity of sample was transferred to a 50 mL volumetric flask, dissolved in 4 ml methanol, 25 ml water, sonicated for 15 minutes and diluted to the final volume of 50 mL with water. Following the filtration, a series of dilution was prepared quantitatively with water from this solution to obtain standard solutions to reach the concentration ranges of calibration curves graphed for each of the proposed methods. All solutions were recorded against methanol - water (1:9, v/v) as a reference solution.

RESULTS

METHOD DEVELOPMENT

UV spectrophotometric method was developed for the analysis of P. Methanol - water (1:9, v/v) was used as the solvent for the preparation of P solutions.

The UV spectrum of P is shown in Figure 2. The spectrum shows a single well-defined peak with maximum absorption at 295 nm in the measuring wavelength range 200 - 350 nm. This wavelength was used for the UV spectrophotometric analysis of P.

The first derivative UV spectrum (^{1}D) of P standard

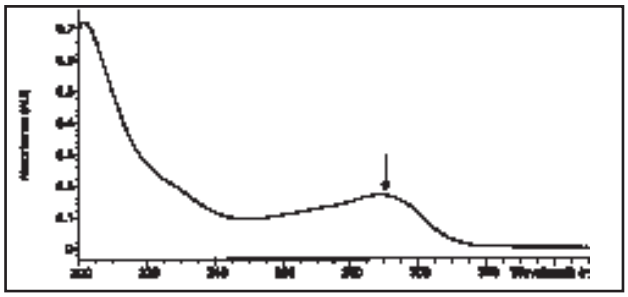

Figure 2. UV spectrum of 5 µg mL^{-1} standard P in methanol - water (1:9, v/v).

is shown in Figure 3. The spectrum shows a single sharper and well-defined peak with maximum absorption at 303 nm in the measuring wavelength

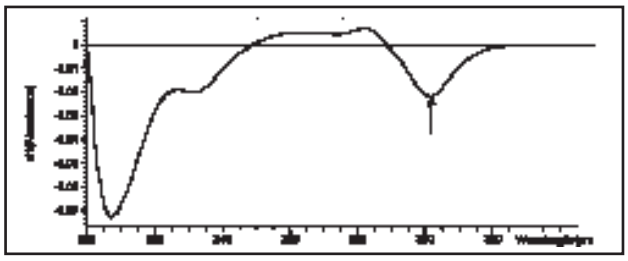

Figure 3. First-derivative UV spectrum of 5 µg mL^{-1} standard P in methanol - water (1:9, v/v).

range 200 - 350 nm. 303 nm was selected as the optimum working parameter for the first order derivative UV spectrophotometric analysis of P. To determine the optimized conditions, different solution, wavelengths, derivative orders, N values (a kind of smoothing factor) and the derivative wavelengths difference ($\Delta\lambda$) parameters were examined for derivative UV spectrophotometric method. $\Delta\lambda$ dependence was based on measuring wavelength range. Generally, the noise decreases by increasing $\Delta\lambda$.

UV and first derivative UV spectra of Pantpas®, tablet solution (at the same concentration with P standard) in methanol: water (1:9, v/v) are shown in Figures 4 and 5, respectively. As no difference was observed between spectra of P standard and tablet solutions and in the maximum wavelengths of all spectra, it was suggested that the developed methods allowed complete elimination of the background absorption due to the tablet excipients at the chosen wavelengths both in UV and first derivative UV spectra of P.

METHOD VALIDATION

Linearity Range

For quantitative analysis of P in methanol - water (1:9, v/v), the calibration curves were plotted for each spectrophotometric method over the concentration ranges cited. The peak to zero method for calibration curve in the first derivative UV spectrophotometric method was used. The statistical parameters and regression equations which were calculated from the calibration curves along with the standard error of the slope and the intercept are given in Table 1. Regression analysis indicated a linear relationship between absorbance and concentration.

The linearity ranges were found to be 2.50 - 80.00 µg mL^{-1} and 0.5 - 70 µg mL^{-1} for UV and first derivative UV spectrophotometric method, respectively (Table 1).

Sensitivity

In accordance with the formula given by International Conference on Harmonization (ICH)[19], LOD is defined as 3 s/k and LOQ is defined as 10 s/k, where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, the slope of the calibration curve.

LOD were calculated as 0.69 µg mL^{-1} and 0.15 µg mL^{-1} and LOQ were calculated as 2.31 µg mL^{-1} and 0.5 µg mL^{-1}, for UV and derivative spectrophotometric methods, respectively (Table 1).

Table 1. Optical characteristics of proposed UV and first-derivative UV spectrophotometric method (n =7).

Parameters	UV Spectrophotometry	First-Derivative UV Spectrophotometry
Wavelength (λ) (nm)	295	303
Regression equation[a] of calibration curve method	y = 0.0349x - 0.0156	y = 0.0020x + 0.0011
Correlation coefficient (r)	0.999	0.999
Standard error on slope	3.7736×10^{-4}	1.5117×10^{-4}
Standard error on intercept	1.8868×10^{-3}	7.1807×10^{-4}
Linearity range (μg mL^{-1})	2.50 - 80.00	0.5 - 70
Limit of quantitation (LOQ) (μg mL^{-1})	2.31	0.5
Limit of detection (LOD) (μg mL^{-1})	0.69	0.15

[a]y =bx + a, where x is the concentration in μg mL^{-1}, y is amplitude for UV and first- derivative UV spectrophotometry.

Selectivity /Specificity

Comparison of the UV spectra of standard P and tablet solutions showed that the wavelength of maximum absorbance did not change (Figs. 2 and 4, Figs. 3 and 5). It was concluded that excipients did not interfere with quantitation of P in these methods.

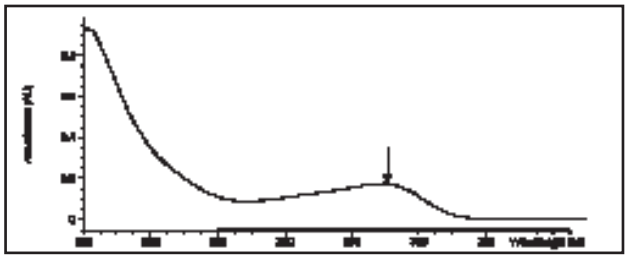

Figure 4. UV spectrum of 5 μg mL^{-1} P in tablet solution.

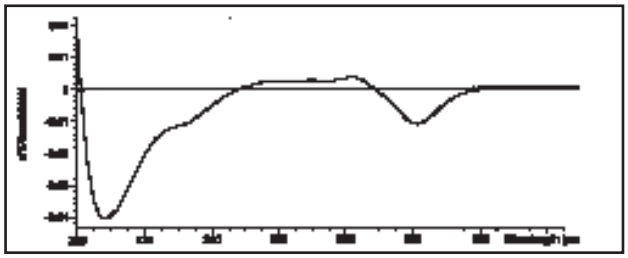

Figure 5. First-derivative UV spectrum of 5 μg mL^{-1} P in tablet solution.

In order to evaluate the effect of excipients in these methods, the standard addition method was applied. y and r values of the developed methods were calculated as y = 0.0313x + 0.1465 and r = 0.9979 for the UV spectrophotometric measurements and y = 0.0023x + 0.0071 and r = 0.9970 for the derivative spectrophotometric measurements. Since the slopes of the calibrations and standard addition curves were identical (Table 1), it was concluded that there was no spectral interaction in the analysis of P in pharmaceutical preparations by the proposed methods. Therefore, the calibration curve method, which is easier and quicker than the standard addition method, was used in quantitative analysis. In the proposed methods there was no need for pre-separation, and only centrifugation was applied to make the solution clear.

Recovery

Recovery experiments were conducted to determine the accuracy of the proposed methods. These studies were performed at a concentration of 40 μg mL^{-1} standard P solution in methanol - water (1:9, v/v) (n=12). The mean recovery and relative standard deviation were found to be 101.77% and 1.17% for UV spectrophotometric method and 101.28% and 1.19% for first derivative UV spectrophotometric method, indicating very good reproducibility of these methods (Table 2).

Table 2. The results of percentage recovery value 40 μg mL^{-1} reference standard solutions by the two developed spectrophotometric methods (n = 12).

Found P (μgmL^{-1})	
UV Spectrophotometry	First-Derivative UV Spectrophotometry
39.96	39.03
40.16	41.76
40.81	39.27
41.09	40.46
41.17	38.76
41.07	41.35
41.18	40.41
40.08	39.35
41.09	38.73
40.15	39.82
40.87	39.42
40.86	39.22
$\bar{X}$ = 40.71 ± 0.14	$\bar{X}$ = 40.51 ± 0.14
SD = 0.47	SD = 0.48
RSD % = 1.17	RSD % = 1.19
CL = 40.40 - 41.02	CL = 40.20 - 40.82

$\bar{X}$ = Mean ± standard error, SD = Standard deviation,
RSD = Relative standard deviation, CL = Confidence levels (α = 0.05).

The other recovery studies were conducted on the synthetic mixture (placebo) prepared by adding accurately weighed amounts of P to the excipient mixture and calculating the percentage recovery in each case (Table 3). The percentage recovery of P was calculated by comparing the added and found concentrations (C_{found} / C_{added} x100) and expressed as mean recoveries and relative standard deviations (RSD%) in each case (Table 3).

Table 3. The results of percentage recovery value in synthetic mixture of P by two spectrophotometric methods (added P for tablet; 40 mg) (n = 7).

UV Spectrophotometric Method		Derivative UV Spectrophotometric Method	
Found (mg)	Recovery	Found (mg)	Recovery
39.62	99.05	40.08	100.17
39.56	98.89	41.16	102.94
40.35	100.89	40.84	102.08
40.34	100.86	40.28	100.68
40.56	101.40	39.96	99.93
40.56	101.40	39.92	99.8
39.53	98.83	39.68	99.21
$\bar{X}$ = 40.07 ± 0.18	100.19 ± 0.18	40.27 ± 0.20	100.68 ± 0.51
SD = 0.48	1.20	0.54	1.34
RSD % = 1.20	1.20	1.34	1.33

$\bar{X}$ = Mean ± standard error, SD = Standard deviation, RSD = Relative standard deviation.

Accuracy and Precision

In this study accuracy was determined by analyzing the recoveries of known amounts of P added into excipients (Table 3). To determine the precision of the methods, P solutions at a concentration of 40 µg mL^{-1} were analyzed 12 times and the mean P values were found as 40.71 ± 0.14 for UV spectrophotometric method and 40.51 ± 0.14 for first derivative UV method. The standard deviation values were found as 0.47 and 0.48 for UV and first derivative UV methods, respectively, and the developed methods had good precision.

The intra - assay precision (repeatability) and accuracy were studied by analyzing repeatedly (7x) in one laboratory on the same day, three different concentration levels (5, 30, 70 µg ml^{-1}) of P. The results are shown in Table 4.

Table 4. Inter-day and intra-day precision and accuracy of P (n =7).

	Inter-day			Intra-day		
UV Spectrophotometric Method						
Added (µg mL^{-1})	Found[a] (µg mL^{-1})	Precision (SD), (RSD%)	Accuracy[b] (Bias%)	Found[a] (µg mL^{-1})	Precision (SD),(RSD%)	Accuracy[b] (Bias%)
5	4.97	0.13, 2.62	-0.60	4.84	0.10, 2.07	-3.20
30	30.03	0.56, 1.87	0,10	29.71	0.59, 1.99	-0.97
70	70.66	0.88, 1.24	0.94	69.73	1.00, 1.43	-0.39
First-Derivative UV Spectrophotometric Method						
5	5.04	0.10, 1.98	0.80	5.03	0.09, 1.79	0.60
30	30.33	0.74, 2.44	1.10	30.22	0.76, 2.51	0.73
70	70.29	0.61, 0.87	0.41	70.62	0.71, 1.01	0.89

Found[a] =$\bar{X}$, mean values represent seven P standard solutions for each concentration; SD = Standard deviation; RSD = Relative standard deviation; Accuracy[b] (Bias %) = (Found-Added / Added) x 100.

The inter - day precision (reproducibility) and accuracy were studied by analyzing the three different concentration levels of P by seven different runs over a week period and results were expressed as RSD%[20] (Table 4). Notice that the intra- and inter-assay RSD% values were satisfactory (≈ 2 %).

The results indicated that the proposed methods were accurate and precise.

Robustness and Ruggedness

The tests mentioned hereunder were used to determine the robustness and ruggedness of the analytical methods. The robustness of the proposed methods was tested by changing parameters such as wavelength range, the degree of derivation and slit width[19]. None of these variables significantly affected the absorbance of P indicating that the proposed methods could be considered as robust.

The ruggedness of the developed methods was expressed as RSD% of the same procedures applied by two different operators in different laboratories by different instruments on different days for same standard and tablet dosage forms of P. The results showed no statistical differences between the different operators and instruments suggesting that the developed methods were rugged (Table 5).

Table 5. The results of analysis from pharmaceutical preparations and standard of P by two different analysts and instruments (n= 6).

	UV Spectrophotometric Method					
	Different analyst			Different instrument		
	$\bar{X}$	SD	RSD %	$\bar{X}$	SD	RSD %
Standard of P (40 µg mL^{-1})	39.82 ± 0.05	0.09	0.23	40.71 ± 0.15	0.33	0.81
Tablet (40 mg P)	39.97 ± 0.40	0.79	1.98	40.28 ± 0.22	0.40	0.99
First-Derivative UV Spectrophotometric Method						
Standard of P (40 µg mL^{-1})	40.72 ± 0.21	0.58	1.42	40.52 ± 0.28	0.57	1.41
Tablet (40 mg P)	40.48 ± 0.31	0.75	1.85	40.37 ± 0.36	0.81	2.01

$\bar{X}$ = Mean ± standard error, SD = Standard deviation, RSD % = Relative standard deviation.

Stability

The stability of the P stock solutions was tested by keeping them in the dark at 4°C; analysis was done daily for one month. Results showed that P in methanol - water (1:9, v/v) solutions was stable at least for a week.

Analysis of P in Tablets

The proposed methods were successfully applied for the determination of P in tablet dosage form. The results concerning the analysis of Pantpas® tablets containing 40 mg of P are presented in Table 6.

Table 6. The results of pharmaceutical preparations containing P analyzed by each spectrophotometric method (n=7).

Found P (µg mL^{-1})		
UV Spectrophotometry	Derivative UV Spectrophotometry	Compared UV Spectrophotometry
39.86	39.92	40.88
40.78	40.72	39.95
39.27	39.76	41.12
39.81	40.88	40.11
40.63	40.24	40.65
40.39	39.60	40.34
40.71	40.24	39.80
$\bar{X}$ = 40.21 ± 0.22	$\bar{X}$ = 40.19 ± 0.18	$\bar{X}$ = 40.41 ± 0.18
SD = 0.57	SD = 0.48	SD = 0.49
RSD % = 1.42	RSD % = 1.19	RSD % = 1.21
CL = 39.67 - 40.75	CL = 39.75 - 40.63	CL = 39.97 - 40.85
t_c = 11, t_t = 2	t_c = 9, t_t = 2	t_c = 13.5, t_t = 2

$\bar{X}$ = Mean ± standard error, SD = Standard deviation, RSD = Relative standard deviation, CL = Confidence intervals (a = 0.05).
t_c, calculated t value; t_t, tabulated t value (t_t = 2.179 for n = 7).
Ho hypothesis: no statistically significant difference exists between the two developed methods and between the developed methods and the compared method. $t_c > t_t$; Ho hypothesis is accepted (p>0.05).

Performances of the Proposed Methods

The performances of the developed methods were statistically compared with test results in the literature one involving another spectrophotometric method (DDQ method)[16]. The methods in the literature were based on charge transfer complexation reaction of pantoprazole, where they act as n-donors, with either π acceptor 2,3 - dichloro- 5,6 - dicyano - 1,4 - benzoquinone (DDQ) and with σ acceptor as iodine. A third method was investigated depending on ternary complex formation with eosin and copper (II). The colored products were quantified spectrophotometrically using absorption bands at 457 nm for DDQ, at 293 nm for iodine and at 549 nm using ternary complex formation for pantoprazole. The obtained results were compared by Wilcoxon test. There was no significant difference between each of the developed methods and the compared spectrophotometric methods with respect to mean values and the standard deviations at 95% confidence level

($p>0.05$) (Table 6). However, no significant difference was found between the spectrophotometric methods indicating that the developed methods were relatively more sensitive. The LOD and LOQ values for the proposed methods were lower than those of the compared UV spectrophotometric method. Additionally, these developed methods do not involve procedural steps as in the spectrophotometric method in the literature.

CONCLUSION

In this study, UV spectrophotometric and first derivative UV spectrophotometric methods were developed for the determination of P in tablet dosage forms. P can be directly determined in tablets in presence of excipients without sample pre-treatment procedures by using spectrophotometric methods. The apparatus and reagents used seem to be accessible even for the simple laboratories.

However, no significant difference was found between the proposed spectrophotometric methods ($t_c = 13.5 > t_t = 2$) indicating that the first derivative spectrophotometric method was relatively more sensitive. The LOD and LOQ values for the proposed first derivative spectrophotometric method were lower than for the proposed UV spectrophotometric method.

It can be concluded that the proposed methods are fully validated. They were found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive, and they give an acceptable recovery of the analyte. The developed methods can be recommended for routine and quality control analysis of P.

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