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Development and validation of UV- spectrophotometric method for the determination of doxazosin mesylate in pharmaceutical formulations

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ABSTRACT

A simple, sensitive, selective rapid spectrophotometric method was developed for the determination of post synaptic α-1 Adrino receptor antagonist Doxazosin mesylate in pure form and pharmaceutical formulations based on the oxidation of Fe^{3+}ion in presence of drug and followed by orange red colored complex formation with 1,10- Phenantrolline reagent at PH 5 which are extractable at 510 nm. Beer's law is obeyed in the concentration range 10-60 μg /ml. The developed method was applied directly and easily for the analysis of the Pharmaceutical formulations. R.S.D was found to be 0.5318^0 and recovery 99.32% respectively. The method was completely validated and proven to be rugged. The interferences of the other ingredients and excipients were not observed. The repeatability and the performance of the proved method were established by point and internal hypothesis and through recovery studies.

KEYWORDS

Spectrophotometry;
Doxazosin mesylate;
$FeCl_3$;
1, 10-Phenantrolline;
Oxidation followed by complex formation.

INTRODUCTION

Doxazosin[(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-yl-carbonyl) piperazine] is a postsynaptic α_1- adrenoreceptor antagonist used either alone or in combination with diuretics or α_1-adrenergic-receptor-antagonist for the treatment of hypertension and benign prostatic hyperplasia. It is structurally related to prazosin (Figure1), but Doxazosin shows a more gradual onset of hypertensive effect and a longer half-life, making it a possible candidate for once-daily oral dosing[2,3]. For determination of Doxazosin in human plasma, several analytical methods have been reported, mainly chromatographic methods coupled with fluorescence[4,10], UV[11] mass spectrometry[12], or MS–MS detection[13,14]. Sample treatment involves primarily liquid–liquid extraction of doxazosin from plasma[4,5,8,10,12,14], although offline and online solid phase extraction[6,11], as well as protein precipitation[7], have also been applied Most of the developed methods have been applied to human pharmacokinetic studies after oral administrations of 4–10 mg doses of doxazosin tablets[6,9,12]. Volta metric methods (cyclic, linear sweep, differential pulse and square-wave voltammetry) were developed for the determination of the Alfuzosin in tablet dosage form, human serum and sim-ulated gastric juice at a glassy carbon disk electrode in Britton-Robinson and phosphate buffers over the pH range 2.0

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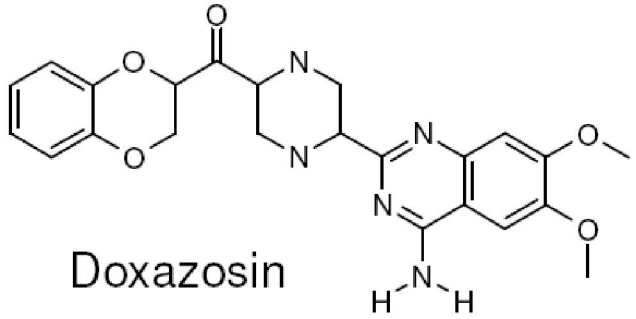

Figure 1

to 7.5. Detection limits were found to be 1.56 x10 $^{-7}$ M for differential pulse voltammetry and 6.2 x 10 $^{-8}$M for square-wave voltammetry[27]. The empirical formula for Doxazosin mesylate is $C_{23}H_{25}N_5O_5$ and the molecular weight is 451.47grams. It has the following structure.

In the present study an attempt has been made to develop simple UV spectrophotometric method for quantitative estimation of Doxazosin in its technical grade, formulations and biological sample (Blood). The functional group used for the color development of Doxazosin was primary amine. The results obtained in this method were based on complex formation reaction of Doxazosin with 1, 10- Phenanthrolline/$Fecl_3$.

An attempt has been made to develop and validate to ensure their accuracy, precision, repeatability, reproducibility and other analytical method validation parameters as mentioned in various gradients.

EXPERIMENTAL

Apparatus

Shimadzu model UV-Visible spectrophotometer model 2450 with 1 cm matched quartz cells was used.

Materials

Pure sample

The pure sample was collected from CIPLA Pharmaceuticals, Avalahalli, VirgoNagar, Bangalore, 560049.

a. Solvent

Methanol

b. Preparation of standard stock solution

Accurately weighed 100mg of Doxazosin was dissolved in 40ml of methanol in 100ml volumetric flask and volume was made up to the mark with methanol. i.e. 1000µg/ml (Stock solution A)

From the above stock solution A 10ml of solution was pipette out into 100ml volumetric flask and the volume was made up to the mark with methanol to obtained the final concentration of 100 µg/ml (Stock solution B)

CHEMICALS

1,10-Phenantrolline (0.01M)

0.234 g of 1, 10- Phenantrolline was accurately weighed and dissolved in Distilled water in 100ml volumetric flask and volume was made up to the mark with Distilled water.

0.2%$Fecl_3$ solutions

0.2g $FeCl_3$ reagent was accurately weighed and dissolved in distilled water in 100ml volumetric flask and made up to the mark with distilled water.

Orthophosphoric acid (0.2M)

1.3ml of Orthophosphoric acid was accurately taken and dilute in Distilled water in 100ml Volumetric Flask and made up to the mark with Distilled water

Preparation of calibration curve

Fresh aliquots of Doxazosin ranging from 1 to 6 ml were transferred into a series of 10ml volumetric flasks to provide final concentration range of 10 to60 µg/ml. To each flask 1ml of (0.01M) 1, 10-phenantrolline solution was added followed by 1ml of (0.2%) Ferric chloride solution and resulting solution was heated for 15min at100^{0}C and finally 1ml (0.2M) Orthophosphoric acid solution was added. The solutions were cooled at room temperature and made up to mark with distilled water. The absorbance of orange red colored chromogen was measured at 510nm against the reagent blank. The color species was stable for 24hrs. The amount of Doxazosin present in the sample solution was computed from its calibration curve.

Procedure for formulations

Twenty tablets containing Doxazosin mesylate were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100mg of Doxazosin was dissolved in a 100ml of methanol and mixed for about 5min and then filtered. The methanol

was evaporated to dryness. The remaining portion of solution was diluted in a 100ml volumetric flask to the volume with methanol up to 100ml to get the stock solution A. 10ml of aliquots was pipette out into 100ml volumetric flask and the volume was made up to the mark with methanol to obtained the final concentration of 100µg/ml (Stock solution B).

Subsequent dilutions of this solution were made with methanol to get concentration of 10to 60 µg/ml and were prepared as above and analyzed at the selected wavelength, 510nm and the results were statistically validated

Procedure for blood sample

After collection of Blood sample it will be centrifuged. For isolation of Doxazosin from plasma sample, Methanol was used for protein precipitation. Liquid-Liquid extraction was performed with plasma by alkalinization with 1M NaOH, followed by extraction with 30% dichloromethane in Hexane. The upper organic layer was evaporated to dryness, and reaming dry residue was dissolved in 100ml Methanol. From the above solution 1ml is taken into a 100ml of Volumetric flask and made up to the mark with methanol.(10 ng/ml)

From the above solution ranging from 0.1-0.7 (10-70ng/ml) were transferred in to each flask 1ml of (0.01M) 1, 10-phenantrolline solution was added followed by 1ml of (0.2%) Ferric chloride solution and resulting solution was heated for 15 min at100^{0}C and finally 1ml (0.2M) Orthophosphoric acid solution was added. The solutions were cooled at room temperature and made up to the mark with distilled water. The absorbance of orange red colored chromogen was measured at 510nm against the reagent blank. The

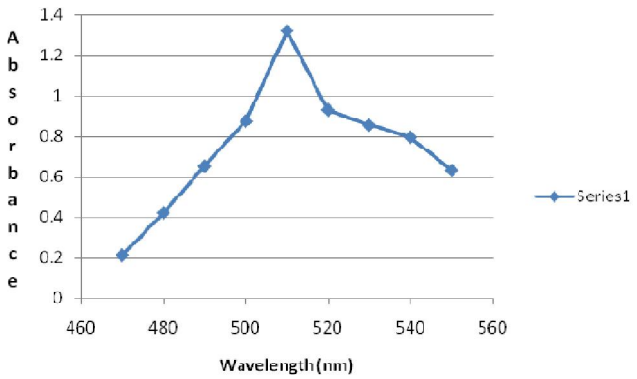

Figure 2 : Absorption spectrum of Doxazosin with 1,10-Phenantrolline/FeCl$_3$

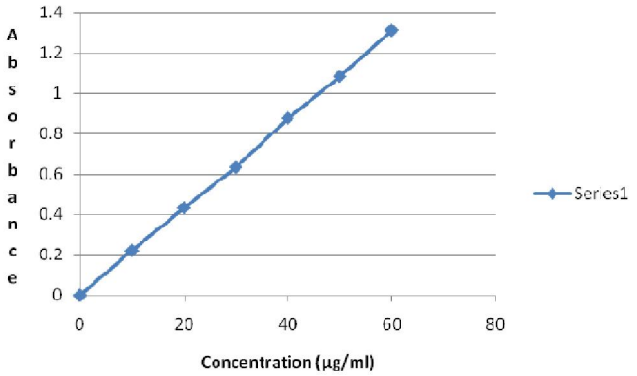

Figure 3 : Beer's law plot of Doxazosin with 1,10-Phenantrolline/FeCl$_3$

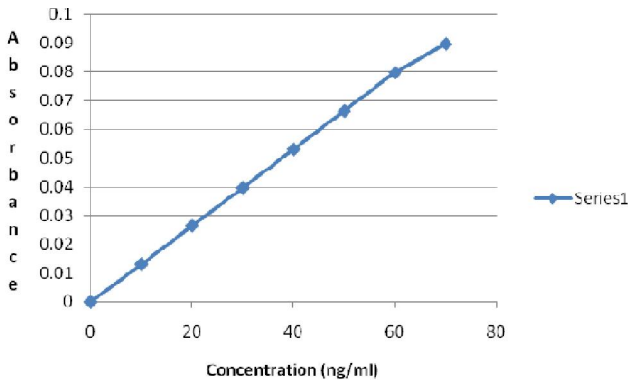

Figure 4 : Beer's law plot for Doxazosin in blood sample

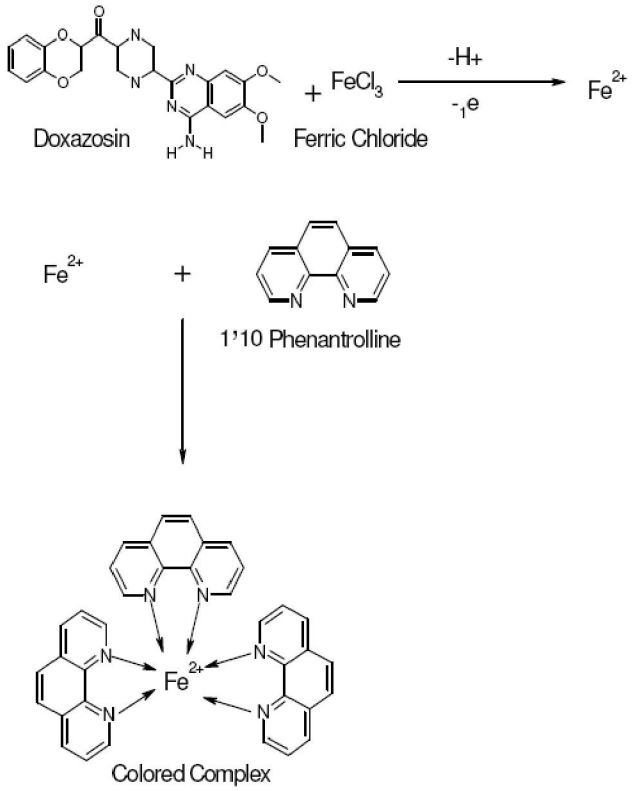

Figure 5 : A schematic seaction mechanism of doxazosin with 1'10 – phenantroline

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TABLE 1 : Optical characteristics and precision by (1, 10 – PT)

Parameter	Visible method
Color	Orange red
Absorption maxima(nm)	510
Beer's law limits (μg ml^{-1})	10 to 60
Molar absorptivity (L $Mol^{-1}Cm^{-1}$)	1.0995×10^4
Sandell's Sensitivity (μg $cm^{-2)}$	0.04106
Regression eqvation (Y*)	
Slope (b)	0.02194
Intercept(a)	0.00662
Standared deviation(SD)	0.00405
Correlation coefficient (r2)	0.9997
%RSD (Relative standared deviation)	0.5318
Range of errors	
Confidence limits with 0.05 level	0.00324
Confidence limits with 0.01 level	0.00425
Limits of detection (LOD)(μg ml^{-1})	0.60916
Limits of quantification (LOQ) (μg ml^{-1})	1.845

RSD of 6 independent determinations
RSD of 9 independent determinations (3 independent samples per day for 3 days

TABLE 2 : Assay results of doxazosin in formulations by visible method

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method	Amount found by the reference method (mg)	% Recovery
DOXACARD	250	249.43	244.5	99.77
		T=0.498		
		F=0.9998		
DURACARD	250	248.3	246	99.32
		T=o.4986		
		F=0.996		

T and F- values refer to comparison of the proposed method with reference method.
Theoretical values at 95% confidence limits t= 0.543 and F= 0.999

color species was stable for 24 hrs. The amount of Doxazosin present in the sample solution was computed from its calibration curve.

The color was developed using 10μg/ml of Doxazosin solution and 1ml of (0.2M) 1,10-Phenantrolline solution was added followed by 1ml of (0.2%) Ferric chloride solution and resulting solution was heated at 100 ^{0}c for 15 min. The solutions were

TABLE 3 : Determination of accuracy of doxazosin

Level of % Recovery	Amount of Dox in formulation (mg)	Amount of Standard Dox added (mg)	Total amount found (mg)	% Recovery
80%	248.65	200	447.57	99.46
	247.35	200	445.23	98.94
	247.12	200	444.81	98.85
100%	248.32	250	496.64	99.32
	248.3	250	496.6	99.32
	247.9	250	495.8	99.16
120%	248.31	300	546.28	99.32
	247.99	300	545.57	99.2
	247.6	300	544.72	99.04

TABLE 4 : Statistical data for accuracy determination

Level of % Recovery	Total amount found (mean)	Standard deviation	% RSD
80%	445.87	0.825	0.185
100%	496.34	0.236	0.0475
120%	545.52	0.3555	0.0651

The results are the mean of five readings at each level of recovered

TABLE 5 : Repeatability data for DOX at 510 nm

Conc. (μg/ml)	Abs 1	Abs2	Abs3	Mean	Std. deviation	(%)RSD
10	0.221	0.22	0.219	0.22	0.001	0.454
20	0.433	0.432	0.433	0.432	0.0057	1.319
30	0.635	0.634	0.636	0.635	0.001	0.157
40	0.878	0.877	0.876	0.877	0.001	0.114
50	1.0842	1.083	1.084	1.0837	0.00064	0.055
60	1.318	1.317	1.32	1.318	0.00152	0.115

Average of five determinations

cooled to room temp and added 1ml (0.1M) of orthophosphoric acid solution and made up to mark with distilled water in 10ml volumetric flask. The absorbance of orange red colored species was measured at 510nm against the reagent blank.

RESULTS AND DISCUSSION

Optical parameters

In order to ascertain the optimum wavelength of maximum absorption (□max) formed in UV spectrophotometric method and of the colored species formed in each so the four visible spetrophotometric methods, specified amount of Doxazosin in final solution 10μg/ml

TABLE 6 : Assay results of doxazosin in blood sample

Conc. in µg/ml	Time in Hours							
10	4	8	12	16	20	24	28	32
	0.223	0.213	0.209	0.203	0.198	0.195	0.189	0.182

Color stability data for 1, 10-phenantrolline method

TABLE 7 : Assay results of doxazosin in blood sample

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method in (mg)	Amount found by the reference method	% of Recovery
Doxacard	2 mg	1.242	1.23	99.4%
		T=0.00844		
		F=0.002979		
Duracard	2mg	1.225	1.23	99.75%
		T=0.00865		
		F=0.003211		

T and F values refer to comparison of the proposed method with reference method. Theortical values at 95% confidence limits T=0.00796 and F=0.0029

TABLE 8 : Determination of accuracy of doxazosin

Name of the Formulation in (mg)	Amount of Drug in Blood sample(mg)	Amount of Standard Drug added in (mg)	Total amount found (mg)	% Recovery
Doxacard(2mg)	1.24	2	3.26	81.5%
Duracard(2mg)	1.25	2	3.255	81.37%

The results are the mean of five readings at each level of recovered

TABLE 9 : Repeatability data for doxazosin at 510nm

Concentration in (ng)	Abs1	Abs2	Abs3	Mean	Std. Deviation	(%) RSD
10	0.0132	0.0133	0.0134	0.0133	0.0001	0.7518
20	0.0265	0.0265	0.0267	0.0265	0.0001	0.3773
30	0.0398	0.0397	0.0396	0.0397	0.0001	0.2518
40	0.0531	0.0531	0.0545	0.0535	0.0008	0.1495
50	0.0664	0.0665	0.0667	0.0665	0.000152	0.2255
60	0.0797	0.0798	0.0797	0..0797	0.00014	0.1756

Average of five determinations

was taken and the colors were developed following the above mentioned procedures individually the absorption spectra were scanned on spectrophotometer in the wavelength region of 380-800 nm (for Method) against corresponding reagent blanks. The regent blank absorption spectrum of each method was also recorded against distilled water /methanol. The results are graphically represented in (figure 2)

Parameters fixation

In developing these methods, a systematic study of the effects of various relevant parameters in the methods concerned were under taken by verifying one parameter at a time and controlling all other parameter to get the maximum color development, reproducibility and reasonable period of stability of final colored species formed. The following studies ware conducted.

Method

The results obtained in this method were based on oxidation followed by complex formation reaction of Doxazosin with 1,10-phenanthrolline, Ferric chloride and Orthophosphoric acid to form an orange red colored chromogen that exhibited maximum absorption at 510 nm against the corresponding reagent blank. The functional group used for the color development for this method was primary amine group. A schematic reaction mechanism of DOX with 1, 10-Phenantrolline reagent was shown in (figure 5) The effect of various parameters such as concentration and volume of 1, 10- Phenantrolline and strength of acid order of addition of reagents, solvent for final dilution were studied by means of control experiments varying one parameters at a time

Optical characteristics

The reference method adhere to beer's law the absorbance at appropriate wave length of a set of solutions contains different amounts of Doxazosin and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blank.

The beers law plot of the system illustrated graphically (figure 3) least square regression analysis was carried out for the slope. Intercept and correlation coefficient. Beer's law limits molar absorptivity, sandells sensitivity for Doxazosin with each of mentioned reagents was calculated. The optical characteristics were present in the TABLE 1.

In order to test whether the colored species formed in the method adhere the beer's law the absorbance at appropriate wavelength of a set of solutions contain dif-

ferent amounts of Doxazosin and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blanks or distilled water. The beers law plots of the system illustrated graphically figure 3, 4 least square regression analysis was carried out for the slope, intercept and correlation coefficient, beer's law limits molar absorptivity Sandells sensitivity for Doxazosin with each of mentioned reagents were calculated. The optical characteristics are presented in the TABLE 1.

Precision

The precision of each one among the five proposed spectrophotometric methods were ascertained separately from the absorbance values obtained by actual determination of a fixed amount of Doxazosin (10,20,30,40,50,60 µg/ml) in final solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods and presented in TABLE 1.

Analysis of formulations

Commercial formulations of Doxazosin were successfully analyzed by the proposed methods. The values obtained from the proposed and reference methods were compared statistically by the t and F tests and were found that those proposed methods do not differ significantly from the reported methods and they were presented in TABLES 2. The proposed methods also applied for Biological Samples (Blood) for good recoveries are obtained which were recorded in TABLE 6, 7, 8.

Accuracy

Recovery studies were carried by applying the method to Drugs sample present in formulations to which known amount of Doxazosin corresponding to 80%,100%and 120%, of label claim was added (standard addition method) in 80% recovery study. Amount of recovery study the amount of standard added is 250 mg of Doxazosin (i.e.100% addition). In 120% recovery study the amount of standard added is 300mg of Doxazosin (i.e. 120% addition). Like this recovery studies were carried by applying the method to Biological sample (Blood) to which known amount of Doxazosin correspond to 2mg Formulations taken by the patient. By the follow of Standard addition method 2mg of label claim was added. After the addition of these standards the contents were transferred to 100ml volumetric flash and dissolved in solvent. Finally the volume was made up to the mark with solvent. The solution was filtered through Whitman No. 41filter paper. The mixed sample solutions were analyzed and their absorbance value was determined. At each level of recovery five determinations were performed and present in TABLES4, 8. The results obtained were compared with expected results and were statistically validated in TABLES 3, 4.

Linearity and range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyze in sample with in a given range. The range of analytical method is the interval between the upper and lower levels of analyze that have been demonstrated within a suitable level of precision, accuracy and linearity.

Specificity and selectivity

Specificity is a procedure to detect quantitatively the analyse in the presence of components that may be expected to the present in the sample matrix. While selectivity is a procedure to detect the analyse qualitatively in presence of components that may be expected to present in the sample matrix. The excipient in formulations was spiked in a pre weighed quantity of Drugs and then absorbance was measured and calculations were done to determine the quantity of the Drugs.

Repeatability

Standard solutions of Doxazosin were prepared and absorbance was measured against the solvent as the blank. The observance of the same concentration solution was measure five times and standard deviation was calculated and presented in TABLE 3, 9.

Interferences studies

The effect of wide range of inactive, ingredients usually present in the formulations for the assay of Doxazosin under optimum conditions was investigated. None of them interfered in the proposed methods even when they are present in excess fold than anticipated in formulations.

CONCLUSION

The proposed method can be used for determination of Doxazosin in Formulations. The method is rapid, simple and has great sensitivity and accuracy. Proposed method makes use of simple reagents, which an ordinary analytical laboratory can afford. Method is sufficiently sensitive to permit determination even down to 10μg ml^{-1}. The proposed method is suitable for routine determination of Doxazosin in its formulation and Blood. The commonly used additives such as Starch, Lactose, Titanium dioxide, and Magnesium state do not interfere with the assay procedures.

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