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Development and validation of UV spectrophotometric method for determination of Doxazosin Mesylate in tablet formulation

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ABSTRACT

Determination of Doxazosin Mesylate in a fixed dosage form was carried out by UV Spectrophotometric method. The absorbance values were observed for different dilutions of drug at 245nm and which were used for the estimation of drug without mutual interference of excipients. The solvent used for the dilution was 0.01 N HCl. This method obeys Beer- Lambert's law in the concentration range of 2-10 µg/ml. The results of analysis have been validated statistically and the recovery studies confirmed the accuracy of this proposed method.

Key words: Doxazosin Mesylate, UV Spectrophotometry, Method validation.

INTRODUCTION

Doxazosin mesylate is is indicated for the treatment of the signs and symptoms of benign Prostatic Hyperplasia (BPH). Chemically it is a quinazoline derivative. Its IUPAC name is 1-(4-Amino-6, 7-dimethoxyquinazolin-2-yl)-4-[(2*RS*)-2, 3-dihydro-1, 4-benzodioxin-2-ylcarbonyl] piperazine methanesulphonate. HPLC methods were reported for determination of Doxazosin mesylate. The review of literature revealed that no method is reported for the Doxazosin mesylate determination by UV spectroscopy method. The present paper describes a simple, rapid, accurate and reproducible method for the estimation of Doxazosin Mesylate in tablet formulation.

MATERIALS AND METHODS

Materials

Doxazosin Mesylate was procured as a gift sample from Aurobindo Pharma, Hyderabad. Freshly prepared 0.01N HCl, other chemicals and reagents were of analytical grade. Double distilled water was used for the study. The commercially available marketed tablet brand containing Doxazosin Mesylate, 4 mg in each tablet have been used for estimation.

Instruments

A Shimadzu UV-Visible Spectrophotometer (UV-1800) with a matched pair of 10 mm quartz cuvettes and Analytical Weighing Balance (BSA 224 S, Sartorius) were used for experimental purpose.

Preparation of Standard Stock Solution and Calibration Curve
Standard stock solution was prepared by dissolving Doxazosin Mesylate in 0.01 N HCl to make final concentration of 100 µg/ml. Different aliquots were taken from stock solution and diluted with 0.01 N HCl separately to prepare series of concentration from 2 – 10 µg/ml. An independent stock solution of 5 µg/ml was also prepared. The λ max was found by UV spectrum of Doxazosin Mesylate in 0.01 N HCl, in range of 200 – 400 nm and it was found to be 245 nm as shown in Fig 1. Absorbance was measured at 245 nm against 0.01 N HCl as blank. The calibration curve was prepared by plotting absorbance versus concentration of Doxazosin Mesylate.

Analysis of Marketed preparation [2]
The proposed procedure was adopted for determination of Doxazosin Mesylate in tablets in following manner. The marketed tablet formulation Doxacard® (4mg) of Doxazosin Mesylate was used for the purpose of analysis. Twenty tablets were weighed and average weight was calculated and crushed to fine powder. The powder equivalent to 4 mg Doxazosin Mesylate was accurately weighed and taken in 100 ml volumetric flask and dissolved in 0.01 N HCl by intermittent shaking. Volume was then made up to 100 ml using 0.01N HCl. The solution was then filtered through Whatmann filter paper no.41. This solution was used as stock solution. The solution was further diluted to prepare 5 ppm solution and then analyzed using UV spectrophotometer.

Method Development [2,3,4,5,8]
This method was validated with respect to linearity, range, accuracy, precision, specificity, Ruggedness.

Specificity
Identification:
The UV absorption spectrum of the sample preparation for assay is concordant with the reference spectrum of standard sample from assay preparation.

Placebo Interference:
Placebo solution was prepared in the same manner as standard and sample preparation. No interference of placebo was found.

Linearity and Range:
The prepared aliquots i.e. series of dilutions 2 – 20 µg/ml were prepared from the stock solution and were scanned for absorbance at λmax 245 nm. . Least square regression analysis was performed on the obtained data.

Accuracy:
Accuracy of the method is closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentration - lower concentration (LC, 80%), intermediate concentration (IC, 100%) and higher concentration (HC, 120%) were prepared from independent stock solution of 5 µg/ml and analyzed (n=10). Accuracy was assessed as the percentage relative error and mean percentage recovery. To provide an additional support to the accuracy of the developed assay method, a standard addition method was employed, which involved addition of different concentration of pure drug (2,4 ppm) to a known pre-analyzed formulation sample and the total concentration was determined using the proposed method,(n=10). The percent recovery of the added pure drug was calculated as percent recovery= [(Ct-Cs)/Ca] x 100, where Ct is the total drug concentration in the formulation sample; Ca is the drug concentration added to the sample.

Precision:
Repeatability studies were done by repeatedly observing the absorbance of standard solution containing 5 µg/ml. Inter- day and intra-day variation were studied to determine intermediate precision of the proposed analytical method.

Inter- day precision:
Inter-day precision was found out by preparing 5 µg/ml concentration of Doxazosin mesylate solution for three days and standard deviation was calculated.

Intra-day precision:
Intra-day precision was found out by preparing 5 µg/ml concentration of Doxazosin mesylate solution for six times in a day and then analyzed each time. Standard deviation was calculated.

Stability:
The sample was subjected for stability studies under room temperature. Stability studies were conducted to check whether any changes occur in absorbance with the time.

Robustness:
Robustness was determined by analyzing Doxazosin Mesylate concentration on different days by different analysts

RESULTS AND DISCUSSION

As shown in the fig 1. λ max of Doxazosin mesylate was found to be 245 nm.

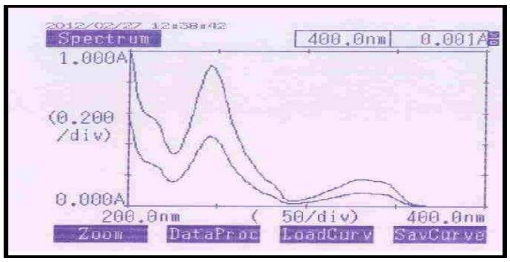


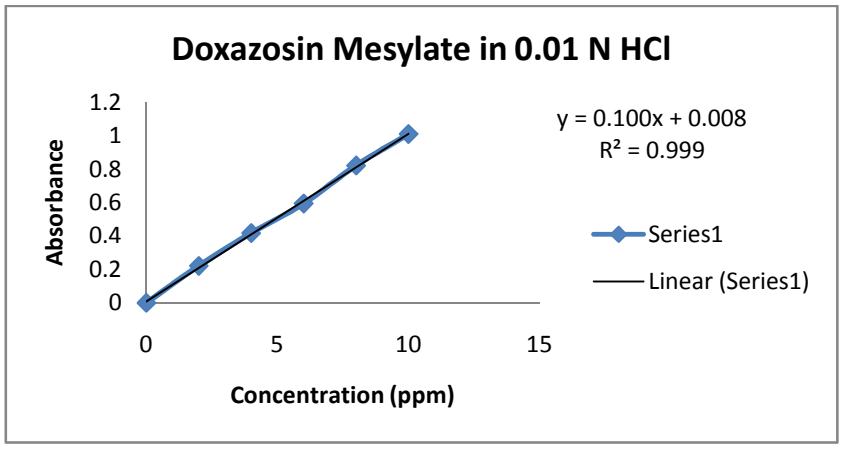
Fig. 1: UV Spectra of Doxazosin Mesylate

Linearity and Range:

Table 2: Linearity

Sr. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.221
3	4	0.417
4	6	0.594
5	8	0.821
6	10	1.009

Fig.2: Beer-Lambert's plot (Absorbance v/s concentration) Calibration curve



From the fig 2. It is clear that the Beer-Lambert's law was obeyed in the concentration range of 2-10µg/ml with regression coefficient (r^2value) 0.9991 with the absorbance ranging from 0.221 – 1.009.

Accuracy:

Table 2: Accuracy data for the developed method

Sr.No.	Level	Estimated concentration (µg/ml)			Mean % Recovery (± S.D)	Accuracy* (%)
		Range	Mean(±S.D)	% RSD		
1	LC-4 ppm	3.96-4.06	4.03 (0.036)	0.9	100.75	0.75
2	IC-5 ppm	4.93-5.02	4.97(0.048)	0.965	99.4	-0.6
3	HC-6 ppm	5.91-6.00	5.96(0.075)	1.26	99.33	-0.66

Table 3: Standard addition of Doxazosin mesylate for accuracy (n=10)

Sr.No.	Drug in formulation (µg/ml)	Pure drug added (µg/ml)	Total drug found (µg/ml)(±S.D)	% Recovery (±R.S.D.)
1	5	0	4.96 ± 0.044	99.2 ± 0.87
2	5	2	6.97 ± 0.037	99.6 ± 0.74
3	5	4	8.92 ± 0.069	99.11 ± 0.98

The excellent mean% recovery values, close to 100% and their low standard deviation values (S.D<1.0) indicate high accuracy of the analytical methods. The validity and reliability of the proposed methods was assessed by the recovery studies. In 0.01 N HCl, mean % recoveries for lower, intermediate and higher concentrations were found to be 100.75, 99.4, and 99.33 respectively. The validity and reliability of the proposed methods was further assessed via recovery studies by the standard addition method (Table 3). The mean% recoveries (%R.S.D) for the intermediate concentration were found to be 99.2 (0.87), 99.6(0.74), 99.11(0.98), respectively. These reveal that any small change in the drug concentration in the solutions could be accurately determined by the proposed analytical method.

Precision:

Table 4: Repeatability data

Doxazosin Mesylate			
Sr.No.	**Concentration (µg/ml)**	**Absorbance**	**% R.S.D.**
1	**5**	**0.514**	**0.613**
2		**0.507**	
3		**0.506**	
4		**0.512**	
5		**0.508**	
6		**0.508**	

Table 5: Inter-day Precision

Day	Doxazosin Mesylate (5 µg/ml)	
	Absorbance	**% R.S.D.**
1st day	**0.515**	**0.49**
	0.512	
	0.510	
2nd day	**0.511**	**0.39**
	0.509	
	0.513	
3rd day	**0.508**	**0.49**
	0.511	
	0.513	

The precision was determined by studying the repeatability and intermediate precision. The repeatability results indicated the precision under the same operating conditions over a short interval of time. Intermediate precision was expressed within different days i.e. both intra- day and inter-day precision was observed. R.S.D. values for the proposed analytical method were well within the acceptable range, indicating that the method has excellent repeatability and intermediate precision

Table 6: Intra – day Precision

Doxazosin Mesylate			
Sr.No.	**Concentration (µg/ml)**	**Absorbance**	**% R.S.D.**
1	**5**	**0.514**	**0.94**
2		**0.505**	
3		**0.504**	
4		**0.516**	
5		**0.508**	
6		**0.508**	

Stability:
The sample was subjected for stability studies under room temperature. The solution was stable for up to 5 hours with % R.S.D. less than 1 as shown in Table 7.

Table 7: Stability data

Sr.No.	**Time (Hrs.)**	**Doxazosin Mesylate**		**% R.S.D.**
		Concentration(µg/ml)	**Absorbance**	
1	**1**	**5 µg/ml**	**0.513**	**0.22**
2	**2**		**0.514**	
3	**3**		**0.512**	
4	**4**		**0.513**	
5	**5**		**0.511**	

Robustness:

Table 8: Data for Robustness test

Sr.No.	**Variable Parameters**	**Assay results (%)**
1	Analyst 1	**98.3**
	Analyst 2	**99.1**
2	Day 1	**99.4**
	Day 2	**99.8**

Estimation of formulation:
The estimated drug content with low values of standard deviation established the precision of the proposed method.

Table 9: Estimation of formulation

Sr.No.	**Brand Name**	**Amount of Drug (mg)**		**% Recovery (±S.D)**	**Accuracy* (%)**
		Labeled	**Estimated (±S.D.)**		
1	Doxacard® 4	4	3.93 (±0.015)	98.25(±0.375)	-1.75

* accuracy given in % relative error $= (\frac{Predicted\ concentration - Nominal\ concentration}{Nominal\ concentration}) \times 100$

CONCLUSION

A UV-Spectrophotometric method was developed for Doxazosin Mesylate determination. The analytical method is simple sensitive, rapid and specific and it can be conveniently employed for the routine analysis and the quality control of Doxazosin mesylate in pharmaceutical dosage forms. The method was suitable to determine concentrations precisely and accurately. The sample recovery from the formulation was in good agreement with its respective label claim.

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