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DEVELOPMENT AND VALIDATION OF THE HPLC METHOD FOR THE ANALYSIS OF DOXAZOSIN IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORMS

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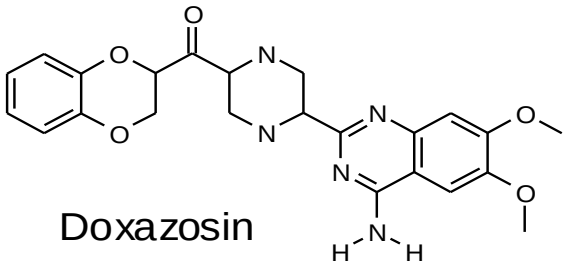
ABSTRACT

A simple, economic, selective, precise, and accurate High Performance liquid Chromatographic method for the analysis of Doxazosin in bulk drug and pharmaceutical formulations was developed and validated in the present study. The mobile phase consists of a mixture of Methanol and Potassium Dihydrogen Orthophosphate in the proportion 60: 40. And adjust the pH to 5.0 ± 0.05 with sodium hydroxide solution. This was found to give a sharp peak of Doxazosin at a retention time of 4.484 min. HPLC analysis of Doxazosin was carried out at a wavelength of 251nm with a flow rate of 1.0mL/min. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient of 0.999 in the concentration range of 50 ppm to 150 ppm. The linear regression equation was y =28.34x-16.91. The developed method was employed with a high degree of precision and accuracy for the analysis of Doxazosin. The developed method was validated for accuracy, precision, robustness, detection and quantification limits as per the ICH guidelines. The wide linearity range, accuracy, sensitivity, short retention time and composition of the mobile phase indicated that this method is better for the quantification of Doxazosin.

INTRODUCTION: Doxazosin mesylate (4-amino-2-[4-(1,4-benzodiaxan-2-carbonyl)- piperazine-1-yl]-6,7-methoxyquinazoline myselate), a quinazoline derivative, is effective and well known for treatment of hypertension and benign prostatic hyperplasia by selectively blocking α-1 Adrino receptor [1–3]. It is structurally similar to prazosin (**Figure 1**).



Different analytical methods have been reported for determination of Doxazosin in biological fluids [4–6] and pharmaceutical formulations [7–10].

For analysis of human plasma, almost all of these methods were based on a high-performance liquid chromatographic (HPLC) system with fluorescence detection ([4–6]).

Fouda *et al*., reported the method using an alumina - based column and glass-bead guard column (4); both column systems were expensive and may not be available for purchase. In addition, the latter technique used a high-pH mobile phase, which may deteriorate the HPLC instrument.

Moreover, in the previous studies, either the usage of solvent extraction procedure [4–6] or solid-phase extraction (SPE) [6] prior to HPLC determination seems to be a more complicated and lengthy method. Generally, sensitive HPLC methods with fluorescence detection that have been reported for Doxazosin are stated to detect levels no lower than 1ng/ml [5–6].

The empirical formula for Doxazosin mesylate is $C_{23}H_{25}N_5O_5$ and the molecular weight is 451.47 grams. It has the following structure.

The HPLC method described here is simple, sensitive, and reproducible for Doxazosin determination in Formulations with low background interference. An attempt has been made to develop and validate to ensure their accuracy, precision and other analytical method validation parameters as mentioned in various gradients. One method reported for the HPLC determination for developed based on the use of a C-18 column, with a suitable mobile phase, without the use of any internal standard. For pharmaceutical formulation the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations.

Experimental:

Instrumentation: HPLC Analytical column Chromolith RP-C18, 100 mm x 10 µ (C-098 & C-099)

CHROMATOGRAPHIC CONDITIONS FOR DOXAZOSIN

Stationary phase	Mobile phase	Flow rate (ml/min)	Run time (min)	Column Temp/ ^oC	Volume of injection loop (µl)	Detection wavelength (nm)	Retention time (min)
C8 waters symmetry	Buffer: Methanol (60:40)	1.0	10	25	20	251	4.484

1. **Preparation of Mobile phase**: For isocratic system, prepare a mixture of Methanol and buffer in the proportion 60:40, and mixed well. Adjust the P^H to 5.0±0.05 with Sodium hydroxide solution. Filter through 0.2 µm Nylon membrane filter paper and degassed prior to use.

2. **Preparation of diluents:** Weigh accurately and transfer 6.8 g of Potassium Dihydrogen Orthophosphate to a 1000 ml volumetric flask. Add about 980 ml of water, dissolve and dilute to volume with water.

3. **Chromatographic conditions**: Separation was performed on Chromolith RP-C18; 100 mm X 4.6 mm X 10 µm column. Mobile phase consists of mixture of Methanol and Potassium Dihydrogen Orthophosphate in the ratio 60:40. Injection volume of 20 µl was used. Mobile phase was filtered before use through 0.2 µm Nylon membrane filter paper and degassed with helium purge for 30 min. The components of the mobile phase were pumped from solvent reservoir to the column at flow rate 1.0 ml/min and wavelength was set to 251 nm. The column temperature was set at 25^{o}C.

4. **Preparation of Doxazosin mesylate Standard Solution**: Weighed accurately about 20 mg of Doxazosin mesylate working standard and transferred to a 20 ml volumetric flack. Add 10 ml of diluents and sonicated to dissolve. Dilute to volume with diluents and mixed. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluents and mixed.

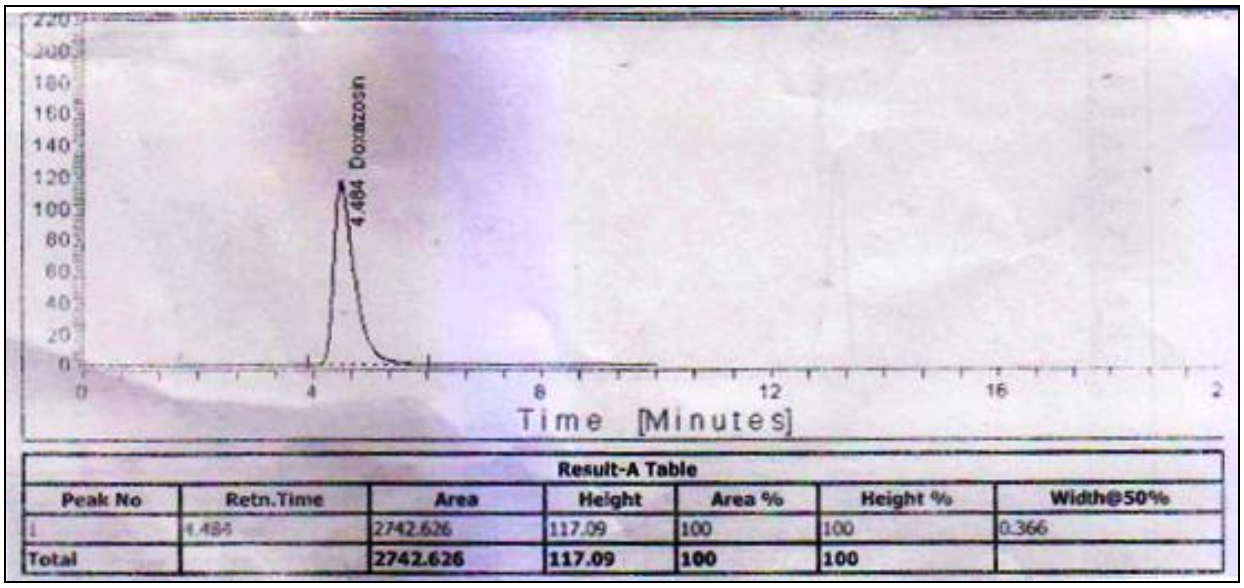
(Dilution Scheme: 20 mg→20.0 ml → 5 ml/10.0 ml)

5. **Preparation of Test Solution**: Weighed and transferred 139.7 mg of sample powder into a 20 ml volumetric flask. Added about 10 ml of diluents and shaken for 20 minutes by manually and further sonicate for 30 minutes. Diluted up to the mark with diluents. Centrifuge this solution at 8000 rpm for 10 minutes. Decanted the supernatant solution into another test tube and transferred 1.0 ml of supernatant solution into another 10 ml volumetric flask and make up the volume with diluents. Further transfer 1.09 ml of solution into another 10 ml volumetric flask and make up the volume with diluents. Filter the solution through 0.45 µm Nylon membrane filter paper.

(Dilution scheme: 139.7 mg→1 ml/10.0 ml→1 ml/10.0 ml)

Assay procedure: The column was equilibrated for at least 30 minutes with mobile phase flowing through

the system with a flow rate of 1.0 ml/min. Detector was set at a wavelength of 251 nm. Five sets of the Drug solutions were prepared in diluents containing Doxazosin at a concentration range of 50 ppm - 150 ppm. Then, 20 µl of each standard and sample solution were injected for five times separately. The retention time for Doxazosin was found to be 4.484 **(Fig. 2)**. The peak areas of the Drug concentrations were calculated. The Regression of the Drug concentration over the amount of Drug in formulations. The linearity plot of peak area of Doxazosin mesylate vs. standard concentration in percentage is presented in **Fig. 2.**



Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	4.484	2742.626	117.09	100	100	0.366
Total		2742.626	117.09	100	100	

FIG. 2: CHROMATOGRAM OF DOXAZOSIN

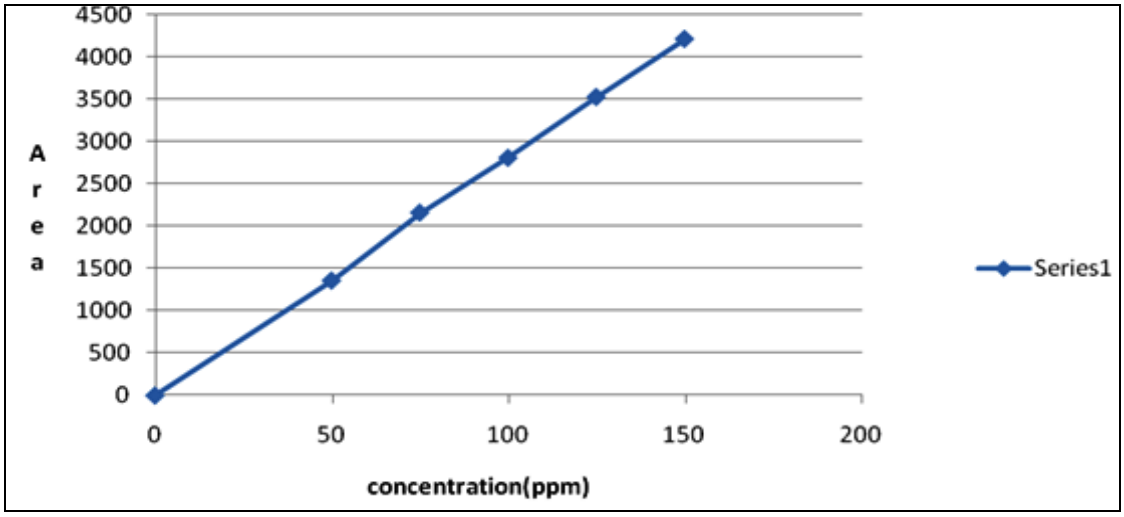


FIG. 3: LINEARITY OF DOXAZOSIN

TABLE 1: SYSTEM SUITABILITY – LINEARITY OF STANDARD

Sr. No.	Area of Doxazosin mesylate	Tailing factor	Theoretical plates
1	2812.03		
2	2821.82		
3	2815.78		
4	2742.63		
5	2801.14	1.17	4352
Mean	2798.68		
Standard Deviation (±)	32.23		
(%) Relative Standard Deviation	1.15		

TABLE 2: RESULTS OF LINEARITY OF STANDARD

Linearity Level	Sample Concentration (in %)	Sample Concentration (in ppm)	Average Area (n=3)	Correlation Coefficient
Level-1	50	50	1359.98	
Level-2	75	75	2163.54	
Level-3	100	100	2812.36	0.999
Level-4	125	125	3531.65	
Level-5	150	150	4218.6	

Table 3: Results of linearity of sample

Linearity Level	Sample Concentration (in %)	Sample Concentration (in ppm)	Average Area (n=3)	Correlation Coefficient
Level-1	50	50	1320.68	
Level-2	75	75	2123.88	
Level-3	100	100	2819.31	0.999
Level-4	125	125	3538.55	
Level-5	150	150	4228.77	

The linearity plot of peak area of Doxazosin mesylate Vs. Sample concentration in percentage

TABLE 4: RESULTS OF LINEARITY OF STANDARD IN PRESENCE OF PLACEBO

Linearity Level	Sample Concentration (in %)	Sample Concentration (in ppm)	Placebo added to the standard solution (mg)	Average Area (n=1)	Correlation Coefficient
Level-1	50	50	137.2	1338.38	
Level-2	75	75	137.2	2122.58	
Level-3	100	100	137.2	2786.42	0.999
Level-4	125	125	137.2	3467.2	
Level-5	150	150	137.2	4205.67	

The linearity plot of peak area of Doxazosin mesylate Vs. Standard concentration in presence of placebo in percentage

TABLE 5: RESULTS OF METHOD PRECISION

Test solution	% Assay of Doxazosin
1	101.03
2	100.68
3	101.13
4	100.80
5	101.78
6	101.13
Mean	101.09
Standard Deviation(±)	0.38
(%) Relative Standard Deviation	0.38

Five test solutions of Doxazosin were prepared as per the analytical method. The % of RSD of Five test solutions was calculated.

TABLE 6: RESULTS OF INTERMEDIATE PRECISION

Test solution	% Assay of Doxazosin
1	101.69
2	100.12
3	101.68
4	101.06
5	100.57
6	101.41
Mean	100.92
Standard Deviation(±)	0.58
(%) Relative Standard Deviation	0.57

Five test solutions of Doxazosin were prepared as per the analytical method. The % of RSD of Five test solutions was calculated

TABLE 7: RESULTS FOR ACCURACY (% RECOVERY)

Level of addition	Amount of Doxazosin Mesylate added (in mg)	Amount of Doxazosin Mesylate found (in mg)	Recovery (%)
First Level (Rec -50%)	9.9	9.83	99.29
Second Level (Rec -75%)	15.7	15.59	99.30
Third Level (Rec -100%)	20.5	20.47	99.85
Fourth Level (Rec -125%)	25.6	25.47	99.49
Fifth Level (Rec -150%)	31.1	30.9	99.36
	Mean		99.46
	Standard Deviation(±)		0.23
	(%) Relative Standard Deviation		0.24

These test solutions were prepared by adding a quantity of Doxazosin mesylate API to excipient blend to produce three different concentration solution equivalent to 50 %, 75 %, 100 %, 125 % and 150 % of test concentration

TABLE 8: ROBUSTNESS

TABLE (8.1): ROBUSTNESS WITH CHANGE IN COLUMN LOT

Flow rate	Same column	Diff column
Sample	**% Assay**	
Test solution	101.25	99.62
Average assay result from method precision	100.09	100.09
Mean	100.67	99.86
Standard Deviation(±)	0.82	0.33
(%) Relative Standard Deviation	0.81	0.33

TABLE (8.2): ROBUSTNESS WITH CHANGE IN FLOW RATE

Flow rate	0.8 mL/minute	1.2mL/minute
Sample	**% Assay**	
Test solution	101.56	101.66
Average assay result from method precision	100.09	100.09
Mean	100.83	100.88
Standard Deviation(±)	1.04	1.11
(%) Relative Standard Deviation	1.03	1.10

TABLE (8.3): ROBUSTNESS WITH CHANGE IN WAVELENGTH

Wavelength	249 nm	253 nm
Sample	**% Assay**	
Test solution	100.92	99.56
Average assay result from Method precision	100.09	100.09
Mean	100.51	99.83
Standard Deviation(±)	0.59	0.37
(%) Relative Standard Deviation	0.58	0.38

TABLE (8.4): ROBUSTNESS WITH CHANGE IN PH OF MOBILE PHASE

pH	4.8	5.2
Sample	**%Assay**	
Test solution	100.81	101.27
Average assay result from method precision	100.09	100.09
Mean	100.45	100.68
Standard Deviation(±)	0.51	0.83
(%) Relative Standard Deviation	0.51	0.83

TABLE 9: RESULTS FOR SOLUTION STABILITY

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Doxazosin mesylate**
0^{th}hr	101.67
12^{th}hr	98.57
24^{th}hr	101.13
36^{th}hr	100.74
48^{th}hr	99.57
Mean	100.34
Standard Deviation(±)	1.25
(%) Relative Standard Deviation	1.25

RESULTS AND DISCUSSION: The appropriate wavelength in UV region has been selected for the measuring of active ingredient in the proposed method. This method was validated by linear fit curve and all the parameters were calculated.

Parameters Fixation: In developing methods, systematic study of the effects of various parameters was undertaken by varying one parameter at a time controlling all other parameters. The following studies were conducted for this purpose.

A) **Mobile phase characteristics**: In order to get sharp peaks and baseline separation of the components, carried out number of experiments by varying different components like percentage of organic phase in the mobile phase, total pH of the selected mobile phase and flow rate by changing one at a time and keeping all other parameters constant. The optimum conditions obtained were included in the procedure proposed.

B) **Detection Characteristics**: To test whether Doxazosin had been linearly eluted from the column, different amounts of Doxazosin were taken and analyzed by the above mentioned procedures. The peak area ratios of component areas were calculated and the values are graphically represented in Fig - 2, the linear fit of the system was illustrated graphically. Least square regression analysis for the method was carried out for the slope, Intercepts and correlation coefficient. The results are presented in **Table 2**.

C) **Performance Calculations:** To ascertain the system suitability for the proposed method, a number of statistical values have been calculated with the observed readings and the results are recorded in **Table 1**.

D) **Method validations**: The UV absorption maximum for Doxazosin was fixed at 251 nm respectively. As the final detection was made by the UV absorption spectrum, each method was validated by linear fit curve.

E) **Precision**: The precision of the method was ascertained separately from the peak area ratios obtained by actual determination of a fixed amount of Drug. The percent of Relative Standard deviation calculated for Doxazosin and are presented in **Table 5**. The precision of the assays was also determined in terms of intra and inter-day variation in the peak areas for a set of Drug solution was calculated in terms of coefficient of variation (CV) and the results are presented in **Table 6**.

F) **Accuracy**: To determine the accuracy of the proposed methods, different of technical grade samples of Doxazosin within the linearity limits were taken and analyzed by the proposed methods. The results (percent error) are recorded in **Table 7**.

G) **Interference Studies**: The effect of wide range of excipients and other additives usually present in the formulations of Doxazosin in the determinations under optimum conditions were investigated. The common excipients such as colloidal Silicon dioxide, ethyl cellulose, hydroxyl propyl methyl cellulose, magnesium state, microcrystalline cellulose provide have been added to the sample solutions and injected. They have not disturbed the elution or quantification of Drug. In fact many have no absorption at this UV maximum.

H) **Analysis of Formulation**: To find out the stability of the proposed methods for the assay of formulations containing Doxazosin was analyzed by the proposed and reference methods. The proposed method does not differ significantly in precision and accuracy from reference method. The results are recorded in Table 7.

I) **Ruggedness and Robustness**: Ruggedness of the proposed method was determined by carrying out the analysis by two different analysts using similar operational i.e. Robustness with Change in Column Lot, Change in Flow rate, Change in wavelength and Change in p^H of the Mobile phase . The results were indicated by % CV in **Table 8.1, 8.2 & 8.4**. Robustness of the method was determined by carrying out the analysis at two different wavelengths i.e. at 249 nm and 253 nm and the results were indicated by % CV in Table **8.3**.

J) **Recovery Studies:** Recovery studies were conducted by analyzing each formulation in the first instance for the active ingredient by the proposed methods known amounts of pure Drug was then added to each of the previously analyzed formulations and the total amount of the Drug was once again determined by the proposed methods after bringing the active ingredient concentration within the linearity limits. The results are recorded in Table 7.

K) **Solution Stability**: The stability of the solutions under study was established by keeping the solution at room temperature for 48 Hours. The results indicate no significant change in assay values indicating stability of Drug in the solvent used during analysis. The results are recorded in **Table 9**.

CONCLUSION: The method was found to be accurate and precise, as indicated by recovery studies close to 100 and % RSD is not more than 2. The summery of validation parameters of proposed HPLC method is given in tables. The simple, accurate and precise HPLC method for the determination of Doxazosin mesylate as bulk and in tablet dosage form has been developed. The method may be recommended for routine and quality control analysis of the investigated drug in bulk and pharmaceutical formulations. The analytical solution is found to be stable up to 48 Hrs at room temperature. Hence, it is concluded that the analytical method is validated and can be used for routine analysis and for stability study.

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