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Development of Spectrophotometric Method for the Determination of Atenolol in Normoten Drug

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Abstract

This research aimed to develop inexpensive, safe, rapid, efficent spectrophotometric method for the assay of atenolol in some antihypertensive drugs namely Normoten in its pharmaceutical formulation. The studied method is depend on the reaction of the drug with phenol red in acidic medium, at pH 3.0. The analytical parameters have been investigated. The maximum absorbance was obtained at 429 nm and the molar absorptivity of 0.054 L mol^{-1} cm^{-1}. Beer's law is linear in the concentration range of 0.5-100 μg/mL for atenolol in Normoten. The detection and quantification limits were found to be 0.038 and 0.113μg/mL for the atenolol in Normoten respectively, and with a linear regression correlation coefficient of 0.997. The recovery was found to be 98.94 to 100.31%. The studied method is can be applied for the determination of atenolol (active ingredient) of the antihypertensive drugs in their pharmaceutical formulations.

Keywords: Normoten, antihypertensive drugs, atenolol, validation, UV-Visible spectrophotometer

1. Introduction

Normoten is categorized as cardiovascular system drugs which contains atenolol. Chemically atenolol known as 4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy) benzeneacetamide as shown in Fig. 1 (O'Neil & Budavari, 1989). Atenolol used for the treatment of hypertension, prevention of cardiovascular diseases (Gotardo, Sequinel, Pezza & Pezza, 2008), treatment of antiangina (Hegde, Kumara Swamy, Sherigara & Nandibewoor , 2008) and control of cardiac arrithymia (Hoffman, Hardman, Limbird, Gilman (eds), Goodman & Goodman¥s, 1987).

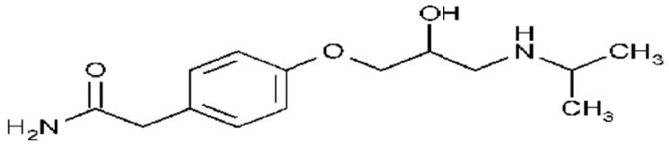

Figure 1. Chemical structure of atenolol

Several methods were used for the quantification of atenolol in bulk and pharmaceuticals. The United States Pharmacopeia uses HPLC method with UV detector for determination of atenolol in tablets (United States Pharmacopeia 2003), whereas the British Pharmacopoeia uses UV spectrophotometry (British Pharmacopoeia, 2001). Atenolol was determined by HPLC (Zarapkar, Kolte & Rane, 1997; Radulovic, Zivanovic & Velimirovic, 1991; Gong, 1989; Ceresole, Moyano, Pizzorno & Segall, 2006; Rapado-Martinez, Garcia-Alvarez-Coque .& Villanueva-Camanas, 1997), high-performance thin-layer chromatographic (HPTLC) (Argekar & Powar, 2000; Argekar & Sawant, 1999), gas liquid chromatography (Sadana & Ghogare, 1990; Rao, Avadhanulu, Giridhar, Pantulu & Kokate, 1990), spectrofluorometry (Gajewska, Glass & Kostelecki, 1992; Zhao, Yan & Guo, 1994), atomic absorption spectrometry (El Ries, 1995), nuclear magnetic resonance spectrometry (Iorio, Mazzeo-Farina & Doldo, 1987). Atenolol was determined spectrophotometrically with by using 2,4-dinitrophenol, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and 2,4,6 trinitrophenol (Prashanth & Basavaiah, 2012), with hydroxylamine hydrochloride (Agrawal, Raman, Rajpu & Menon, 1992), sodium nitroprusside (Bashir, Shah, Bangesh & Riazullah, 2011), chloranil and propan-2-ol (Korany, Abdel-Hay, Galal & Elsayed, 1984), permanganate in alkaline medium (Hiremath, Mulla & Nandibewoor, 2005), metol and sulphanilic acid (Basavaiah, Chandrashekar & Nagegowda, 2004), bromate-bromide mixture (Basavaiah, Chandrashekar & Nagegowda, 2006), cerium(IV) sulphate (Basavaiah, Chandrashekar & Nagegowda, 2003), choranilic acid (Agarwal, Singhal & Prakash, 1998), 1,2-naphthoquinone-4-sulfonic (Ali & Elbashir, 2013) and neutralization reaction with phenol red in acetone (Basavaiah, Chandrashekar, Somashekar, & Ramakrishna, 2005). The objective of

the current investigation is to validate cheap, safe, fast, precise and accurate spectrophotometric method for the analysis of some Antihypertensive drugs, using phenol red (PR) in ethanol as a chromogenic reagent. The method was depend on the complexation of the investigated drugs with phenol red (PR) to give coloured complex species measured at the visible region.

2. Materials and Methods

2.1 Chemicals, Reagents and Apparatus

Reagents and Chemicals of high grade of were used. Deionized water was used for solution preparation. The standard of Normoten was provided by (Jazeera Pharmaceutical Industries, Saudi Arabia. Normoten-50 (Atenolol) tablets was labeled to contain 50 mg Atenolol per tablet. The analysis was carried out with a UV-VIS Spectrophotometer (SP- 3000, Optima, Japan) with 1-cm quartz cells, Digital Water Bath (Daihan Labtech Co. Ltd., Indonesia) and pH-meter (Jenway Ltd., U.K.).

2.2 Preparation of Phenol Red (PR) Solution

An accurately weighed 1.0 g of phenol red were dissolved in 20.0 ml ethyl alcohol, put into a 100 ml volumetric flask and completed to the volume with deionized water and thoroughly mixed to prepare (0.1 - 1.0% w/v). The solution was kept in amber glass bottle to prevent it from light.

2.3 Preparation of Buffer Solutions

A preparation of buffer solutions were carried out by mixing acetic acid with sodium acetate solution, Na_2CO_3 solution with $NaHCO_3$ solution and NaH_2PO_4 solution with NaOH solution to obtain pH of range 3.0- 11.0.

2.4 Preparation of Stock Solutions of Normoten (1000 µg/mL)

An exactly 0.1 g of the drug was dissolved in methanol, put into 100 mL volumetric flask and completed to the volume with methanol and thoroughly mixed to obtain a solution of 1000 µg/mL. The stock solution of Normoten (Atenolol) was further diluted to get solutions of 0.5- 120 µg/mL.

2.5 Preparation of Sample Solutions of Normoten

The contents of each of 3 tablets for the Normoten drug were ground and homogenized . An exact quantity equivalent to weight stated for each of the drug (Normoten 50 mg) was dissolved in methanol and transferred to a 25 mL volumetric flask, and then completed to the volume with the same solvent. Suitable dilutions of this solution with methanol were made for the analysis.

2.6 Procedure for the Analysis Using Phenol Red as a Chromogenic Reagent

About 2 mL of the prepared solutions of Normoten were put into 10 mL volumetric flask followed by addition of 2 ml of pH 3.0 and 1 mL of 0.80 % (PR), the solutions were then heated to 40°C for 10.0 min, the mixtures was diluted with methanol. The absorbance were read at 429 nm for the drug solution versus the blank.

2.7 Account of the Molar Ratios

The account of the molar ratios was determined by application of continuous variation method (Job, 928). Equal concentrations of 10.0 µg/mL for each of Normoten and (PR) were prepared. A volume of 10-mL of Normoten and (PR) were made up by the following ratios 0:10, 1:9, 2:8,3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 and 10:0.

3. Results and Discussion

3.1 Analysis of Absorption Spectra

The maximum absorption spectrum of the reaction of Normoten with (PR) was obtained as shown in (Figures 2). The maximum absorption wavelength peak λmax at 340 nm for Normoten, and the λmax 460 nm for (PR), while the λmax for the drug with (PR) is 429 nm against the reagent blank.

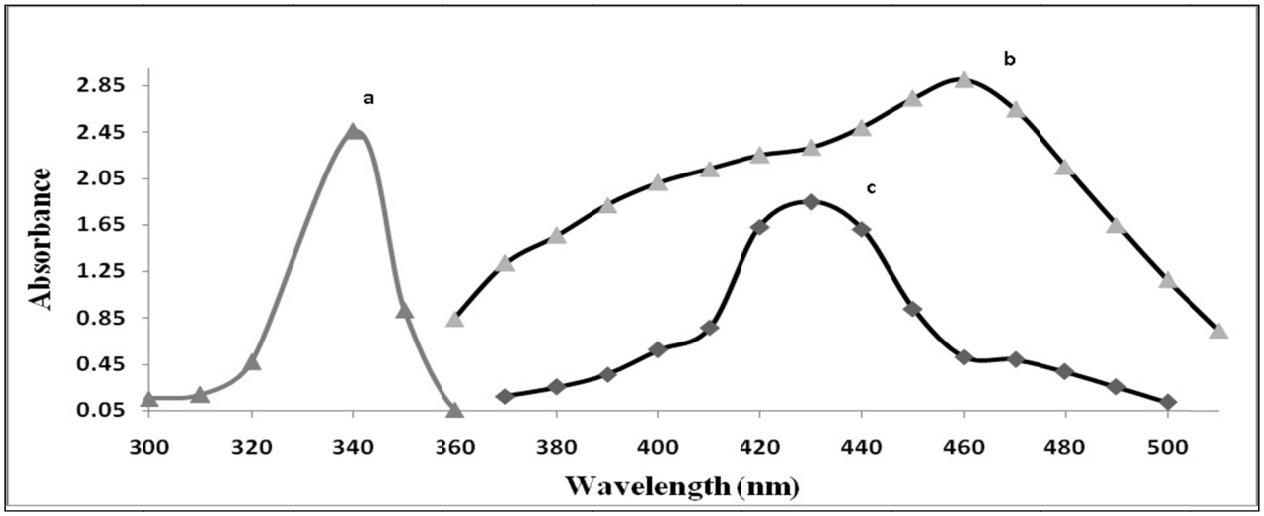

Figure 2. a- λmax of Normoten (10 µg/mL) against methanol, b- λmax of (PR) (0.8%) against water, c- λmax of reaction of Normoten (10 µg/mL) with (PR) (0.8%)

3.2 Optimization of the Reaction Variables

The optimum conditions for the development of studied method were established by changing the amount of pH, buffer volume, reagent (PR) concentration, temperature and standing time and the effect obtained on the absorbance was recorded, and they were found to be 3.0, 2.0, 0.80 %, 40 °C and 10 minutes respectively as shown in (Fig 3).

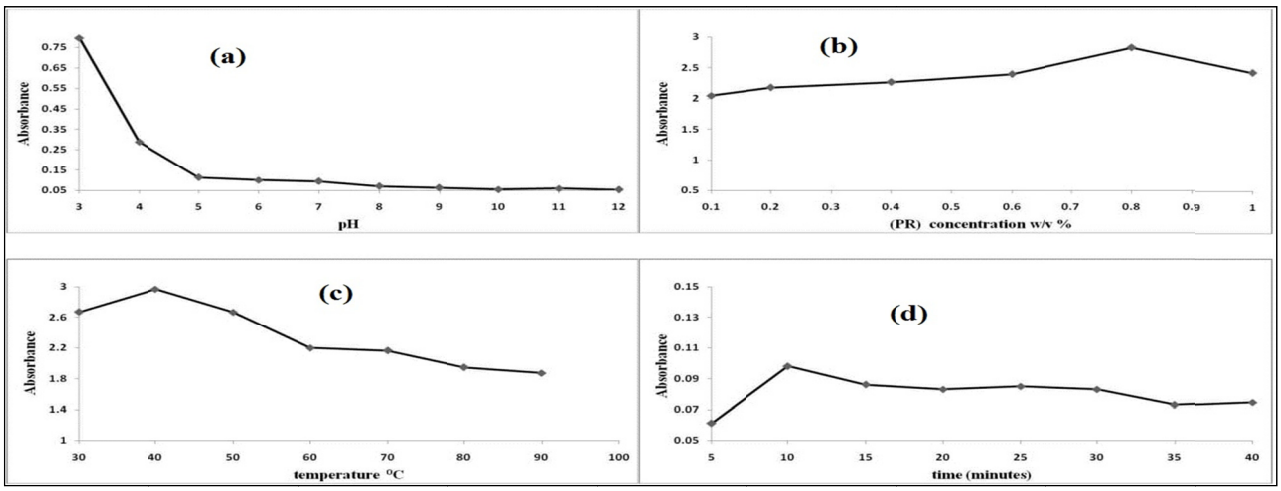

Figure 3. Effect of analytical parameters on the reaction of Normoten with (PR): (a) Effect of pH, (b) Effect of (PR) concentrations, (c) Effect of temperature, and (d) Effect of standing time

3.3 Soichiometric Ratio of the Reaction

The continuous variation of the method was carried out (Job, 928). Similar concentrations of Normoten drug solution and (PR) were prepared. Sets of 10 mL volumes of the solutions of drug and (PR) were carried out by the following ratios (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0), The solution was further analyzed by the same procedure. The Job's graph was constructed which indicated that the ratio of (PR): Normoten drug reaction (Figure 4).

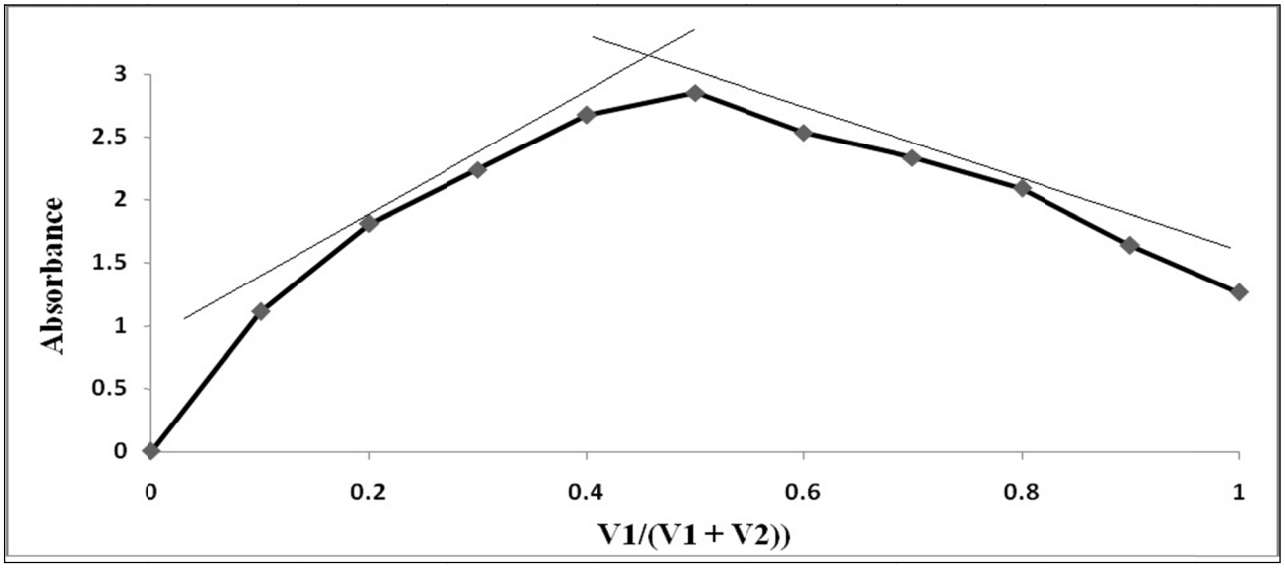

Figure 4. Stoichiometry by Job's method for (PR) with Normoten drug (V1:(PR) and V2 : drug sample)

3.4 Method Validation

Calibration curve for the analysis of studied drug samples (Normoten) and (PR) was setup by plotting the absorbance versus corresponding concentrations as shown in (Fig. 5). Linear graph with good R^2 value was obtained in 0.5- 100 µg/mL. The detection limit (LOD) was determined from standard deviation of five determinations of blank ((n = 5, SD = 0.0005) and the slope of the calibration graph.

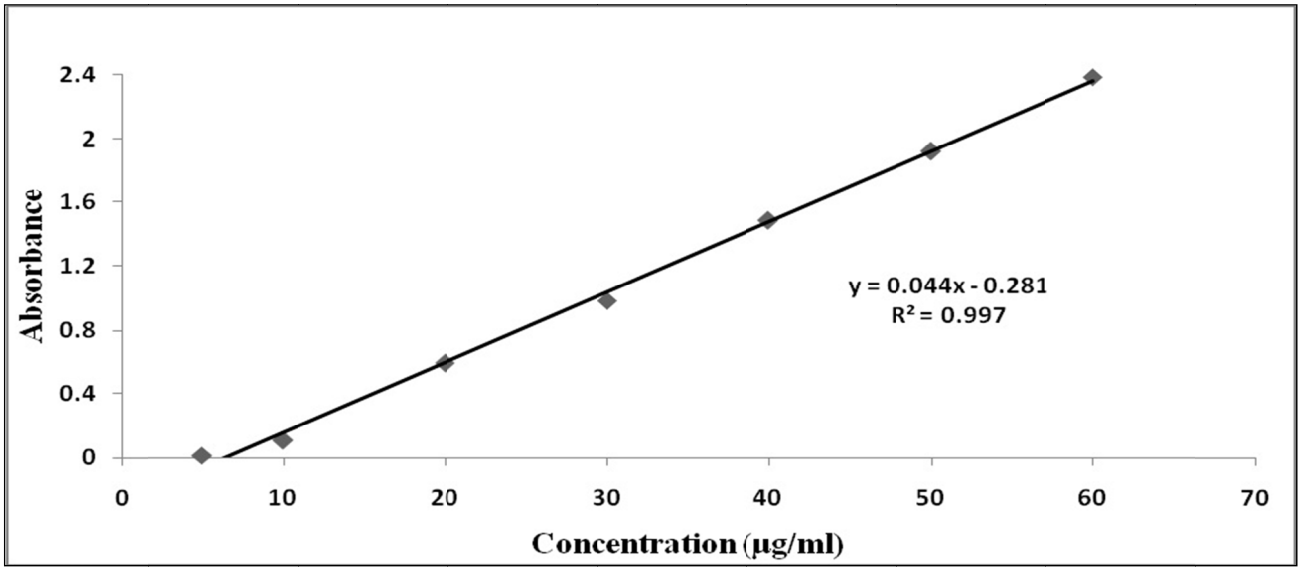

Figure 5. Calibration Curve of Normoten (Atenolol)

The equation of the linear line was found to be Y = 0.044 X + 0.281. The LOD and quantification limit (LOQ) were calculated as the guidelines of The International Conference of Harmonization for method validation (ICH Guideline, 2005). The analytical parameters were outlined in (Table 1).

Table 1. Analytical parameters of the studied method

Parameter	Normoten drug sample and (PR)
λmax /nm	429 nm
Beer's linearity (µg/mL)	0.5 - 100
Molar absorptivity (L mol^{-1} cm^{-1})	0.054
R^2 value	0.997
Regression equation (Y)	Y = 0.044 X + 0.281
Slope	0.044
Intercept	0.281
LOD (µg/mL)	0.038
LOQ (µg/mL)	0.113

Robustness is determined by changing with the small variations in the analytical parameters. The results revealed that the small changes did not influence the procedure as seen in (Table 2).

Table 2. Assay parameters of PR method on the suitability test parameters and sensitivity

Condition	Normoten concentration (10.0 µg/mL)	Recovery % ± RSD*
pH	2.80	100.68 ± 0.23
	3.20	99.39 ± 0.35
(PR) concentration	0.78	99.55 ± 0.46
(w/v %)	0.82	100.68 ± 0.46
Temperature (°C)	38	99.39 ± 0.35
	42	99.77 ± 0.23
Reaction time (min.)	8	101.14 ± 0.23
	12	100.30 ± 0.13

- • Each analysis was average of three readings (n = 3)., * RSD is the relative standard deviation..

The accuracy and precision of the studied method were carried out by using three different concentrations of studied drug solutions. The percentages f the relative error were found to be 0.227- 0.645, and the intraday and interday evaluations reveal that the method is of high accuracy and precision as shown in (Tables 3 and 4).

Table 3. Accuracy and precision

Sample	Taken	Found	% Relative error	SD	% RSD
Normoten (µg/mL)	10.00	10.045	0.450	0.046	0.458
	20.00	19.871	0.645	0.035	0.176
	30.00	30.068	0.227	0.045	0.150

Values are mean of 3 readings. (n = 3).

Table 4. Evaluation of Interday and Intraday Accuracy

Added (µg/mL)	Interday (n = 3)				Intraday (n = 3)			
	Found	Recovery %	± SD	%RSD	Found	Recovery %	± SD	%RSD
10	9.955	99.55	0.023	0.231	9.932	99.32	0.046	0.463
20	20.098	100.49	0.035	0.174	19.939	99.70	0.035	0.176
30	30.091	100.30	0.068	0.226	30.060	100.20	0.056	0.182

• Each analysis was average of three readings (n = 3).

Three different concentrations were added to a fixed amount of the prepared drug sample, standard Normoten, and the total was analyzed by the studied method. Each analysis was carried out three times. The recoveries were found to be in the range of 98.94 to 100.31% for the drug sample as shown in (Table 5).

Table 5. The recovery

sample	Normoten (µg/mL)	Standard Normoten Added (µg/mL)	Found (µg/mL)	% Recovery ± RSD
Normoten	10.0	5.0	14.841	98.94 ± 0.31
(µg/mL)	10.0	10.0	20.061	100.31 ± 0.17
	10.0	20.0	29.818	99.52 ± 0.16

• Each analysis was average of three readings (n = 3).

It is obvious from the findings gained that the investigated method was of good analysis with respect of the analysis of atenolol in the antihypertensive drugs. Thus the sample of the drug (Normoten) was subjected to the analysis of its atenolol content by the proposed method and the percentage of recovery was found to be 99.50 ± 0.05 as (Percentage ± SD, and n = 3).

4. Conclusions

The current investigation showed the reproducible findings of phenol red (PR) reagent in the validation of simple,

precise, accurate and fast spectrophotometric analysis for atenolol in antihypertensive drugs namely Normoten in its dosage forms. The investigated method is preferable to the past research spectrophotometric method for evaluation of Normoten as it is very simple. Moreover, the chemicals and reagents used in the analysis are cheap and available. Beside that the investigated method compromise an easy analysis of chromogenized species and no extraction processes was carried out. The studied method can be applied for any antihypertensive drugs containing atenolol.

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