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A Validated Stability-Indicating HPLC Method for the Determination of Terazosin in Pharmaceutical Dosage Forms

Ramachandra Bandi¹, C. Raja Kumar², B. Ramakrishna³, N. Pravallika⁴

Department of Chemistry, Annamacharya Institute of Technology and Sciences, Tirupati, A.P., India¹

Department of Chemistry, Annamacharya Institute of Technology and Sciences, Kadapa, A.P., India^{2,4}

Department of Chemistry, Government Degree College, Rajampet, A.P., India³

ABSTRACT:

Objective: The objective of this study was to develop and validate a stability-indicating reverse-phase HPLC method for the accurate and precise quantification of terazosin in bulk drug substances and pharmaceutical dosage forms, even in the presence of degradation products.

Methods: A reverse-phase HPLC method was developed using a Hypersil C₈ column (150 × 4.6 mm, 5 µm) with Waters Alliance HPLC system equipped with Empower software. Isocratic elution was performed using a mobile phase consisting of acetonitrile and buffer in a 40:60 (v/v) ratio, adjusted to pH 6.80 ± 0.1, at a flow rate of 1.0 mL/min. Detection was carried out at 254 nm, and terazosin showed a retention time of 3.675 minutes.

Results: The developed method demonstrated excellent linearity over the concentration range of 5–30 µg/mL, with a correlation coefficient of 1.0 and a linear regression equation of $Y = 214106x + 2860.2$. Forced degradation studies under acidic, alkaline, photolytic, and thermal stress conditions revealed significant degradation of terazosin, with all degradation products well resolved from the parent compound. The percentage recovery of terazosin from pharmaceutical formulations ranged between 99.98% and 100.27%.

Conclusion: The validated HPLC method proved to be reliable and suitable for routine quality control and stability testing of terazosin. Comprehensive validation including linearity, accuracy, precision, system suitability, specificity, robustness, and forced degradation studies confirms the method's effectiveness as a stability-indicating analytical tool.

KEYWORDS: Terazosin; HPLC method development and validation; stability-indicating method; forced degradation studies; degradation profile.

I. INTRODUCTION

Terazosin is an α₁-adrenoceptor antagonist widely used in the treatment of hypertension and benign prostatic hyperplasia (BPH) [1,2], providing effective symptom relief in both conditions. In comparison with other α₁-blockers such as alfuzosin and tamsulosin, terazosin has demonstrated greater efficacy in several clinical aspects, including improvement of BPH symptoms [3], regulation of blood pressure, reduction of platelet aggregation, and enhancement of endothelial function in patients with BPH. Furthermore, terazosin has been shown to be both safe and effective in managing lower urinary tract symptoms (LUTS) in both male and female patients [4,5].

As an antihypertensive agent, terazosin—administered either as monotherapy or in combination with other antihypertensive drugs—produces a significant reduction in blood pressure, particularly diastolic blood pressure, in patients with mild to moderate hypertension [6]. A clear dose-response relationship exists, with optimal antihypertensive efficacy typically observed at doses ranging from 1 to 10 mg. Beyond its use in hypertensive and BPH populations, terazosin has also been reported to lower blood pressure in healthy individuals [7]. However, treatment with terazosin may be associated with adverse effects such as syncope and orthostatic hypotension, especially following the initial dose, a phenomenon commonly referred to as the “first-dose effect” [8]. Alpha-1 adrenoceptor

antagonists, including terazosin, are commonly used in the treatment of patients with lower urinary tract symptoms (LUTS) and prostatic enlargement due to their significant effects on urinary symptoms, quality of life, urinary flow rate, and post-void residual urine volume [9,10]. However, these agents may produce vasodilatory adverse effects such as dizziness and orthostatic hypotension, attributed to their action on α_1 -adrenoceptors in the systemic vasculature [11]. Despite these effects, several studies advocate the use of α_1 -blocker therapy in patients with concomitant hypertension and benign prostatic hyperplasia (BPH) [12].

Terazosin hydrochloride dihydrate is a long-acting α_1 -adrenoceptor antagonist officially listed in multiple pharmacopoeias [13–15] and is indicated for the management of mild to moderate hypertension and benign prostatic hyperplasia [16,17]. Various analytical techniques have been reported for the determination of terazosin in pharmaceutical formulations, including potentiometry [18], voltammetry [19,20], spectrophotometry [21,22], fluorimetry [23,24], and thermal analysis [25,26]. Additionally, high-performance liquid chromatography (HPLC) methods, with or without fluorescence detection, have been employed for the simultaneous estimation of terazosin and other related drugs in dosage forms [27–29]. Despite the availability of numerous analytical methods for terazosin determination, there is limited literature describing stability-indicating HPLC methods specifically designed for this drug. The present study addresses this gap by developing and validating a stability-indicating reverse-phase HPLC assay for the quantification of terazosin. The method was established in accordance with International Conference on Harmonisation (ICH) guidelines, utilizing a symmetry column without the use of an internal standard. Comprehensive validation confirmed the method's accuracy, precision, repeatability, reproducibility, and other essential analytical performance characteristics.

II. MATERIALS AND METHODS

The Waters HPLC 2695 system, equipped with a quaternary pump, autosampler, UV–Visible detector, and a thermostatted column compartment, was used for the analysis. The system was operated and controlled using Waters (Alliance) Empower software. Chromatographic separation was achieved on a Hypersil C8 column (150×4.6 mm, 5 μm particle size). Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and acetonitrile of analytical grade (99.99% purity) were procured from Sigma-Aldrich and used throughout the study.

Table-1: Chromatographic conditions of Terazosin

Stationary phase	Mobile phase	Flow rate (ml/min)	Run time (min)	Column Temp (0°C)	Volume of injection loop(μl)	Detection wavelength (nm)	Retention time (min)
Hypersil C ₈	Phosphate buffer Acetonitrile in the ratio (60:40) v/v	1.0	10	30	20	254	3.675

2.1 Mobile Phase Preparation

The mobile phase for isocratic elution was prepared by mixing acetonitrile and phosphate buffer in a volumetric ratio of 40:60. The pH of the mixture was adjusted to 6.80 ± 0.1 using potassium hydroxide solution. Prior to use, the mobile phase was filtered through a 0.4 μm nylon membrane filter and degassed to remove dissolved gases.

2.2 Preparation of Buffer

A buffer solution was prepared by accurately weighing 1.36 g of potassium dihydrogen phosphate and 0.3 g of dipotassium hydrogen phosphate and dissolving them in distilled water in a 1000 mL volumetric flask. The solution was mixed thoroughly until complete dissolution.

2.3 Chromatographic Conditions

Chromatographic separation was performed using a Hypersil C8 column (150 × 4.6 mm, 5 µm particle size). The mobile phase consisted of acetonitrile and phosphate buffer in a 40:60 (v/v) ratio. Prior to use, the mobile phase was filtered through a 0.4 µm nylon membrane filter and degassed by helium purging for 15 minutes. The eluent was delivered at a flow rate of 1.0 mL/min, and detection was carried out at 254 nm using a UV detector. The column temperature was maintained at 30 °C throughout the analysis.

2.4 Preparation of Terazosin Standard Stock and Working Solutions

Approximately 20 mg of terazosin working standard was accurately weighed and transferred into a 100 mL volumetric flask. About 30 mL of diluent was added, and the mixture was sonicated until complete dissolution. The volume was then made up to the mark with diluent to obtain a stock solution of 1000 µg/mL (Stock Solution A). From this stock solution, 10 mL was transferred into a 100 mL volumetric flask and diluted to volume with diluent to obtain a working standard solution of 100 µg/mL (Standard Solution B). Aliquots ranging from 0.5 to 3.0 mL of this solution were further diluted to 10 mL to prepare calibration standards in the concentration range of 5–30 µg/mL.

2.5 Preparation of Test Solution (Formulation)

Twenty tablets containing terazosin were finely powdered, and an amount equivalent to 20 mg of terazosin was accurately weighed and transferred into a 100 mL volumetric flask. Approximately 10 mL of diluent was added, and the mixture was shaken manually for 20 minutes, followed by sonication for an additional 10 minutes. The volume was then made up to the mark with diluent and centrifuged at 8000 rpm for 10 minutes. The clear supernatant was carefully transferred to obtain a solution with a concentration of 1000 µg/mL. From this solution, 10 mL was pipetted into a 100 mL volumetric flask and diluted with diluent to obtain a concentration of 100 µg/mL. Further aliquots ranging from 0.5 to 3.0 mL were transferred into 10 mL volumetric flasks and diluted to volume to achieve final concentrations of 5–30 µg/mL. All solutions were filtered through a 0.45 µm nylon membrane filter prior to analysis. For chromatographic evaluation, 20 µL of the blank solution, placebo solution, and three replicates of the standard solution were injected. Peaks corresponding to the blank and placebo were excluded from analysis.

2.6 Assay Procedure

The column was equilibrated for at least 30 minutes with the mobile phase flowing at a rate of 1.0 mL/min. The detector wavelength was set at 254 nm. Six sets of terazosin standard solutions were prepared over a concentration range of 5–30 µg/mL. Each standard and test solution was injected six times using an injection volume of 20 µL. The retention time of terazosin was approximately 3.675 minutes (Fig. 4.14). Peak areas were recorded, and a calibration curve was constructed by plotting drug concentration against peak area. Linearity was evaluated using regression analysis.

2.7 System Suitability Solution

The terazosin standard working solution was used as the system suitability solution.

2.8 System Suitability Procedure

Equal volumes of the blank solution were prepared, and six replicate injections of the system suitability solution were performed. Subsequently, two replicate injections of the test solution were analyzed. Chromatograms were recorded, and any peaks arising from the blank were excluded. The percentage relative standard deviation (%RSD) of the six replicate injections was calculated. In addition, the tailing factor and number of theoretical plates were evaluated based on the peak obtained during the sixth injection of the system suitability solution.

2.9 System Suitability Acceptance Criteria

Tailing factor: Not more than (NMT) 2.0

Theoretical plates: Not less than (NLT) 2000

2.10 Procedure for Forced Degradation Studies

Stability testing is an essential component of pharmaceutical development and provides insight into the behavior of drug substances under various environmental conditions such as temperature, light, humidity, and storage. These studies support the determination of shelf life and retest periods by evaluating assay stability and identifying degradation products.

2.11 Preparation of Degraded Samples

Acidic Degradation

5 mg sample of the drug was dissolved in a phosphate buffer–acetonitrile mixture (60:40, v/v) and subjected to degradation using 0.1 N HCl at ambient temperature. Aliquots of 10 mL were withdrawn at regular intervals over 48 hours.

Alkaline Degradation

5 mg drug sample was dissolved in phosphate buffer–acetonitrile (60:40, v/v) and exposed to 0.1 N NaOH at room temperature. Aliquots were collected at predetermined intervals over 48 hours.

Thermal Degradation

Thermal degradation was performed by exposing 10 mg of the drug to elevated temperature in an oven maintained at 75 °C. After degradation, a working solution was prepared using phosphate buffer and acetonitrile (60:40, v/v). The sample was analyzed on the 10th day following preparation.

Photodegradation

The drug sample was exposed to UV light at 254 nm for 48 hours. After exposure, a working solution was prepared and diluted to the required concentration using a phosphate buffer–acetonitrile mixture (60:40, v/v). The degraded sample was then subjected to chromatographic analysis.

2.12 Specificity

The specificity of the developed HPLC method was evaluated by its ability to accurately quantify terazosin in the presence of potential interfering substances, including degradation products. Forced degradation studies were conducted under acidic (0.1 N HCl), alkaline (0.1 N NaOH), thermal (75 °C), and photolytic (254 nm) conditions. The effectiveness of the method in separating terazosin from its degradation products was assessed, and peak purity was evaluated using a PDA detector for all stressed samples. The results confirmed the stability-indicating capability of the method.

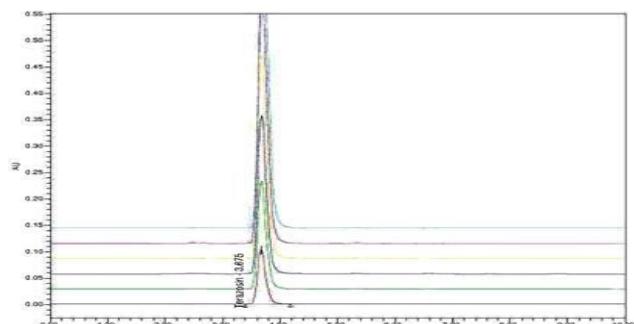


Figure-1: Overlaid Linearity Chromatogram of Terazosin

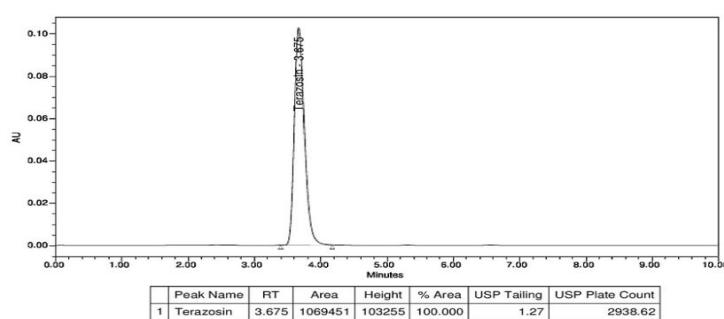


Figure -2: Sample Chromatogram of Terazosin

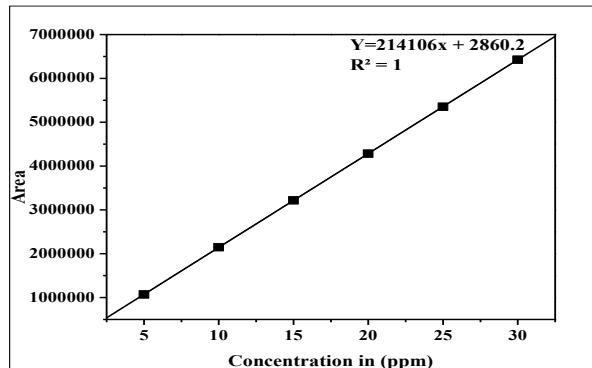


Figure -7: Linearity of Terazosin standard

Table -2: Performance calculations, detection characteristics precision and accuracy of the proposed method for Terazosin

Parameter	HPLC Method
Wavelength (nm)	254
Retention time (t) min	3.675
Linearity range ($\mu\text{g ml}^{-1}$)	5-30
LOD ($\mu\text{g ml}^{-1}$)	0.5882
LOQ ($\mu\text{g ml}^{-1}$)	1.7823
Regression equation (y=bc+a)	---
Slope (b)	214106
Intercept (a)	2860.2
Correlation coefficient(r^2)	1
Relative Standard deviation (%RSD)	0.31
Intermediate Precision (%RSD)	1.110

%RSD of five independent determinations

Table -3: Results of linearity of sample

%	Conc.(mcg)	Area
25	5.0000	1069451
50	10.0000	2148170
75	15.0000	3217831
100	20.0000	4281981
125	25.0000	5353986
150	30.0000	6426826

Table –4: Accuracy Data (Triplicate values at 50,100 &150 percent levels)

S.No	Spike level	Peak area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Avg	% RSD
1	50%	2147946	10	10.04	100.4	100.27	0.12
		2145518	10	10.027	100.27		
		2143044	10	10.015	100.15		
2	100%	4276825	20	19.97	99.94	99.81	0.14
		4271452	20	19.96	99.81		
		4265630	20	19.93	99.67		
3	150%	6423210	30	30.018	100.06	99.98	0.09
		6418916	30	29.997	99.99		
		6412235	30	29.97	99.88		

Table- 5: Summary of forced degradation results for Terazosin

Stress conditions	time / h	Assay of active	Degradation (%) Substance	Peak purity
Acid hydrolysis	48	94.91	5.09	1
Base hydrolysis	48	91.98	8.02	1
Thermal	75°C	84.5	15.5	1
UV	10 th Day	88.5	11.5	1

Table -6: Result of precision of the test method

S No	Analyst 1 (intra-day precision)			Analyst 2 (inter-day precision)		
	Name	RT	Area	Name	RT	Area
1	Sample-1	3.678	4282047	Sample-1	3.677	4251425
2	Sample-2	3.679	4280421	Sample-2	3.679	4266241
3	Sample-3	3.680	4301421	Sample-3	3.681	4187451
4	Sample-4	3.683	4294185	Sample-4	3.685	4295463
5	Sample-5	3.683	4274084	Sample-5	3.685	4310245
6	Sample-6	3.686	4264764	Sample-6	3.689	4312345
	AVG	3.682	4282820	AVG	3.683	4270528
	STDEV	0.003	13295.5	STDEV	0.004	47398.2
	%RSD	0.082	0.31	%RSD	0.121	0.110

Table- 7 Assay Studies

Drug	Amount present/tablet	Amount found	% of Assay
Terazosin	100 mg	99.82 mg	99.82

Table -8: Ruggedness - (Day1&Day2)

S.No	Name	RT	Area
1	Sample-1	3.678	4282047
2	Sample-2	3.679	4280421
3	Sample-3	3.680	4301421
4	Sample-4	3.683	4294185

5	Sample-5	3.683	4274084
6	Sample-6	3.686	4264764
7	Sample-7	3.677	4251425
8	Sample-8	3.679	4266241
9	Sample-9	3.681	4187451
10	Sample-10	3.685	4295463
11	Sample-11	3.685	4310245
12	Sample-12	3.689	4312345
Avg		3.682	4276674.33
SD		0.00368	33804.352
%RSD		0.10	0.79

Table-9: Robustness Study

Drug Name	Parameter	Chromatographic Conditions			
		Retention time	Area	USP Plate Count	USP Tailing
Terazosin	Change of Flow ate				
	1.1ml/min	4.098	4784054	2804.16	1.24
	1.0ml/min	3.365	3914845	2682.64	1.19
	Temperature Change				
	30°C	3.718	4319080	2673.87	1.18
	35°C	3.656	4391575	3060.45	1.21

III. RESULTS AND DISCUSSION

3.1 Optimization of Chromatographic Conditions

The primary objective of developing this stability-indicating HPLC method was to achieve effective separation between terazosin and its degradation products. To accomplish this, a C8 stationary phase was selected along with a mobile phase composed of phosphate buffer and acetonitrile in a 60:40 (v/v) ratio. Efficient separation of terazosin from its degradation products was successfully achieved using a Hypersil C8 column under these optimized chromatographic conditions. The method produced a tailing factor of less than 2.0, with the main terazosin peak eluting at approximately 3.675 minutes, while degradation products eluted within 4 minutes. This optimized retention behavior significantly reduced overall analysis time, improved sample throughput, and enhanced cost efficiency. Forced degradation studies confirmed the high specificity of the method, as all degradation products were well resolved from the principal drug peak. Furthermore, the method was thoroughly validated in accordance with international regulatory guidelines, demonstrating its reliability, accuracy, and suitability for routine analysis.

3.2 Results of Forced Degradation Studies

Forced degradation studies were conducted to evaluate the stability-indicating capability of the developed HPLC method. Terazosin exhibited degradation under acidic, alkaline, thermal, and photolytic stress conditions. The formation of polar degradation products was observed in the chromatograms, as illustrated in Figures 3–6. The extent of degradation was found to be 5.09% under acidic conditions, 8.02% after 48 h of alkaline exposure, 15.5% under thermal stress, and 11.5% following UV exposure for ten days. Peak purity values greater than 990 confirmed the spectral homogeneity of the terazosin peak under all stress conditions, indicating effective separation from degradation products. The assay of terazosin tablets remained unaffected, confirming the stability-indicating nature of the developed method. A summary of the forced degradation results is presented in Table 5. Overall, moderate degradation was observed under acidic, alkaline, and photolytic conditions, while thermal stress resulted in comparatively higher degradation.

3.3 Precision

Method precision (intra-day precision) was evaluated by performing six independent assays of terazosin test samples against a reference standard. The precision of the method was expressed as the percentage relative standard

deviation (%RSD) of the assay values. Intermediate precision (inter-day precision) was assessed by conducting analyses on different days, using different analysts and HPLC systems within the same laboratory. The results demonstrated satisfactory reproducibility, with %RSD values well within acceptable limits. The precision data are summarized in Table 6.

3.4 Accuracy (Recovery Studies)

The accuracy of the method was evaluated by recovery studies using the standard addition technique. Known amounts of terazosin were spiked into the placebo matrix at three concentration levels—50%, 100%, and 150% of the nominal assay concentration corresponding to a 50 mg tablet strength. For each level, 10 mL aliquots were transferred into 50 mL volumetric flasks and diluted to volume with diluent. Three replicate samples were prepared at each concentration level. The percentage recovery was calculated using the calibration curve. Recovery values ranged from 98.0% to 102.0%, with an average recovery of 100.02%, confirming the accuracy of the method. The detailed recovery data are presented in Table 4.

3.5 Linearity

Linearity was evaluated over a concentration range of 25–150% of the target concentration (20 µg/mL). Standard solutions of terazosin were prepared at concentrations ranging from 2.5 to 15 µg/mL. Each solution was injected into the chromatographic system, and calibration curves were constructed by plotting peak area versus concentration using least-squares linear regression analysis. The regression equation obtained was $Y = 21410x + 2860$, with a correlation coefficient (R^2) of 1.000, indicating excellent linearity across the studied range. The calibration curve is shown in Figure 7, and detailed linearity data are summarized in Table 3. Representative chromatograms are provided in Figure 3, while the overlaid linearity chromatogram is shown in Figure 1.

3.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ for terazosin were determined using the calibration curve method. Calibration solutions covering low concentration ranges were prepared and analyzed. The LOD and LOQ values were found to be 0.5882 µg/mL and 1.7823 µg/mL, respectively. Precision at both concentration levels was verified by analyzing six replicate samples, and acceptable %RSD values confirmed method sensitivity and reliability.

3.7 Robustness

The robustness of the analytical method was evaluated by introducing deliberate variations in experimental conditions. These included changes in flow rate (± 0.1 mL/min), column temperature (± 5 °C), and mobile phase composition ($\pm 10\%$ acetonitrile). The resolution between terazosin and its degradation products remained greater than 2.0 under all tested conditions, demonstrating the robustness of the method. Additionally, the stability of standard and sample solutions was assessed at 48-hour intervals and on the 10th day by comparing assay values with freshly prepared solutions. The results are summarized in Table 9.

3.8 Ruggedness

Method ruggedness was assessed through intermediate precision studies involving six independent sample preparations analyzed by two different analysts under similar experimental conditions. The %RSD values for both method precision and intermediate precision were below 2.0%, confirming the ruggedness of the analytical method. The results are presented in Table 8.

IV. CONCLUSION

A robust, precise, and stability-indicating RP-HPLC method has been successfully developed and validated for the quantitative determination of terazosin in bulk drug and pharmaceutical dosage forms. The method demonstrated excellent specificity, accuracy, precision, robustness, and ruggedness. Forced degradation studies revealed moderate degradation under acidic (5.09%), alkaline (8.02%), and photolytic (11.5%) conditions, with higher degradation observed under thermal stress (15.5%). Overall, the method is simple, sensitive, selective, rapid, and reliable, making it highly suitable for routine quality control and stability testing of terazosin in pharmaceutical formulations.

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