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Development and Validation of Stability Indicating Method for Terazosin in Bulk and Pharmaceutical Formulation by UV-Spectrophotometer

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Abstract: Terazosin hydrochloride is indicated in the symptomatic relief of benign prostatic hyperplasia and it is also used as an antihypertensive agent. A rapid, simple, specific, and economic UV-spectrophotometric method has been developed to determine the stability indicating method for terazosin using water as a solvent. The absorption spectrum of terazosin showed maximum absorbance at 250 nm and obeyed Beer's law in concentration range 1.5-9 µg/ml. Linear regression of absorbance on concentration gave the equation y=0.1103x+0.0101 with correlation coefficient 0.999. The method developed is validated for parameters like specificity, linearity, range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness as per Q2A specifications of the ICH guidelines. The drug sample was exposed to acid, alkali, and oxidation by hydrogen peroxide, thermal and photolytic conditions to study the forced degradation of the drug. The obtained results proved that this method can be employed for the routine analysis of terazosin in bulk as well as in the commercial formulations.

Keywords: Terazosin, UV-Spectrophotometry, Maximum absorbance, Beer's law, Correlation coefficient, ICH guidelines, hypertension, Prostatic hyperplasia

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Introduction

Terazosin is a selective alpha1-antagonist used for treatment of benign prostatic hyperplasia (BPH). It also acts to lower blood pressure, so it is a drug of choice for men with hypertension and prostate enlargement. It works by blocking the action of adrenaline on smooth muscle of the bladder and the blood vessel walls. Chemically Terazosin is [4- (4- amino- 6, 7- dimethoxyquinazolin- 2- yl) piperazin-1-yl]-(oxolan-2-yl) methanone (Fig. 1). From the literature survey some supporting information were referred in the present research work such as, validated specific HPLC method for

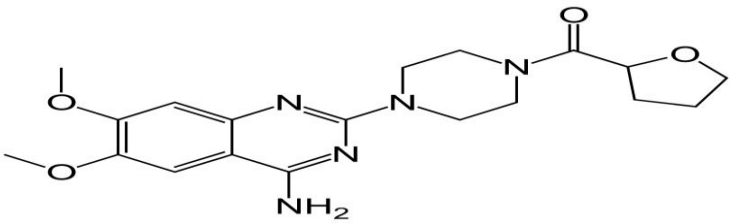

Fig. 1: Chemical structure of Terazosin.

determination of prazosin, terazosin and doxazosin in the presence of degradation products formed under ICH recommended stress conditions (Bakshi *et al,*, 2004), determination of terazosin by MBTH and BPB reagents using spectrophotometric technique (Ramachandra and Naidu, 2016), Spectrophotometry method for the determination of Terazosin in tablet formulation (Shrivastava and Dhakad, 2016), stability indicating RP-HPLC method for the simultaneous determination of Prazosin, terazosin, Doxazosin in pharmaceutical formulations (Shrivastava and Gupta, 2012), stability indicating HPTLC determination of Terazosin in tablet formulation (Shrivastava *et al.*, 2013), Spectrophotometric method development and validation of prazosin (Nikam *et al.*, 2016), a new validated spectrophotometric method for the estimation of rabeprazole sodium in tablet dosage form (Domatoti andKoppula, 2012) and Stress degradation studies on telmisartan and development of a validated method by UV spectrophotometry in bulk and pharmaceutical dosage forms (Patel *et al.*, 2011). There are a few available literatures which explain the method development, validation and stability studies for estimation of Terazosin by UV-Spectrophotometric method. The aim of this investigation was to develop and validate an accurate UV-Spectrophotometric method for Terazosin. The developed UV-Spectrophotometric method was subjected to validation parameters like Precision, Linearity, Robustness, Ruggedness, accuracy was established as per the guidelines recommended by ICH (Shabir, 2003). Stability indicating nature of the method was also performed by exposing the sample under various conditions like acid, base, peroxide, heat and photo stability conditions.

Materials and Methods

Chemicals and reagents:

Terazosin was procured from Hetero Pharma Limited, Hyderabad, India. Hytrin® 2 mg tablet from Abbott Pharmaceuticals, India tablet formulation was used for sample preparation. All the chemicals and reagents used were of analytical grade (Merck, Hyderabad, India).

Instruments:

All absorbance measurements were done with UV-Spectrophotometer with 1 cm matched quartz cell and Borosil glass wares were used for the study. All weighing was done on electronic balance.

Preparation of Standard stock solution of Terazosin:

An accurately weighed quantity of Terazosin 100 mg was transferred to 100ml volumetric flask, dissolved in 100 ml water, the final volume was made with water to obtain standard solution having concentration of 1000 µg/ml. 10 ml of this solution was transferred to 100ml volumetric flask, volume was made with water. It gives 100 µg/ml. These stock solutions were used to prepare further dilutions.

Preparation of working standard solutions:

Six working standard solutions of Terazosin were prepared by pipetting 0.15, 0.3, 0.45, 0.6, 0.75, 0.9 ml of stock solution in 10 ml of volumetric flasks and the volume was adjusted up to mark with distilled water to make the concentrations 1.5, 3, 4.5, 6, 7.5, 9 µg/ml.

Selection of Analytical wavelength:

For selection of analytical wavelength, the standard solution was further diluted and then scanned in UV spectrophotometer from a range of 200-400 nm against water as blank and the wavelength corresponding to maximum absorbance (λmax) was found at 250 nm.

Preparation of sample solution:

Terazosin is available as tablets of extended release containing 2 mg of Terazosin. Terazosin is available in the local market with brand names Hytrin® (2 mg, Abbott Laboratories, India). Twenty tablets of Terazosin were taken and triturated into a fine powder and the powder equivalent to 10 mg of Terazosin was weighed accurately and transferred into a 100 ml standard volumetric flask. The contents were dissolved in water and sonicated for 30 min. This entire solution was filtered through 0.45 micron Whatmann filter paper (No. 41) and the final solution was made up with water to get the solution of 100µg/ml. 10ml of this filtrate solution was transferred into 100ml volumetric flask, volume was made with water to get 10µg/ml. From the above solution 3 ml were pipetted out separately into 10 ml volumetric flask to give 3 µg/ml concentrations. The sample solution absorbance was measured at 250 nm against blank solution of water.

Estimation of drug content in tablet formulation:

Assay procedure:

A series of sample solutions i.e. 3 µg/ml of Terazosin was scanned and the absorbance of sample solutions were measured. The amount of Terazosin in tablet dosage form was determined by fitting the responses into the regression equations of the calibration curve and the results obtained were comparable with the corresponding label claim to obtain % recoveries (Table 1).

Validation of Proposed Method:

The method was validated according to ICH guidelines in order to determine the linearity, precision, accuracy and ruggedness of the method.

Specificity:

The analyte was assessed in the presence of the components and it was found that there was no interaction with the analyte.

Linearity:

Linearity was assessed by performing measurement at several analyte concentration. Varying quantities of stock solution was diluted with the water to give 1.5, 3, 4.5, 6, 7.5, 9 µg/ml of Terazosin. The calibration curve was obtained by plotting absorbance against concentration (µg/ml).

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation.

(a) *Repeatability*:

Repeatability was determined by preparing six replicates of 3 µg/ml of sample and standard separately to determine the system precision and method precision, respectively and the absorbance was measured at 250 nm.

(b) *Intermediate precision*:

Intermediate precision was determined by the typical variations to be studied on different days (interday and intraday) by different analysts (analyst 1 and analyst 2) on different instruments (two different instruments). The tests were carried out by preparing drug solution of concentration 3 µg/ml of Terazosin and analyzing. The results were reported as % RSD. The precision result showed a good reproducibility with per cent relative standard deviation less than 2.

(c) *Reproducibility*:

Reproducibility is assessed by means of an inter-laboratory trail. This is the precision between two laboratories.

Accuracy:

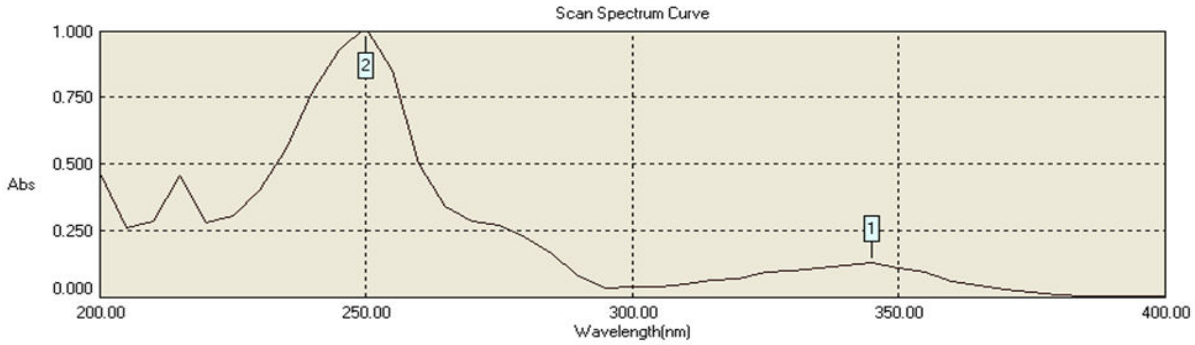

Fig. 2: Wavelength selection at 250 nm.

Table 1: Assay of commercial formulations

Sample no	Formulation	Label claim (mg)	Amount found (mg)	% Recovery
1	Hytrin® Tablet	2	1.99	99.56

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated.

Limit of Detection and Limit of Quantitation:

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined on the basis of response and slope of the regression equation. LOD and LOQ of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines:

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve

Ruggedness Studies:

Ruggedness studies were performed by preparing six replicates of 3 µg/ml of Terazosin, analysing by two different analysts and on two different instruments and the results are reported as % RSD.

Robustness Studies:

The Robustness of the method was determined by making slight changes in the experimental conditions such as the ±2 nm wavelength and temperature and scanning the samples maintained at room temperature and ±2 °C.

Forced Degradation Study:

Drug samples were subjected to alkaline hydrolysis using 0.1N NaOH, Acid hydrolysis using 0.1N HCl and photo degradation under UV radiation, peroxide oxidation using 0.3% H_2O_2 and thermal degradation. Treated samples were scanned and their respective spectra were recorded and the changes in absorbance value were recorded.

Results and Discussion

Wavelength Selection:

The detection of wavelength at 250nm was selected as the drug showed optimal absorbance at that wavelength is shown in Figure 2.

Assay:

Table 1 shows the assay of commercial formulations.

Specificity:

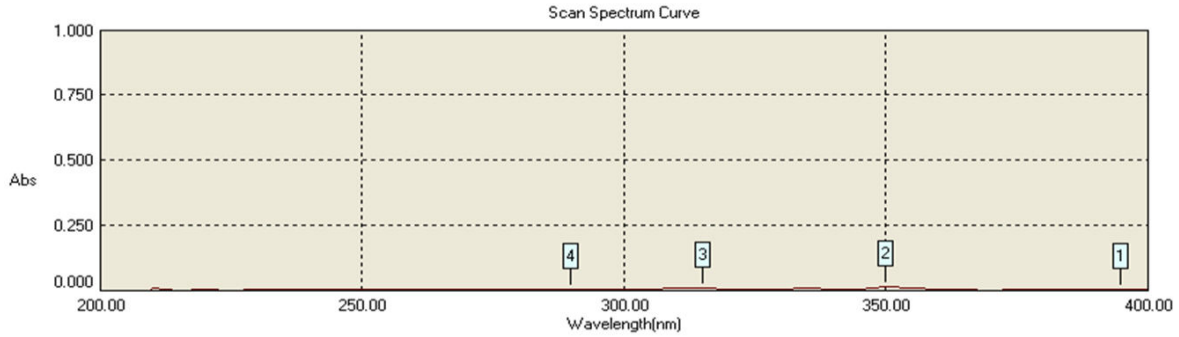

Fig. 3: Specificity.

Table 2: Linearity of Terazosin (Set-1)

Conc. (µg/ml)	Abs
1.5	0.171
3	0.345
4.5	0.499
6	0.654
7.5	0.840
9	0.992

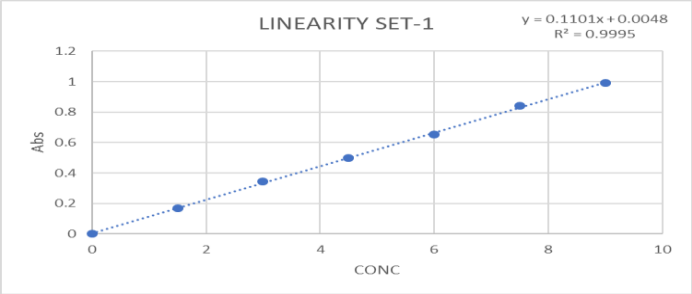

Fig. 4: Linearity of Terazosin (Set-1).

Table 3: Linearity of Terazosin (Set-2)

Conc. (µg/ml)	Abs
1.5	0.184
3	0.351
4.5	0.524
6	0.680
7.5	0.828
9	0.994

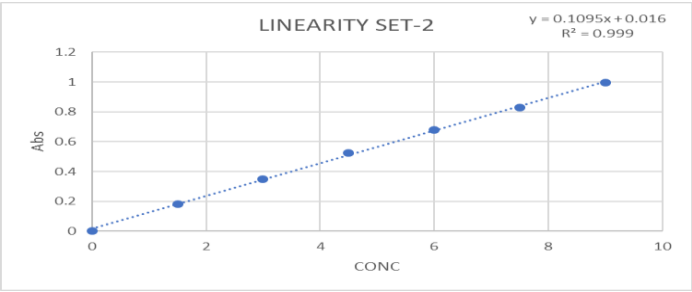

Fig. 5: Linearity of Terazosin (Set-2).

Table 4: Linearity of Terazosin (Set-3)

Conc. (µg/ml)	Abs
1.5	0.177
3	0.357
4.5	0.501
6	0.690
7.5	0.851
9	0.996

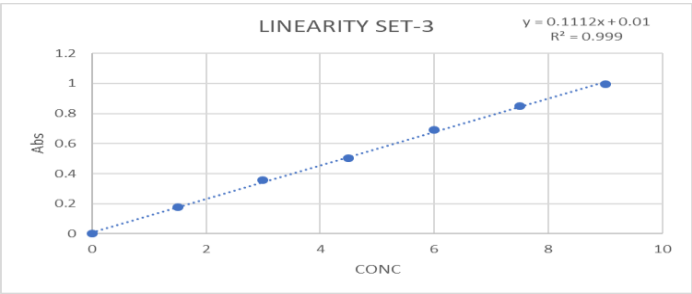

Fig. 6: Linearity of Terazosin (Set-3).

Table 5: Summary of system precision

Conc. (µg/ml)	Abs.	Avg.	S. D	% R.S.D
3	0.350	0.349	0.001	0.347
3	0.349			
3	0.348			
3	0.350			
3	0.347			
3	0.348			

Table 6: Summary of method precision

Conc. (µg/ml)	Abs.	%Assay	Avg.	S. D	% R.S.D
3	0.348	98.40	98.45	0.351	0.356
3	0.347	98.10			
3	0.349	98.70			
3	0.350	99.00			
3	0.348	98.40			
3	0.347	98.10			

The analyte was assessed in the presence of the components and it was found that there was no interaction with the analyte (Fig. 3).

Linearity:

Various standards in the range 1.5 to 9 µg/ml of Terazosin were observed into UV system. A graph of absorbance (on Y-axis) versus concentration (on X-axis) is plotted and the correlation coefficient was calculated and represented in Tables 2, 3 and 4 and Figures 4, 5 and 6.

Limit of detection and limit of quantitation:

Calculation of SD and Slope:

Set-1, $y = 0.1101x + 0.0048$, $R^2 = 0.999$

Set-2, $y = 0.1095x + 0.016$, $R^2 = 0.999$

Set-3, $y = 0.1112x + 0.01$, $R^2 = 0.999$

Average of m = 0.111 (slope)

S.D of c = 0.006 (S.D)

LOD = 3.3×S.D /Slope

= 3.3×0.006/0.111

= 0.178 µg\ml

LOQ = 10×S.D/Slope

= 10×0.006/0.111

= 0.54 µg\ml

Range:

Lowest concentration to highest concentration is 1.5 µg/ml to 9 µg/ml.

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation and is shown in Tables 5 and 6.

(a) *Repeatability*:

(i) *System precision*:

Table 5 illustrates the summary of system precision.

(ii) *Method precision*:

Table 6 shows summary of method precision.

(b) *Ruggedness* (*Intermediate Precision*):

Intermediate precision was determined by the typical variations to be studied on different days (interday and intraday) by different analysts (analyst 1 and analyst 2) on different instruments (two different instruments). The tests were

Table 7: Summary of intermediate precision

Lab-1 % Assay					Lab-2 % Assay			
Day 1			Day 2		Day 1		Day 2	
Conc.	A-1	A-2	A-1	A-2	A-1	A-2	A-1	A-2
3 µg/ml	98.1	98.7	98.4	99.0	98.4	99.0	99.0	98.1
3 µg/ml	99.0	98.4	98.1	98.4	98.1	98.4	98.7	98.4
3 µg/ml	98.4	98.4	98.7	98.1	99.0	98.1	98.4	98.1
3 µg/ml	98.4	98.1	98.4	98.4	98.7	98.4	98.1	98.4
3 µg/ml	98.1	98.4	99.0	98.1	98.4	98.1	98.4	99.0
3 µg/ml	99.0	98.1	98.7	99.0	98.1	99.0	98.1	98.4
Avg	98.5	98.35	98.55	98.3	98.45	98.5	98.34	98.4
SD	0.410	0.226	0.315	0.245	0.351	0.410	0.351	0.329
%RSD	0.416	0.230	0.319	0.249	0.356	0.416	0.357	0.334
Intermediate precision within laboratory variations (N=6)								
Avg			98.43	Avg		98.42		
SD			0.119	SD		0.068		
%RSD			0.121	%RSD		0.070		

Table 8: Summary of reproducibility

Over all Mean %Assay	98.43
Overall SD	0.007
Overall % RSD	0.007

Table 9: Summary of Accuracy studies

Level	**Amount Added (µg/ml)**	**Amount Found (µg/ml)**	**% Recovery**	**Average % Recovery**	**% SD**	**% RSD**
50%	1.5	1.490	99.3	99.30	0.20	0.20
50%	1.5	1.487	99.1			
50%	1.5	1.493	99.5			
100%	3	2.976	99.2	99.53	0.31	0.31
100%	3	2.994	99.8			
100%	3	2.988	99.6			
150%	4.5	4.505	100.1	99.77	0.31	0.31
150%	4.5	4.487	99.7			
150%	4.5	4.478	99.5			

carried out by preparing drug solution of concentration 3 µg/ml of Terazosin and analyzing. The results were reported as % RSD. The precision result showed a good reproducibility with per cent relative standard deviation less than 2 (Table 7).

(c) *Reproducibility*:

Reproducibility is assessed by means of an inter-laboratory trail. This is the precision between two laboratories is shown in Table 8.

Accuracy Studies:

The accuracy of the method, recovery studies were carried out by adding different amounts (50%, 100% and 150%) of bulk samples of Terazosin within the linearity range were taken and added to the pre-analyzed formulation of concentration 3 µg/ml. From that percentage recovery values were calculated. The results were within the range and were found to be highly accurate and shown in Table 9.

Table 10: Summary of -2nm wavelength i.e. 248 nm

Conc. (µg/ml)	Abs.	%Assay	Mean	SD	% RSD
3	0.348	98.40	98.4	0.268	0.273
3	0.347	98.10			
3	0.349	98.70			
3	0.348	98.40			
3	0.347	98.10			
3	0.349	98.70			

Table 11: Summary of +2nm wavelength i.e. 252 nm

Conc. (µg/ml)	Abs.	%Assay	Mean	SD	% RSD
3	0.353	99.79	100.09	0.268	0.268
3	0.354	100.09			
3	0.355	100.39			
3	0.354	100.09			
3	0.353	99.79			
3	0.355	100.39			

Table 12: Summary of (-2°C) i.e. 23°C Temperature

Conc. (µg/ml)	Abs.	%Assay	Mean	SD	%RSD
3	0.350	99.00	98.8	0.363	0.368
3	0.351	99.30			
3	0.349	98.70			
3	0.348	98.40			
3	0.350	99.00			
3	0.348	98.40			

Table 13: Summary of (+2°C) i.e. 27°C Temperature

Conc. (µg/ml)	Abs.	%Assay	Mean	SD	%RSD
3	0.356	100.69	100.87	0.209	0.207
3	0.357	100.98			
3	0.356	100.69			
3	0.358	101.18			
3	0.357	100.98			
3	0.356	100.69			

Robustness:

The Robustness of the method was determined by making slight changes in the experimental conditions such as the temperature and scanning the samples maintained at room temperature, heated and cooled to ±2°C and wavelength ± 2 nm and shown in Tables 10, 11, 12 and 13.

Forced Degradation Study:

Drug samples were subjected to alkaline hydrolysis using 0.1N NaOH, Acid hydrolysis using 0.1N HCl, peroxide oxidation using 0.3% H_2O_2, photo degradation by using longer wavelength UV radiation and thermal degradation treated samples were scanned and their respective

Table 14: Summary of Forced degradation studies

Degradation Parameter (N=3)	Conc. (µg/ml)	Abs.	% Degraded	% Recovered
Acid Degradation	3	0.867	46.71	53.29
		0.867		
		0.864		
Alkali Degradation	3	0.981	79.95	20.05
		0.984		
		0.983		
Peroxide Degradation	3	0.904	56.97	43.03
		0.900		
		0.902		
Thermal Degradation	3	0.828	35.32	64.68
		0.826		
		0.824		
Photolytic Degradation	3	0.886	53.13	46.87

spectra were recorded and the changes in absorbance value recorded is shown in Table 14.

Conclusion

A simple method was developed for the determination of Terazosin in pure and its pharmaceutical formulations. Terazosin exhibited maximum absorption at 250 nm in Water and obeyed linearity in the concentration range of 1.5-9 µg/ml. The proposed method was statistically validated. This study presents a simple stability-indicating UV spectroscopic method for estimation of Terazosin in the presence of degradation products. By the forced degradation studies, it was found that the drug Terazosin is maximum degraded in the presence of alkali as per the studies performed. So, it was concluded that the drug should be stored in cool and dark place, away from air.

Acknowledgements

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