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Stability Indicating HPTLC Determination of Terazosin in Tablet Formulation

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Abstract A simple, rapid and precise HPTLC method was developed for the quantitative estimation of terazosin hydrochloride in pharmaceutical dosage form. Good sharp peak was obtained in mobile phase composition Chloroform:Toluene:MeOH (9:1:6) at Rf ≈ 8. Stationary phase was silica gel precoated aluminum plate 60F-254 plates, [20cm × 10cm with 250µm thickness and wavelength selected was 254nm. Terazosin was exposed to acidic, alkaline, thermal, photolytic and oxidative stress conditions, and the stressed samples were analyzed by the proposed method. The described method showed linearity over a range of 50-2500µg/ml. The correlation coefficient is 0.999. Overall, the proposed method was found to be suitable and accurate for quantitative determination and stability study of terazosin in pharmaceutical dosage form.

Keywords: *terazosin hydrochloride, stability indicating method, stress degradation, validation of analytical method*

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1. Introduction

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, which enables recommendation of storage conditions, retest periods, and establishing shelf life [1]. An ideal stability-indicating chromatographic method should estimate the drug and also be able to resolve the drug from its degradation products [2].

The stability-indicating assay is a method that is employed for the analysis of stability samples in the pharmaceutical industry. It is also required that analytical methods should be validated to demonstrate that impurities unique to the new drug substance do not interfere with or are separated from specified and unspecified degradation products in the drug product [3]. Regulatory agencies recommend the use of stability-indicating methods for the assay analysis of stability samples. Thus, stress studies are required in order to generate the stressed samples, method development and its validation [4].

Benign prostatic hyperplasia (BPH) is a focal enlargement of the periurethral region of the prostate seen in most aging men, which results in symptoms requiring clinical intervention in approximately a third of men over the age of 60. BPH has been tied to a larger collection of symptoms including frequency of urination, urgency, urinary incontinence, waking up multiple times at night to void (nocturia), weakened urinary stream, straining to void and a sense of incomplete emptying of the bladder. These morbidities have been grouped together under the general descriptor-lower urinary tract symptoms (LUTS) [5].

Presently, α_1-adrenoreceptor antagonists (α_1- blockers that include doxazosin, terazosin, tamsulosin, and alfuzosin) are common for treating BPH related LUTS. They treat the dynamic component of BPH by blocking α_1-receptormediated sympathetic stimulation to relax the smooth muscle in the prostate. All these agents produce their effects on voiding within hours of administration, regardless of prostate size, without altering serum prostate specific antigen or volume [6]. The alpha 1A receptors are prominent in prostrate, prostatic capsule, prostatic urethera and bladder [7]. Alpha 1-adrenoceptor antagonists (α-blockers) remain the most widely used pharmacologic agents for treating bladder outflow resistance caused by BPH [8].

Terazosin hydrochloride dehydrate RS-1-(4-amino-6, 7-dimethoxy-2-quinazolinyl)-4-[(tetra-hydro-2-furanyl) carbonyl]- piperazine monohydrochloride [9] a once a day specific α_1 blocker was approved by food and drug administration in 1987 for the treatment of hypertension and in 1993 for the treatment of BPH [10]. The United States experience has unequivocally demonstrated the short-term safety and efficacy of terazosin therapy in BPH [11].

High-performance thin layer chromatography is one of the sophisticated instrumental techniques based on the full

capabilities of thin layer chromatography. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, and so on enable it to be a powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples, food stuffs, and so on [12]. HPTLC is a valuable tool for reliable identification because it can provide chromatographic fingerprints that can be visualized and stored as electronic images [13].

Three spectrophotometry [14,15,16], seven HPLC [9,17,18,19,20,21,22] and two TLC [23] methods are till date available in the literature for the determination of terazosin in various matrixes. Simultaneous determinations of terazosin with other alpha one adrenoreceptor blockers are also published recently [24,25,26]. Negative result of simultaneous UV spectrophotometry method with other alpha one blockers is also reported with available literature [27]. But there is no stability indicating HPTLC method reported till date. Thus the aim of our study is to develop and validate stability indicating HPTLC method for the determination of terazosin and application in pharmaceutical formulations.

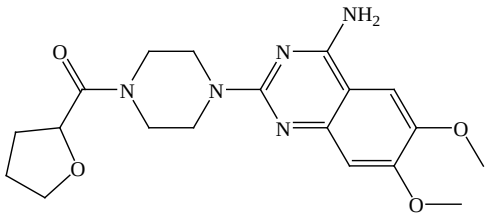

Figure 1. Chemical structure of terazosin

2. Material and Method

Terazosin was provided by Noche Labs Private Limited, Hyderabad and all the solvents purchased from Merke Limited, Mumbai & Thomas Baker, Mumbai and used such. The samples were spotted in the form of bands of width 6mm with a Camag 100microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60F-254 plates, [20cm × 10cm with 250µm thickness; E. Merck, Darmstadt, Germany)] using a Camag Linomat V (Switzerland) sample applicator. Both test and standard samples (5µL each) were applied onto the HPTLC plate as 6mm wide bands and 12mm apart from middle of bands by spray-on technique along with nitrogen gas supply for simultaneous drying of bands, by means of a Camag (Switzerland) Linomat V sample applicator fitted with a 100µL syringe (Hamilton, Bonaduz, Switzerland). Wavelength selected was 254nm.

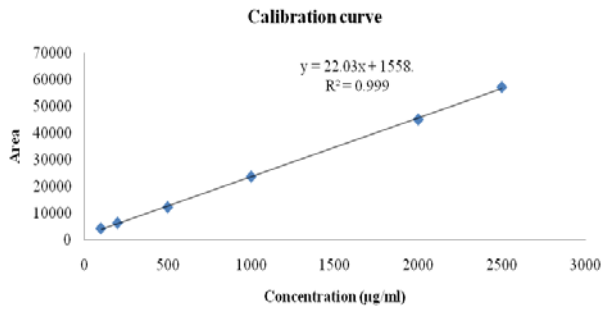

Figure 2. Calibration curve of terazosin

2.1. Preparation of Standard Stock Solution

Reference standard of Terazosin (100mg) was transferred to 10ml volumetric flask and dissolved in methanol:water (1:1). The flask was shaken for 10min and the volume was made up to the mark with the same solvent to obtain standard stock solution of Terazosin (10000µg/ml; stock solution). The working standard solution of Terazosin was prepared from suitable aliquots of stock solution were pipette out and volumes were made up to the mark with mobile phase.

2.2. Preparation of Calibration Curve

From standard stock solution, aliquots are made with diluents to obtain concentration of 100-2500µg/ml of Terazosin. The solution of (20µl) was spotted into plate with the help of syringe. All measurements were repeated three times for each concentration. The calibration curves of the area under curve Vs concentration were recorded for terazosin.

2.3. Validation

2.3.1. Specificity

Specificity of the stability indicating method was established by separation of the principle peak with degradants during forced degradation. The stress conditions utilized were acid hydrolysis, alkaline hydrolysis, oxidation by peroxide, thermal degradation and photo degradation by UV light. Overall these studies were aimed to degrade 10-30% of the drug.

The acid decomposition study was performed by refluxing the solution of drug in 0.1 N HCl solutions at 40°C for 2 days. For alkaline hydrolysis the solution of drug was refluxed in 0.1 N NaOH solution at 40°C for 10hours. For oxidative decomposition study was performed by exposing the solution of drug in 30% H_2O_2 (v/v) solution at 40°C up to 5 days. The photolytic degradation was performed by exposing the solution of drug to UV light for upto 5 days. Lastly, thermal degradation was performed by standing the pure drug in solid form in stability chamber at 40°C and 75% RH for upto 5 days. All of the solutions were filtered through whatman filter paper and properly diluted to prepare 500µg/ml concentration solution. Samples were then runned under optimized chromatographic condition.

2.3.2. Linearity

This parameter was assessed by correlation coefficient, by visualizing calibration curve and plot of residuals.

2.3.3. Precision

Repeatability was assessed by running six times 500µg/ml solution at a time. For interday and intraday precision was assessed by using 400, 500 and 600µg/ml dilutions

2.3.4. Accuracy

Powdered Terapress (Intas Pharmaceuticals) equivalent to 10mg terazosin was transferred to 10ml volumetric flask and ultrasonication was done for 15minutes with approximately 5ml methanol:water (1:1). Solution was then diluted up to the mark with same solvents and filtered

through whatman filter paper. 2ml of this solution was spiked in three different 10ml volumetric flask with 2, 3, and 4ml previously analyzed standard stock solution. Finally volume was made up to the mark with methanol:water (1:1) and estimation of drug content was done by proposed method.

Recovery was investigated for three QC levels (400, 500, and 600) for terazosin.

2.3.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated through standard deviation of intercept of the calibration curve (28). Limit of detection and quantitation were calculated by using following equation.

$$LOD = \frac{3.3 \times SD}{\text{Slope}}, LOQ = \frac{10 \times SD}{\text{Slope}}$$

2.3.6. Robustness

The robustness of the method was evaluated by the changing the experimental condition such as mobile phase composition, amount of mobile phase and time from spotting to chromatography etc.

Following parameter were considered during the course of robustness study:

A: Mobile phase composition change

B: Amount of mobile phase change

C: Time from spotting to chromatography change.

3. Results and Discussion

3.1. Optimization of Mobile Phase

Mobile phase was optimized by varying proportions of methanol, toluene and chloroform. It was found that mobile phase composition Chloroform:Toluene:MeOH (9:1:6) gives sharp peak at Rf ≈ 8. Chromatogram of optimized chromatographic condition is presented under Figure 3.

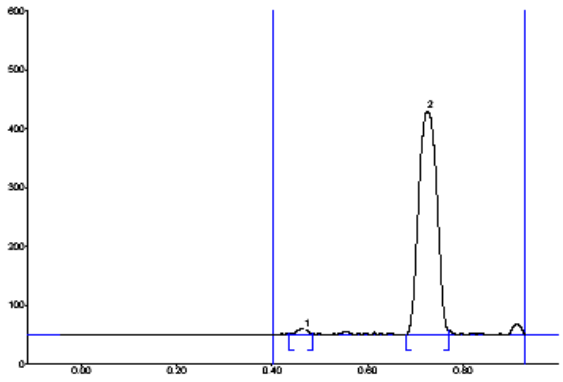

Figure 3. Chromatogram of optimized chromatographic condition

3.2. Validation

3.2.1. Specificity

Specificity of the stability indicating method was established by separation of the principle peak with degradants during forced degradation. The stress conditions utilized were acid hydrolysis, alkaline hydrolysis, oxidation by peroxide, thermal degradation and photo degradation by UV light. No degradation was observed by thermal degradation. Degradants were found to be properly separated by parent peak of terazosin in each case. Product obtained by acidic and alkaline degradation was 2-piperazinyl-6,7-dimethoxy-4-aminoquinazoline confirmed by LC-MS studies and a mass of 290.4 was obtained in positive ESI mode (Bakshi et al.). Terazosin forms tetrahydrofuroic acid, which decomposes further to tetrahydrofuran and carbon dioxide (26). Chromatograms related to stress studies are presented under Figure 4, Figure 5, Figure 6.

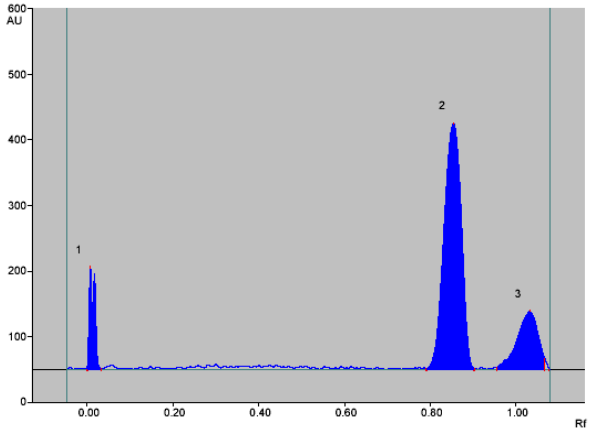

Figure 4. Chromatogram of acidic hydrolysis

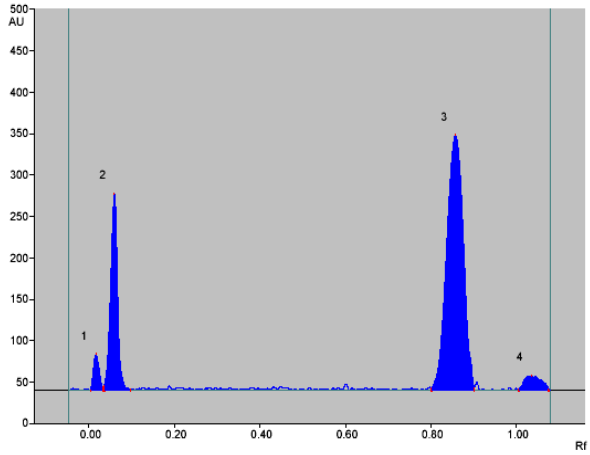

Figure 5. Chromatogram of alkaline hydrolysis

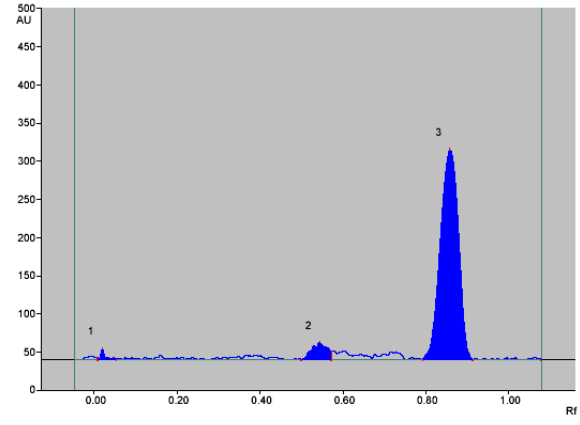

Figure 6. Chromatogram of degradation by UV light

This is clear in the study that all of the potential interferents are well separated from the parent peak proves method sufficient specific for the determination of Terazosin in pharmaceutical formulations.

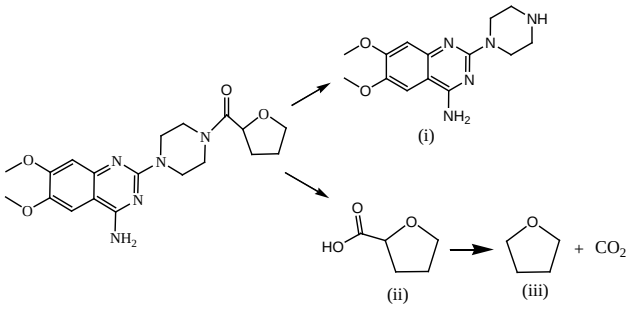

Figure 7. Possible degradation pathway of terazosin (i) 2-piperazinyl-6,7-dimethoxy-4-aminoquinazoline (ii) tetrahydrofuroic acid (iii) tetrahydrofuran

3.2.2. Linearity

Correlation coefficient of 0.999 shows good linearity. By visualizing calibration curve (Figure 2) this is clear that all of the points are closed to regression line also proves linearity of the method. The residuals are distributed both sides equally confirming the linearity of the proposed method.

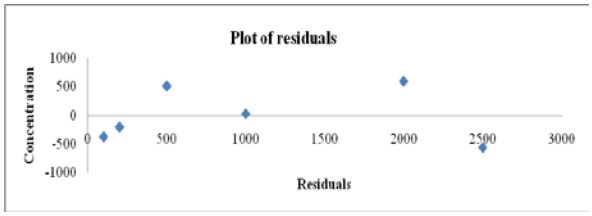

Figure 8. Plot of residuals

3.2.3. Precision

Repeatability RSD was found to be 0.331. Intraday and interday precision RSD was found to be between 0.337-0.486 and 0.378-0.885 respectively.

Values (RSD) in each case found was less than 1, shows method is precise in nature.

3.2.4. Accuracy

Accuracy studies were performed on terapress tablets. Results are presented under Table 1.

Table 1. Recovery study of Terazosin

Area found before spiking (C_1)	Area of Std added (C_2)	Area found after spiking (C_3)	% recovery (C_3-C_1)*100/C_2	Mean ± SD	RSD
6169	5964	12117	99.73	99.86 ± 0.619	0.620
		12129	99.93		
		12146	100.22		
6142	8167	14336	100.33		
		14361	100.64		
		14326	100.21		
6157	10370	16439	99.15		
		16387	98.65		
		16513	99.86		

Percentage recovery found was between 99.15-100.64% proves method to be accurate.

3.2.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ of the method were found to be 18.06µg/ml and 54.72µg/ml.

Robustness

In each case there is no significant change is found (as RSD values are lower than 2) shows method sufficient robust for the analysis of terazosin.

Table 2. Data for robustness

Parameters	Area						SD	RSD
Mobile Phase Composition Chloroform:Toluene:MeOH (9:1:6) v/v	S_1	S_2	S_3	S_4	S_5	S_6		
9:1:6	10081	10056	9989	10089	10132	10037	48.89	0.48
9.5:1:5.5	10307	10524	10423	10348	10471	10453	80.57	0.77
8.5:1:6.5	10285	10503	10346	10258	10425	10403	91.81	0.88
Amount of Mobile Phase	S_1	S_2	S_3	S_4	S_5	S_6		
8ml	10267	10241	10229	10447	10533	10365	123.7	1.19
10ml	10276	10532	10324	10247	10231	10463	113.1	1.09
12ml	10529	10319	10243	10237	10447	10263	121.3	1.17
Time from Spotting to chromatography	S_1		S_2		S_3			
1 minute	10457		10369		10523		77.26	0.74
5 minute	10537		10359		10444		89.03	0.85
8 minute	10451		10532		10351		90.66	0.87

3.3. Analysis in Formulations

The tablet powder equivalent to 2mg terazosin was transferred to a 10 ml volumetric flask and diluted with methanol:water (1:1) to about 50ml. The solution was ultrasonicated for 15 minute and diluted up to the mark with methanol:water (1:1). The solution was then carefully filtered with filter paper before spotting on TLC plate in HPTLC system.

Table 3. Results of analysis of formulation by proposed method

Name of formulation	Drug	Assay (n=3)	± SD	Percentage
Terapress 2 (Intas pharmaceuticals)	Terazosin	1.95 1.96 1.95	± 0.0057	97.5 %

4. Conclusion

HPTLC is one of the most widely applied methods for the analysis in pharmaceutical industries, clinical chemistry, forensic chemistry, biochemistry, cosmetology, food and drug analysis, environmental analysis, and other areas. It is due to its numerous advantages, for example, it is the only chromatographic method offering the option of presenting the results as an image. Other advantages include simplicity, low costs, parallel analysis of samples, high sample capacity, rapidly obtained results, and possibility of multiple detection [12]. Increased sensitivity of methods like HPLC-EMS and UPLC-MS/MS is mostly compromised with complicated instrumentations, procedures, and mobile phases [29]. Thus simple and economical HPTLC method development was attempted here.

Thus a simple, fast, economical and accurate HPTLC method was developed including forced degradation studies. Method was optimized by using mobile phase chloroform:Toluene:MeOH in ratio 9:1:6. Regression equation and correlation coefficient found was y = 22.03x + 1558, respectively. Linearity was assessed by analyzing correlation coefficient, visualizing calibration curve and plot of residual. Method was found to be sufficient linear, precise and accurate. Possible degradation pathway of terazosin is presented under Figure 7. The proposed method can be used for the determination of terazosin in pharmaceutical preparations even in the presence of potential degradants due to environmental conditions.

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Competing Interests

The authors declare no conflict of interest.

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