1 INTRODUCTION:

Ultra performance Liquid Chromatography (UPLC) is a well-known and widely used analytical technique which is used for analysis of drug product and drug substance a result of significant improvements in instrumentation and column pickings i.e. low particle size column, Ultra performance liquid chromatography (UPLC) has emerged as the preferred method for the separation and quantitative analysis of a wide range of samples. Terazosin was determined by spectroscopic method, fluorimetry method, high-performance liquid chromatography (HPLC) with fluorescence detection [4] HPLC, x-ray fluorescence spectrometry based on the formation of ion-pair associates with zinc thiocyanate [6] and electrochemical method potentiometric sensor and the other a voltammetric technique. Terazosin Hydrochloride Di hydrate is white to yellow [8] coloured crystalline powder soluble in water and in Methanol, slightly soluble in 0.1N Hydrochloric acid and very slightly soluble in chloroform, practically insoluble in Acetone and hexane. Terazosin Hydrochloride Di hydrate is a synthetic chemotherapeutic antihypertensive of the Quinazoline drug class and is used to relax the muscles in the prostate and bladder neck, making it easier to urinate. Terazosin is in a group of drugs called alpha-adrenergic blockers [9]. Terazosin relaxes veins and arteries so that blood can more easily pass through them. Terazosin Hydrochloride Dihydrate available in tablet form as Terazosin Hydrochloride Dihydrate is white crystalline powders. Molecular weight of Terazosin Hydrochloride Dihydrate is 459.93g/mole and the molecular formula is $C_{19}H_{25}N_5O\ 2H_2O$. Melting Point of Terazosin Hydrochloride Di hydrate is 271 – 274°C.

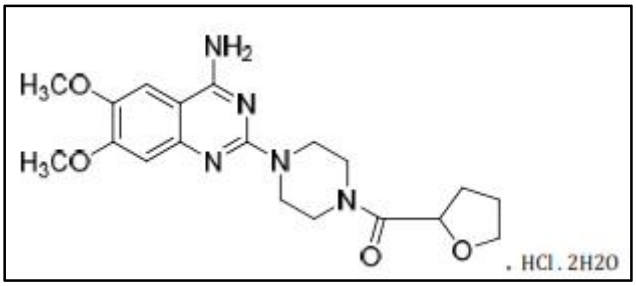


Figure 1: Structure of terazosin hydrochloride

Stability testing forms an important part in the process of drug development. The purpose of stability indicating method is to provide the effect of variety of factor such as Temperature, light, heat, acidic condition, basic condition and oxidative condition on drug product. The best Stability indicating method is one in which all impurity, which

may be generated during the degradation study, are well resolved from the main peak of the drug [10]. The stability-indicating assay is a method that is used for the analysis of stability samples in the pharmaceutical industry. It is also required that analytical methods should be validated to demonstrate that impurities do not interfere with or are separated from known and unknown degradation products in the drug product [11]. There is no doubt on the fact that the spectroscopic methods [12-14] are rapid and far more economical than chromatographic methods, but their destructive nature and lack of sensitivity is a huge disadvantage. UPLC method has an advantage on HPLC [15-16] method are small run time, small column particle size, high pressure, high resolution. Main object of this research work is to develop a new RPUPLC stability indicating method for the quantitative analysis of terazosin in pharmaceutical dosage form.

2. LITERATURE REVIEW

The literature review shows that various analytical methods were reported for its determination as API, pharmaceutical formulation. Brief details for the same are as under.

Nageswari A., K.V.S.R., Krishna R., Mukkanti K. Described Stabilityindicating UPLC method for determination of Imatinib Mesylate and their degradation products in active pharmaceutical ingredient and Pharmaceutical dosage forms. The proposed RP-UPLC method utilizes Acquity UPLC BEH 50-mm, 2.1 mm and 1.7 µm C-18 column at 30 °C, with a gradient program of 9.0 min at a flow rate of 0.3 mL/min. The compounds of interest were monitored at 237 nm. Resolution for Imatinib and eight related components was found to be greater than 1.5 for any pair of components. The correlation coefficients ($r^2 > 0.9990$) obtained indicate clear correlations between the concentrations and their peak areas for the investigated compounds. RSD obtained for the repeatability and intermediate precision experiments, was less than 5.0%. Accuracy of the method was further ascertained by performing recovery studies through spiking experiments. The drug substance was subjected to hydrolytic, oxidative, photolytic and thermal stress conditions as per ICH. The developed method was validated according to the current ICH guidelines for specificity, limit of detection, limit of quantitation, linearity, accuracy, precision, ruggedness and robustness. The method is also suitable for the assay determination of IMM in pharmaceutical dosage forms.

Rao J.V.L.N.S., Reddy G.K., Jayasree V., and Chowdary K.P.R. described spectrophotometric method for the determination of Terazosin. A simple and sensitive

spectrophotometric method for the determination of terazosin in pure and dosage forms is proposed. The drug forms a stable green chromogen with ferric chloride and potassium ferricyanide, exhibiting maximum absorption at 740 nm. The chromogen obeys Beer-Lambert's law in the concentration range of 1-10 Î¼g/ml.

Prasad C.V.N., Guatham A., Bharadwaj V. and Praimoo P. described quantitative Determination of Terazosin HCl In Tablet Preparation By Fluorimetry.The method consists of measurement of fluorescence for the drug samples extracted by methanolic 0.1N H2H2SO4. The samples showed an excitation at 246 nm and an emission at 382 nm. The linearity was found in the range of 25 - 150 ng/ml. The effect of change in ionic strength of aqueous sulfuric acid solutions on fluorescence intensity of drug sample was also studied.

Sekhar E.C., Rao T.R., Sekhar K.R., Naidu M.U., Shobha J.C., Rani P.U., Kumar T.V., and Kumar V.P. described determination of terazosin in human plasma, using high-performance liquid chromatography with fluorescence detection. The structurally related compound prazosin was used as an internal standard. The method comprises extraction with methylene chloride followed by chromatography on a C18 reversed-phase column. The compounds were detected using spectrofluorimetry. The absolute recoveries were more than 90% with a minimal detection of 1 ng/ml and calibration curve was linear between 1 and 80 ng/ml.

Bakshi M., Ojha T. and Singh S., described validated specific HPLC methods for determination of prazosin, terazosin and doxazosin in the presence of degradation products formed under ICH-recommended stress conditions. in the presence of degradation products generated from forced decomposition studies. Resolution of drugs from degradation products was obtained using a reversed-phase C-18 column using water/acetonitrile/ methanol/glacial acetic acid/diethylamine (25:35:40:1:0.017) as mobile phase for prazosin and terazosin and acetonitrile/water/glacial acetic acid/diethylamine (65:35:1:0.02) for doxazosin. The detection was done at 254 nm. The methods were validated with respect to linearity, precision, accuracy, specificity and robustness.

Raul A.S. and Adriana N.M. Described sensitive determination of terazosin by x-ray fluorescence spectrometry based on the formation of ion-pair associates with zinc

thiocyanate, The method is based on the precipitation of an ion-associate complex formed between terazosin and $[Zn(SCN)_4]^{2-}$ and the formation of a thin film on a membrane filter. The optimum conditions for ion-associate complex formation, such as pH, ionic strength, shaking time and stoichiometry of the complex, were studied. The complex was retained on the membrane filter, and the Zn Kα line was measured by XRF. The method presents the advantages of the separation of the drug from the matrix (excipients), the preconcentration of Zn, producing enhancement of XRF intensity though increased sensitivity, and the elimination of the matrix effects due to the thin film obtained. The lowest detection limit was 0.732 µg ml^{-1}. The validation studies were realized by the related applications and the results were evaluated statistically. The method was successfully applied to terazosin determination in tablets or in bulk drug.

Faridbod, F.; Ganjali, M. R.; Safaraliee, L.; Riahi, S.; Hosseini, M.; Norouzi, P., described Verapamil Potentiometric Membrane Sensor for **v**erapamil Pharmaceutical Analysis. Computational Investigation. Verapamil is a calcium channel blocker used in the management of angina, arrhythmia and hypertension. In this study a potentiometric liquid membrane sensor is used for simple and fast determination of verapamil in pharmaceutical formulation and urine. Computational studies were done electronically and geometrically on verapamil and tetraphenylborate before and after complex formation. These studies demonstrated tetraphenylbarate fits better with verapamil than potassium tetrakis. Thus, for the membrane preparation VER-tetraphenylborate complexes were employed as electroactive materials in the membrane. The wide linear range (10^{-5}-10^{-2} mol L^{-1}), low detection limit (8.2×10^{-6} mol L^{-1}), and fast response time (~30s) are characterizations of the proposed sensors. Validation of the method shows suitability of the sensors for application in quality control analysis of verapamil in pharmaceutical formulation and urine.

Sapna N. M., Pradeep R. V. described Stability indicating LC method for the estimation of venlafaxine in pharmaceutical formulations. The analysis was done on a Spherisorb C8 (4.6×250 mm, 5 µm) column. The mobile phase consisted of acetonitrile:sodium dihydrogen orthophosphate [0.04 M], pH 6.8 (75:25) at a flow rate of 1.5 ml/min. Detection was carried out at a wavelength of 224 nm. The developed method was found to give good separation between the pure drug and the degraded product. The polynomial regression data for the calibration plots showed good linear

relationship in the concentration range of 1–10 µg/ml with *r*=0.9999. The method was validated for precision, accuracy, ruggedness and recovery. The minimum detectable and minimum quantifiable amounts were found to be 150 and 600 ng/ml, respectively. The drug was stable under basic and oxidative conditions. However, the sample treated with acid showed an additional peak at a retention time of 4.32 min other than the main peak at a retention time of 5.32 min. Statistical analysis proves that the method is reproducible and selective for the estimation of venlafaxine. As the method could effectively separate the drug from the degradation product, it can be employed as a stability indicating one.

Bakshi M, Singh S. described review on stabilityindicating assay methods. The shortcomings of reported methods with respect to regulatory requirements are highlighted. A systematic approach for the development of stability-indicating methods is discussed. Critical issues related to development of SIAMs, such as separation of all degradation products, establishment of mass balance, stress testing of formulations, development of SIAMs for combination products, etc. are also addressed. The applicability of pharmacopoeial methods for the analysis of stability samples is discussed. The requirements of SIAMs for stability study of biotechnological substances and products are also touched upon.

3. AIM OF PRESENT WORK:

The above literature review reveals that there were many methods for the quantitative analysis of terazosin hydrochloride dihydrate as a drug saubstance as well as pharmaceutical dosage form, few methods are there which deals with bionalytical study and stability study. The aim of present work is to developed a shortest and optimized method for quantitative analysis of terazosin hydrochloride dihydrate formulation development and stability testing as well as for routine analysis. The aim and scope of the proposed work are as under.

- To develop rapid RP-UPLC method for quantification of the drug substance with highest selectivity, precision and accuracy.
- Forced Degradation Study to confirm the stability of the drug substance.
- Perform analytical method validation for the proposed method as per ICH guideline [17].

4. METHOD DEVELOPMENT

4.1 Mobile phase and column selection:

Based on literature review and drug information (pKa value 7.04), acetate and phosphate buffer (pKa = 2.1, 7.2 and 12.3) were selected. Many experimental trials were performed using selected buffer to obtained best chromatographic condition for assay analysis of terazosin hydrochloride dihydrate in tablet formulation. During experimental trails different column and organic solvent (i.e. methanol, acetonitrile etc.) used and at last accepted result obtained using developed chromatographic condition.

5. ANALYTICAL METHOD VALIDATION

Chromatographic condition:

Instrument	**:** Waters UPLC with empower software
Column	: ACQUITY UPLC BEH C18 (100*2.1) mm ,1.7 µ
Flow rate	: 0.25 mL/min
Mobile phase	: Buffer:Acetonitrilel (80:20)
Buffer	: 20 mm KH_2PO_4 in 1 lit. Water. (pH 3.0 by OPA)
Oven temperature	: 30°C
Wave length	: 246 nm
Injection volume	: 1µL
Run time	: 6 min
Diluent	**:** Water: Acetonitrile (50:50)

Mobile Phase Preparation:

Weigh 2.62 gm $KH2PO_2$ and transfer into 1000 mL water. Adjust the pH= 3.0 by Ortho phosphoric acid.

Diluent Preparation:

Water: Acetonitrile (50:50)

Blank Preparation:

Diluent is used as a blank

Standard Preparation:

To prepare a stock solution (500 µg/mL) for assay analysis, weigh accurately about 50 mg terazosin hydrochloride di hydrate reference standard and transfer into 100 mL

volumetric flask. Add 70 mL of diluents to dissolve the substance by sonication for one minute and dilute it up to the mark with diluents.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of terazosin hydrochloride di hydrate.

Test Preparation:

To prepare a stock solution (500 µg/mL) for assay analysis, weigh accurately about 50 mg terazosin hydrochloride di hydrate sample and transfer into 100 mL volumetric flask. Add 70 mL of diluents to dissolve the substance by sonication for one minute and dilute it up to the mark with diluents.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of terazosin hydrochloride di hydrate.

Procedure:

Inject blank followed by five replicated injection of standard preparation. Asymmetry of first injection of standard should not more than 2.0. Related standard deviation of replicate standard preparation should not more than 2.0 %. If system suitability pass than make duplicate injection of sample preparation.

5.1 Specificity Study:

The evaluation of the specificity of the method was determined against diluent and stress (forced) degradation. The specificity of the method toward the drug was established by means of the interference of the blank preparation and degradation products against drug during the forced degradation study.

Blank Preparation:

Diluent is used as a blank.

Standard Preparation:

To prepare a stock solution (500 µg/mL) for assay, weigh accurately about 50.02 mg terazosin hydrochloride di hydrate reference standard and transfer into 100 mL volumetric flask. Add 70 mL of diluents to dissolve the substance by sonication for one minute and dilute it up to the mark with diluents.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50.02 µg/mL of terazosin hydrochloride di hydrate.

Test Preparation:

To prepare a stock solution (500 µg/mL) for assay, weigh accurately about 50.24 mg terazosin hydrochloride dihydrate sample and transfer into 100 mL volumetric flask. Add 70 mL of diluents to dissolve the substance by sonication for one minute and dilute it up to the mark with diluents.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 .24µg/mL of terazosin hydrochloride dihydrate.

5.1.1 Forced Degradation Study:

Stress study was carried out by application of chemical and physical forced degradation. To perform forced degradation study, the drug content 50 mg in 100mL volumetric flask was employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After the degradation treatments were completed, the stress content were allowed to equilibrate to room temperature and diluted with diluent to attain 500 µg/ml concentrations, further 5 mL sample solution diluted to 50mL to attained 50 µg/ml concentration of terazosin hydrochloride dihydrate. Pattern of stress (degradation) conditions and preparation for same was described as under:

Acid Degradation:

Acidic degradation study was performed by heating the drug content in 0.1N HCl at about 80° C for 1 hour and after cooling to room temperature it was neutralized with 0.1 N NaOH solution. Further solution was diluted to achieve concentrations 50 µg/ml with diluent.

Alkali Degradation:

Alkaline degradation was performed by heating the drug content in 0.1 N NaOH at around 80˚ C for 1 hour and then the mixture was neutralized with 0.1 M HCl. It was further diluted with diluent to achieve 50 µg/ml concentrations.

Oxidative Degradation Study:

Oxidative degradation study was performed at room temperature for by adding 3% H_2O_2 in the drug content for 30 min then diluted to 50µg/ml with diluent.

Thermal Degradation Study:

Thermal degradation study was performing by keeping powdered drug content at around 80˚C for 1 hour. After this it was allowed to come at room temperature.

Photolytic Degradation Study:

Photolytic degradation study was performed by exposing drug content in sun-light for 48 hour, further it diluted to 50 µg/ml using diluent.

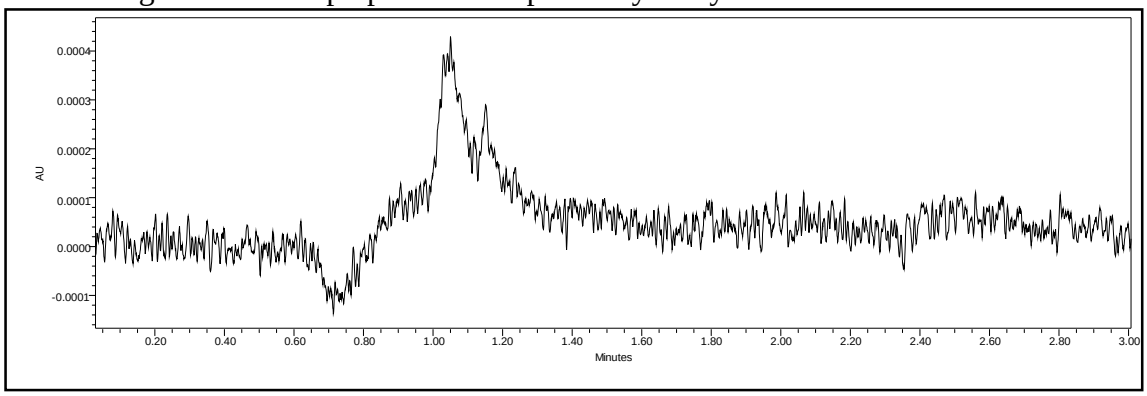
Blank preparation:

Blank preparation was also performed for all degradation to identify the peaks which arise due to blank. Blank was subjected under same all stress condition as that of sample.

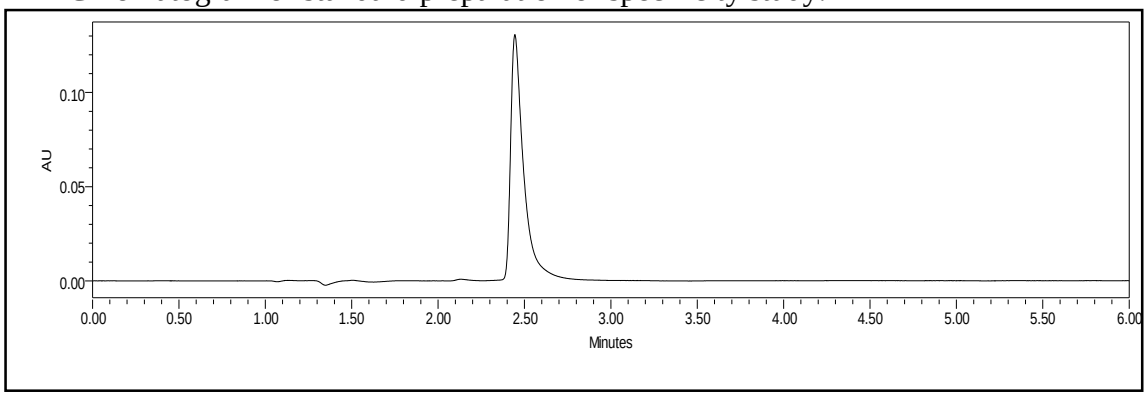
Table-1: Chromatographic sequence for Specificity study

No.	Description	Injection Replicate	Remarks
1	Blank	1	As such
2	Standard Preparation	5	
3	Test Preparation	2	
4	Bracketing Standard	1	
5	Blank preparation of acidic stress	1	Acidic Forced Degradation
6	Test preparation of acidic stress	2	
7	Bracketing Standard	1	
8	Blank preparation of alkali stress	1	
9	Test preparation of alkali stress	2	Alkali Forced Degradation
10	Bracketing Standard	1	
11	Blank preparation of oxidative stress	1	
12	Test preparation of oxidative stress	2	
13	Bracketing Standard	1	Oxidative Forced Degradation
14	Blank preparation of thermal stress	1	
15	Test preparation of thermal stress	2	
16	Bracketing Standard	1	
17	Blank preparation of photolytic stress	1	Thermal Forced Degradation
18	Test preparation of photolytic stress	2	
19	Bracketing Standard	1	

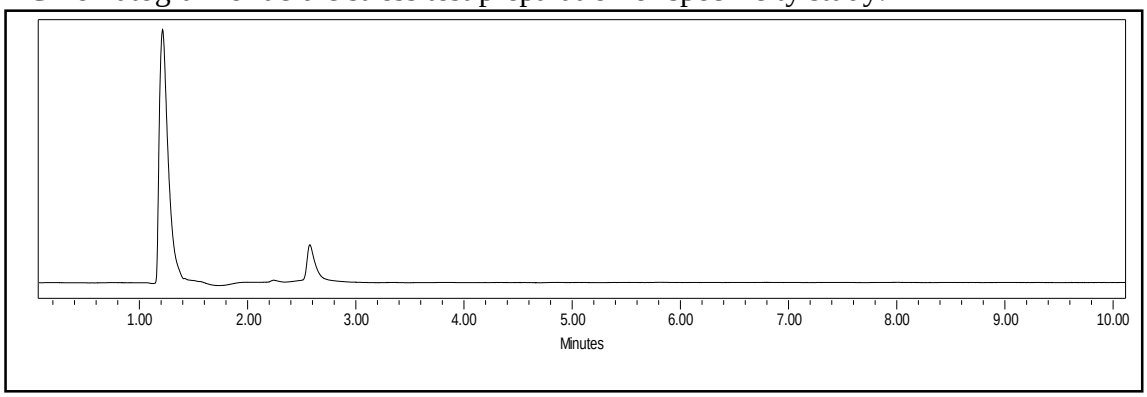
Chromatogram of blank preparation of specificity study:



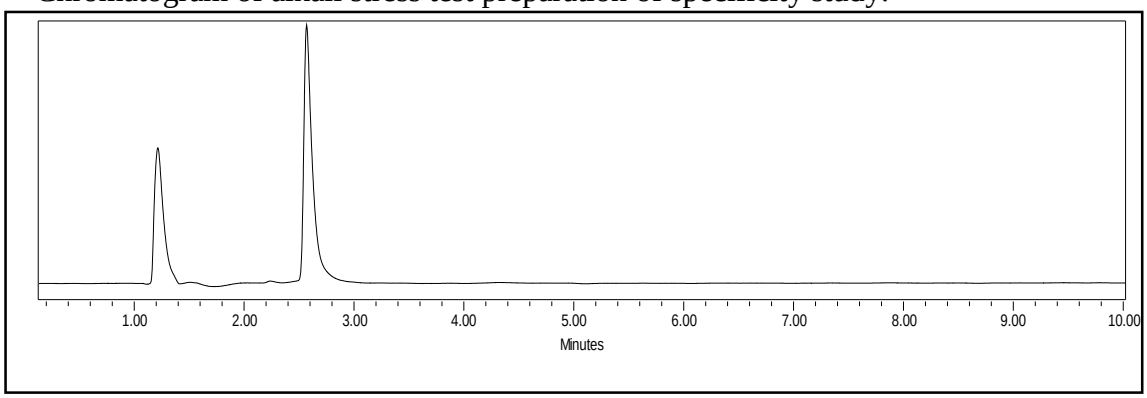
Chromatogram of standard preparation of specificity study:



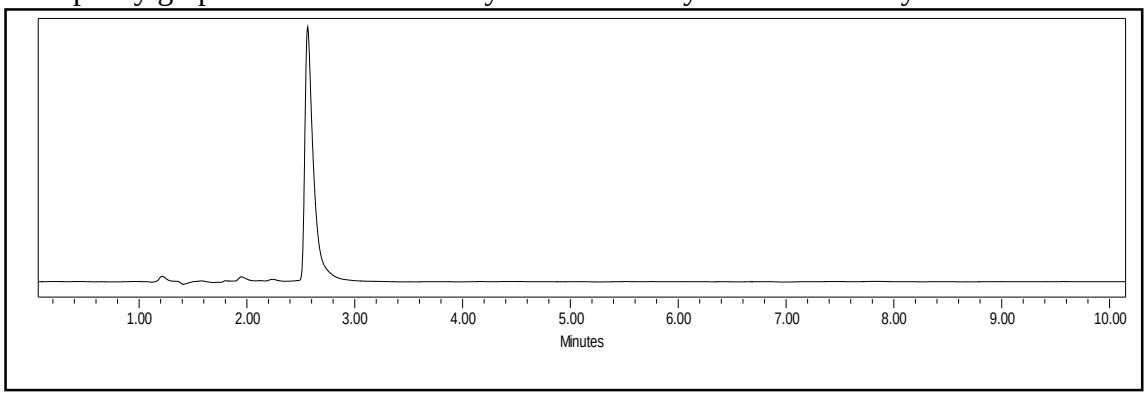
Chromatogram of acidic stress test preparation of specificity study:



Chromatogram of alkali stress test preparation of specificity study:



Peak purity graph of alkali stress study of terazosin hydrochloride dehydrate:



Observations:

(1) Any interference was not observed from blank to the peak of interest, in addition to this peak purity was also within the acceptance criteria proved by the photo diode detector.

(2) From the above chromatogram it can be conclude that there is no interference of any degradation product to the peak of interest and impurity has been generated by each stress condition.

Table-2: Degradation result of stress condition

Degradation Condition	Time	% Degradation
Acidic	1 Hrs at 80°C	90%
Basic	1Hrs at 80°C	39.01%
Oxidative	1Hrs at RT	6.01%
Thermal	1Hrs at 80°C	2.01%
Photolytic	48 hrs	5.05%

5.2 Linearity and Range:

The linearity plot was prepared with 8 concentration levels (20, 30, 40, 50, 60, 70 and 80 µg/ml of Terazosin hydrochloride dihydrate). These concentration levels were respectively corresponding to 40, 60, 80, 100, 120, 140 and 160% of standard solution concentration. The peak areas vs. concentration data were evaluated by linear regression analysis.

Standard solution preparation:

Weigh accurately 50.12mg of terazosin hydrochloride dihydrate standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved

by sonication and dilute up to the mark with diluent. The concentration obtained was 501.2 µg/ml of Terazosin hydrochloride dihydrate.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.12 µg/ml of Terazosin hydrochloride dihydrate.

Linearity Standard Solution Preparation:

Stock Solution:

Weigh accurately 50.10 mg of terazosin hydrochloride dihydrate standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 501.0 µg/ml of Terazosin hydrochloride dihydrate.

Linearity Level 1 (40%):

2 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 20.04 µg/ml of Terazosin hydrochloride dihydrate.

Linearity Level 2 (60%):

3 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 30.06 µg/ml of Terazosin hydrochloride dihydrate.

Linearity Level 3 (80%):

4 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 40.10 µg/ml of Terazosin hydrochloride dihydrate.

Linearity Level 4 (100%):

5 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 50.12 µg/ml of Terazosin hydrochloride dihydrate.

Linearity Level 5 (120%):

6 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 60.14 µg/ml of Terazosin hydrochloride dihydrate.

Linearity Level 6 (140%):

7 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 70.16 µg/ml of Terazosin hydrochloride dihydrate.

Linearity Level 7 (160%):

8 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 80.18 µg/ml of Terazosin hydrochloride dihydrate.

For each linearity level, the solution was injected in duplicate. Linearity was evaluated by linear regression analysis.

Linearity Level 8 (180%):

9 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 90.20 µg/ml of Terazosin hydrochloride dihydrate.

For each linearity level, the solution was injected in duplicate. Linearity was evaluated by linear regression analysis.

Table 3: Sequence of Linearity and range study

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Linearity level-1 (40%)	2
4	Linearity level-2 (60%)	2
5	Linearity level-3 (80%)	2
6	Linearity level-4 (100%)	2
7	Linearity level-5 (120%)	2
8	Linearity level-6 (140%)	2
9	Linearity level-7 (160%)	2
10	Bracketing Standard	1

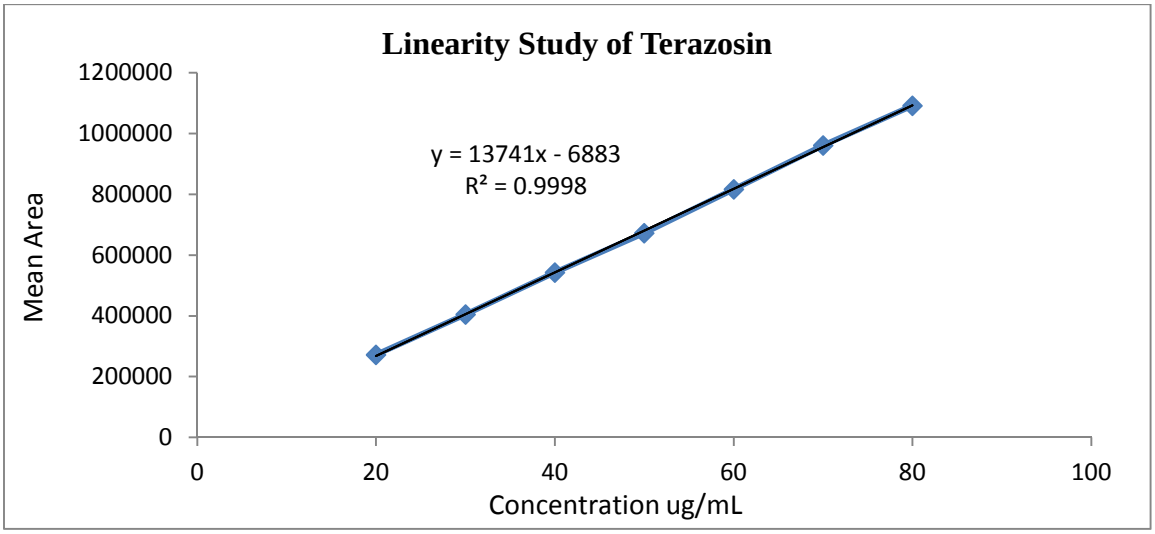
Table 4: Observation of Linearity and range study

Observation				
Data for Standard Preparation				
Replicate	**Area**		**Standard Weight**	50.07
1	323631		Standard Potency	99.42
2	324810			
3	325029			
4	325188			
5	325833			
Average	324898.2			
Stdev	804.44			
%RSD	0.25			
Data for Leniarity Level Preparation				
Linearity Level	Replicate	Area	Mean Area	
Level-1 (40%)	1	131755	132034	
	2	132313		
Level-2 (60%)	1	196785	196260	
	2	195735		
Level-3 (80%)	1	260529	259783	
	2	259037		
Level-4 (100%)	1	324143	324521	
	2	324899		
Level-5 (120%)	1	384971	385689	
	2	386407		
Level-6 (140%)	1	451027	450268	
	2	449509		
Level-7 (160%)	1	516488	516074	
	2	515660		

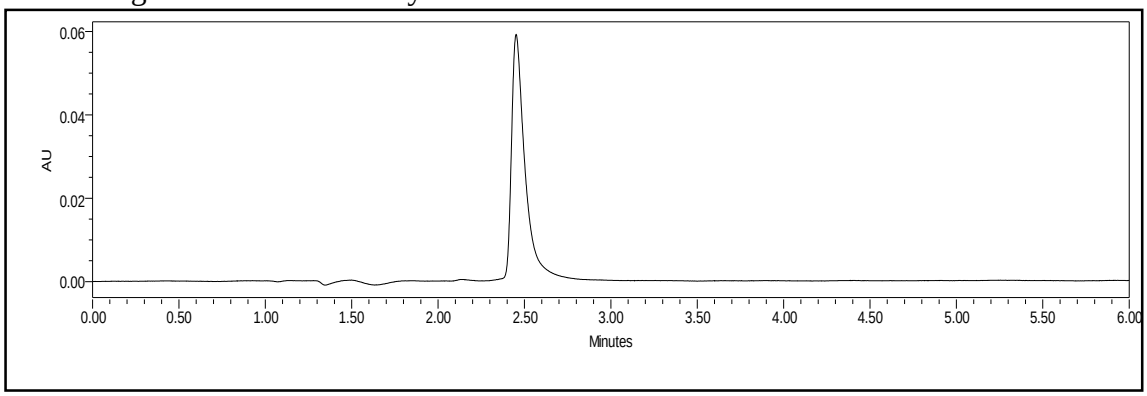
Table-5: Summary of linearity study:

Linearity Level	% of Level	Concentration (µg/ml)	Mean Area
1	40	20.0	132034
2	60	30.0	196260
3	80	40.0	259783
4	100	50.0	324521
5	120	60.0	385689
6	140	70.0	450268
7	160	80.0	516074
Correlation Co-efficient			0.999
Slope			3189
Intercept			4582

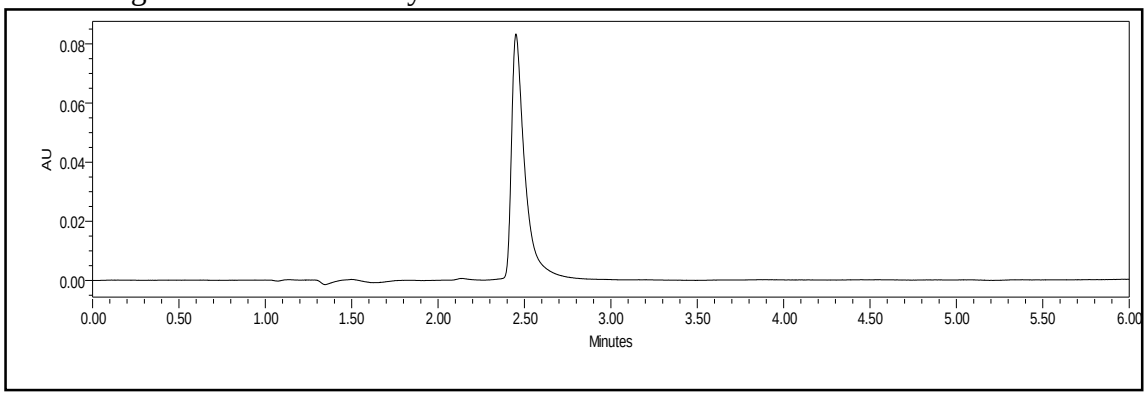
Chart 1: Evaluation of linearity



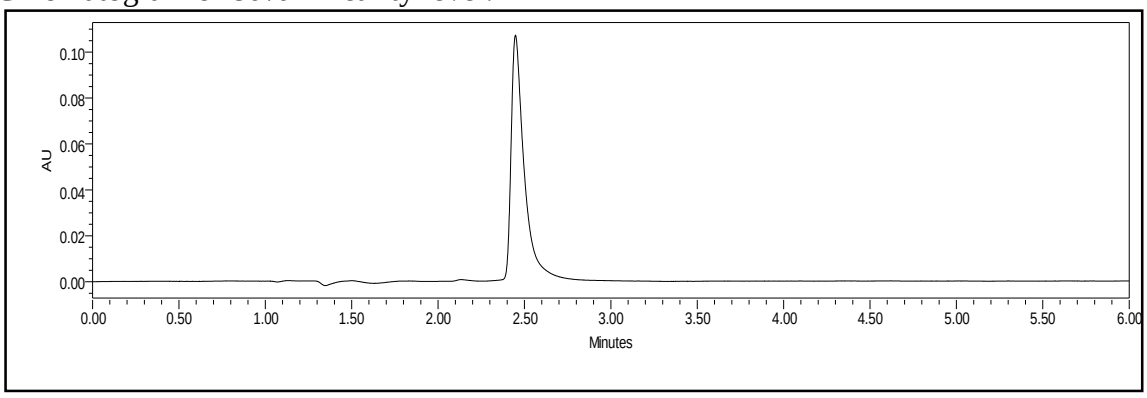
Chromatogram of 40% Linearity level:



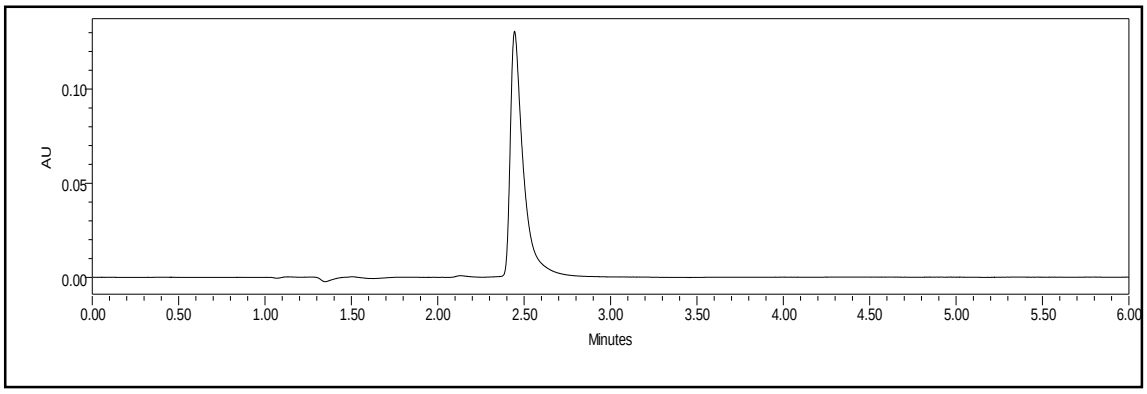
Chromatogram of 60% Linearity level:



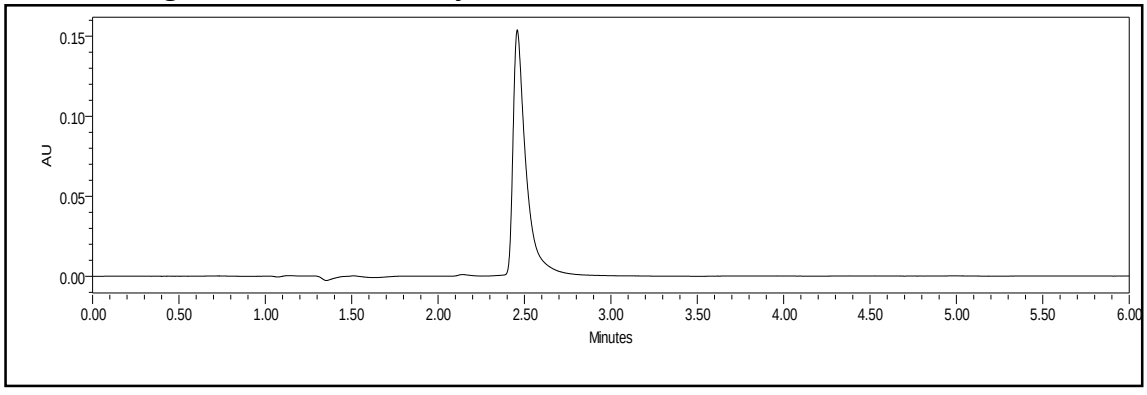
Chromatogram of 80% Linearity level:



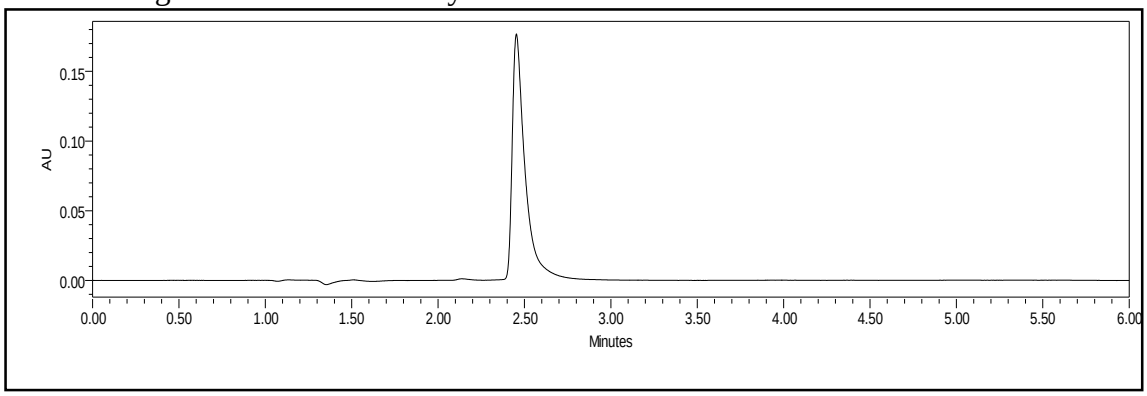
Chromatogram of 100% Linearity level:



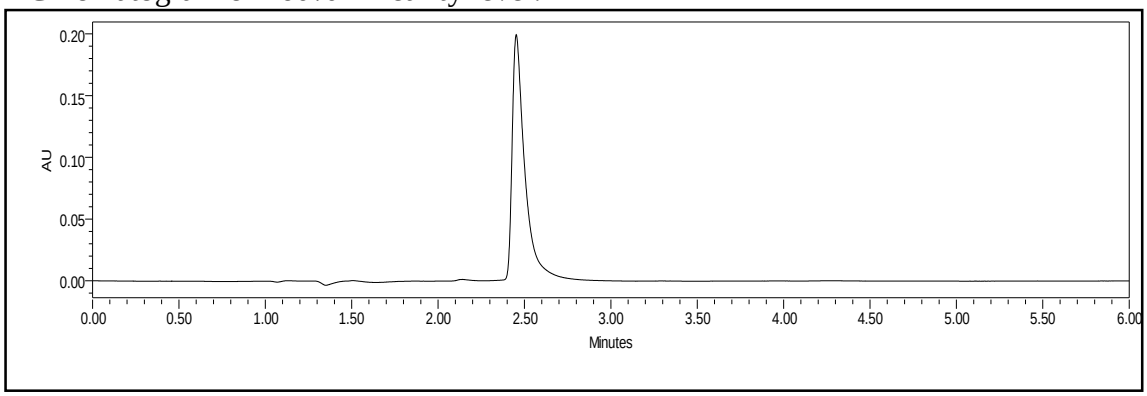
Chromatogram of 120% Linearity level:



Chromatogram of 140% Linearity level:



Chromatogram of 160% Linearity level:



Correlation coefficient of the linearity study was found to R^2 = 0.999 with linear regression equation y = 13741x + 6883, which proves the method is highly linear over the working range 20 – 80 µg/ml.

5.3 Precision Study:

Precision study was established by evaluating method precision and intermediate precision study. Method precision of the analytical method was determined by analyzing six sets of sample preparation. Assay of all six replicate sample preparations was determined and mean % assay value, standard deviation, % relative standard deviation and 95% confidence interval for the same was calculated.

Intermediate precision of the analytical method was determined by performing method precision on another day by another analyst using different make of raw materials under same experimental condition. Assay of all six replicate sample preparations was determined and mean % assay value, standard deviation, % relative standard deviation and 95% confidence interval for the same was calculated. Overall assay value of method precision and intermediate precision was compared and % difference and overall % relative standard deviation was calculated.

For method precision, blank, standard preparation and six sets of test preparations was prepared as per method as under:

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: Weigh accurately 50.16mg of terazosin hydrochloride dihydrate standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 501.6 µg/ml of Terazosin hydrochloride dihydrate.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.16 µg/ml of Terazosin hydrochloride dihydrate.

Test preparation:

Weigh accurately 50.13mg of terazosin hydrochloride dihydrate standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 501.3 µg/ml of Terazosin hydrochloride dihydrate.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.13 µg/ml of Terazosin hydrochloride dihydrate.

The same procedure was used for preparing the six Test preparation Sets. Same approach was applied for the intermediate precision study on the second day with different analyst.

Table-6: Sequence of precision study

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Test preparation (Set-1)	2
4	Test preparation (Set-2)	2
5	Test preparation (Set-3)	2
6	Test preparation (Set-4)	2
7	Test preparation (Set-5)	2
8	Test preparation (Set-6)	2
9	Bracketing Standard	1

Table-7: Summary of precision study

Observation					
Data for Standard preparation					
Replicate	Area		Standard Weight	50.16	
1	671853		Standard Potency	99.42	
2	672286				
3	670961				
4	674811				
5	672214				
Average	672425				
Stdev	1434.17				
%RSD	0.21				
Data for Test preparation					
Set No.	Replicate	Area	Mean Area	Wt. of Sample	% Assay
1	1	672231	671630	50.13	99.36
	2	671029			
2	1	671152	672258	50.03	99.65
	2	673364			
3	1	674855	675624	50.09	100.03
	2	676392			
4	1	683321	682938	50.21	100.87
	2	682554			
5	1	670021	670180	50.01	99.38
	2	670339			
6	1	672265	671677	50.18	99.27
	2	671088			

% Assay calculation is as under:

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{100} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} x P$$

Where,

A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

P = Potency of Working Standard (%).

Table -8: Summary of Intermediate precision study

Observation

Data for Standard preparation

Replicate	Area
1	672287
2	671953
3	675651
4	675214
5	672317
Average	673484
Stdev	1790.77
%RSD	0.27

Standard Weight	50.18
Standard Potency	99.42

Data for Test preparation

Set No.	Replicate	Area	Mean Area	Wt. of Sample	% Assay
1	1	673548	673014	50.07	99.69
	2	672480			
2	1	675336	674675	50.18	99.71
	2	674014			
3	1	674037	674688	50.2	99.67
	2	675339			
4	1	673002	672478	50.03	99.69
	2	671953			
5	1	670021	670952	50	99.52
	2	671882			
6	1	674412	673930	49.96	100.04
	2	673447			

% Assay calculation is as under:

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{100} x \frac{5}{50} x \frac{\mathbf{100}}{W_2} x \frac{50}{5} xP$$

Where,

A_T = Average Area of Test Preparation.

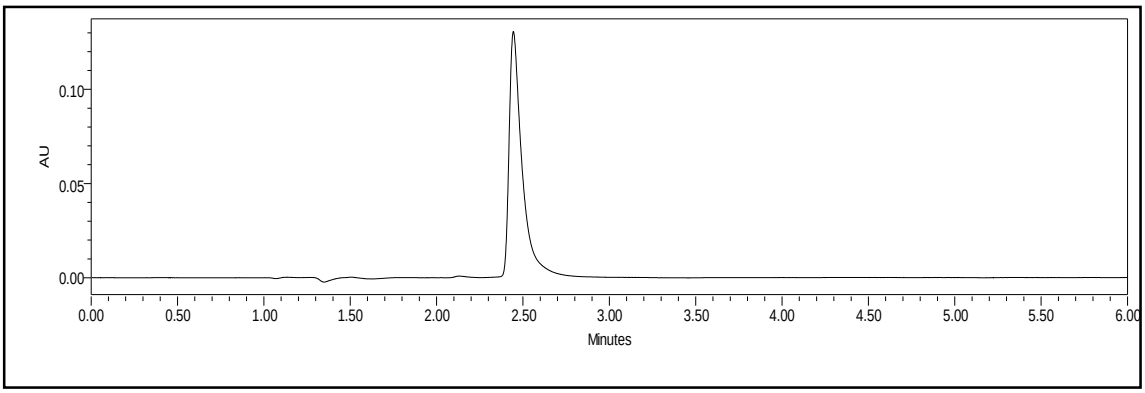
A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

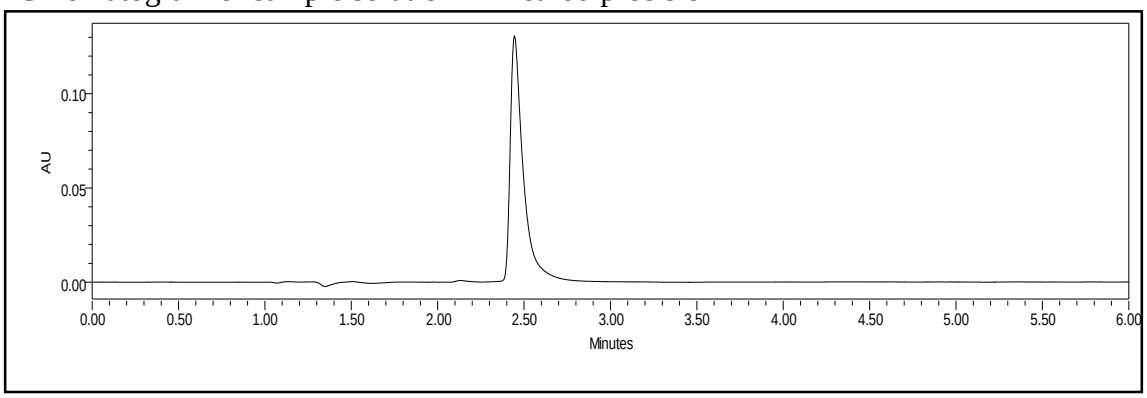
W_2 = Weight of Test Sample (mg).

P = Potency of Working Standard (%).

Chromatogram of Standard preparation in method precision



Chromatogram of sample solution in method precision



Chromatogram of sample solution in intermediate precision

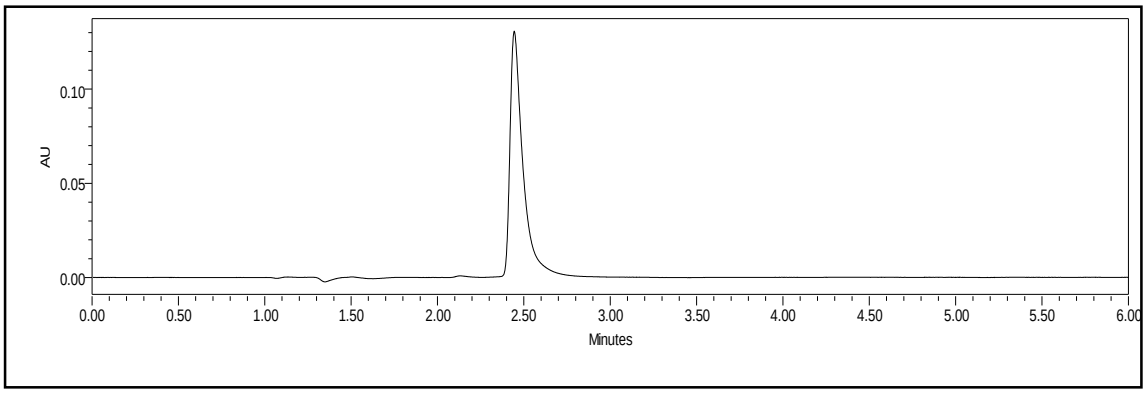


Table-9: Summary of precision study

Summary of precision study:				
Study	**%Assay**	**Mean Assay %**	**Std. Dev.**	**%RSD**
Method Precision	99.36	99.76	0.61	0.61
	99.65			
	100.03			
	100.87			
	99.38			
	99.27			
Intermediate Precision	99.69	99.72	0.17	0.17
	99.71			
	99.67			
	99.69			
	99.52			
	100.04			
Overall	99.74			
	0.43			
	0.43			

Overall the data for the precision study suggest % Assay value for each Test Preparation is between 98 – 102% which is under the acceptance criteria while % RSD of all results are less than 2%. Hence from all the observation it can conclude that the proposed method is highly precise.

5.4 Accuracy Study:

This Experiment can be performed by the recovery test. Recovery of the method was evaluated at 3 different concentration levels (Generally corresponding to 50, 100 and 150% of test solution concentration) by addition of known amounts of standard to placebo preparation. For each concentration level, 3 sets were prepared and injected in duplicate.

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: Weigh accurately 50.14mg of terazosin hydrochloride dihydrate standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 501.4 µg/ml of Terazosin hydrochloride dihydrate.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.14 µg/ml of Terazosin hydrochloride dihydrate.

Sample preparations for accuracy levels are as under:

Accuracy level 1 (50%) :

Test stock solution:

Weigh accurately 24.96 mg of terazosin hydrochloride dihydrate standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 249.6 µg/ml of Terazosin hydrochloride dihydrate.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 24.96 µg/ml of Terazosin hydrochloride dihydrate. The same procedure was applied for preparing the three sets.

Accuracy level 2 (100):

Test stock solution:

Weigh accurately 50.04 mg of terazosin hydrochloride dihydrate standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 500.4 µg/ml of Terazosin hydrochloride dihydrate.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.04 µg/ml of Terazosin hydrochloride dihydrate. The same procedure was applied for preparing the three sets.

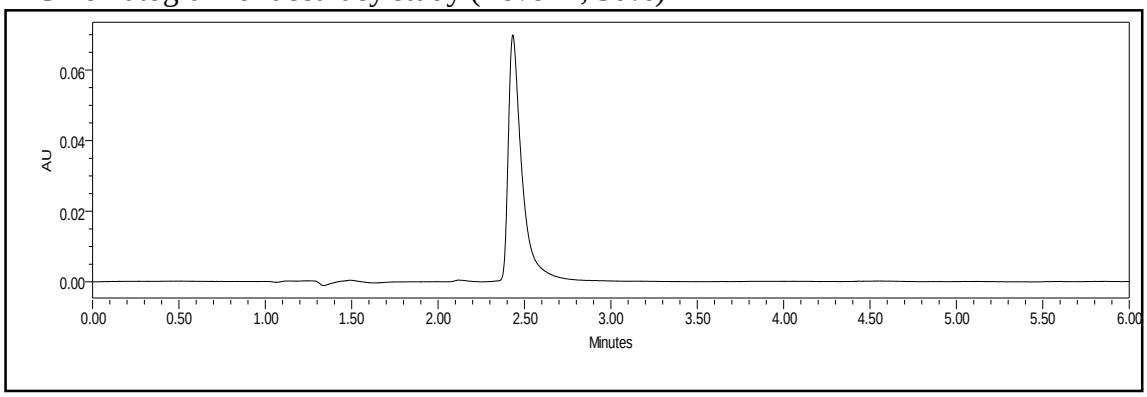
Accuracy level 3 (150 %):

Test stock solution:

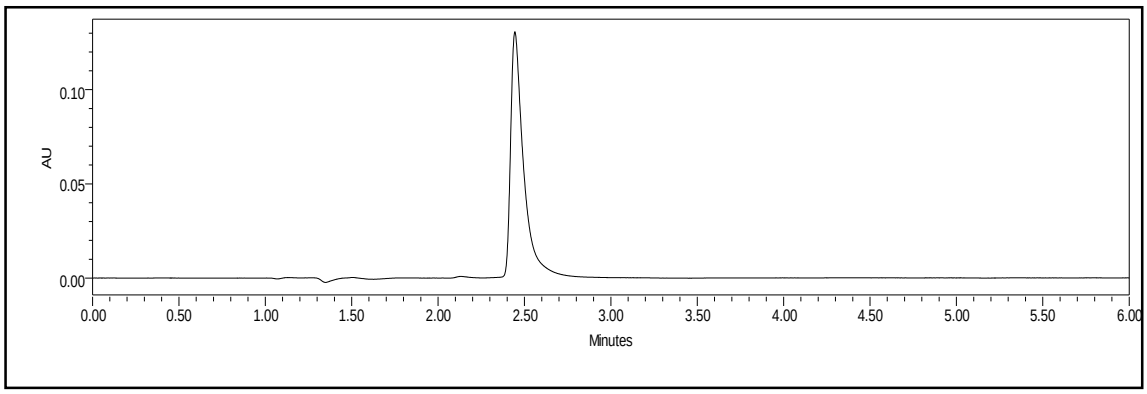
Weigh accurately 75.02 mg of terazosin hydrochloride dihydrate standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 750.2 µg/ml of Terazosin hydrochloride dihydrate.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 75.02 µg/ml of Terazosin hydrochloride dihydrate. The same procedure was applied for preparing the three sets.

Chromatogram of accuracy study (Level-1, 50%)



Chromatogram of accuracy study (Level-2, 100%)



Chromatogram of accuracy study (Level-3, 150%)

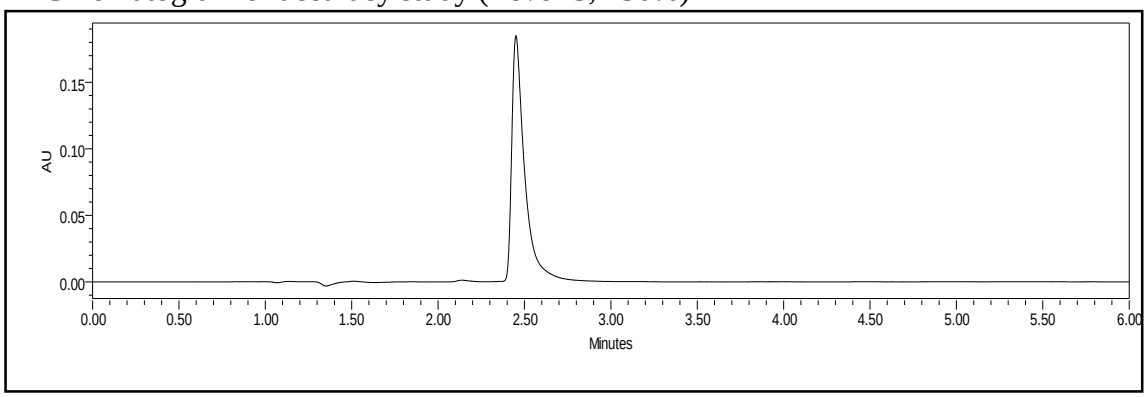


Table-10: Sequence of Accuracy Study

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Accuracy level-1 preparation: Set-1	2
4	Accuracy level-1 preparation: Set-2	2
5	Accuracy level-1 preparation: Set-3	2
6	Accuracy level-2 preparation: Set-1	2
7	Accuracy level-2 preparation: Set-2	2
8	Accuracy level-2 preparation: Set-3	2
9	Accuracy level-3 preparation: Set-1	2
10	Accuracy level-3 preparation: Set-2	2
11	Accuracy level-3 preparation: Set-3	2
12	Bracketing Standard	1

Table-11: Summary of accuracy study:

Observation				
Data for Standard preparation				
Replicate	Area		Standard Weight	50.14
1	671558		Standard Potency	99.42
2	672174		Standard Conc.	50.14
3	674402			
4	678315			
5	669232			
Average	324873			
Std. dev.	3430.80			
%RSD	1.06			
Data for Test preparation				
Accuracy Level	Set No	Replicate	Area	Mean Area
1(50%)	1	1	162547	162825
		2	163103	
	2	1	164338	164834
		2	165330	
	3	1	164841	164963
		2	165085	
2(100%)	1	1	321838	321633
		2	321428	
	2	1	325563	324873
		2	324183	
	3	1	323139	322929
		2	322719	
3(150%)	1	1	488021	488929
		2	489837	
	2	1	488902	488800
		2	488698	
	3	1	488021	488865
		2	489709	

Table-12: Summary of accuracy study:

Accuracy (Recovery) Study							
Accuracy Level	**Set No**	**Amount added (µg/ml)**	**Amount Found (µg/ml)**	**Recovery (%)**	**Average recovery**	**Std Dev.**	**% RSD**
I (50%)	1	24.96	25.13	100.68	101.21	0.47	0.47
	2	25.04	25.44	101.60			
	3	25.12	25.46	101.35			
II (100%)	1	50.04	49.64	99.20	99.67	0.61	0.61
	2	49.96	50.14	100.36			
	3	50.12	49.84	99.44			
II (150%)	1	75.02	75.46	100.59	100.58	0.09	0.09
	2	74.94	75.44	100.67			
	3	75.08	75.45	100.49			

Calculation formulas for recovery study are as under:

$$\text{Amount added } (\mu g/ml) = \frac{Wt.taken}{Volume\,1} x \frac{Volume\,2}{Volume\,3} x\,1000$$

$$\text{Amount found } (\mu g/ml) = \frac{Mean\ area\ of\ test\ preparation}{Average\ area\ of\ standard\ preparation} x\ standard\ conc.$$

Where, Volume = Dilution given for preparing the solution.

From the all above data it has been proven that the % recovery is within the limit of 98 to 102 % this is in the limit of acceptance criteria and % RSD value of % recovery of replicate set is below 2 % .Hence this suggest that proposed method is highly accurate.

5.5 Robustness Study:

Robustness of the method was evaluated by assaying test solutions under slight but deliberate changes in analytical conditions, such as change in flow rate, change in proportions of Buffer-Acetonitrile (82:18 and 78:22,v/v), Change in flowrate and change in column-lot.

5.5.1 Change in flow rate:

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: Weigh accurately 50.21 mg of terazosin hydrochloride dihydrate standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 502.1 µg/ml of Terazosin hydrochloride dihydrate.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.21 µg/ml of Terazosin hydrochloride dihydrate.

Test preparation:

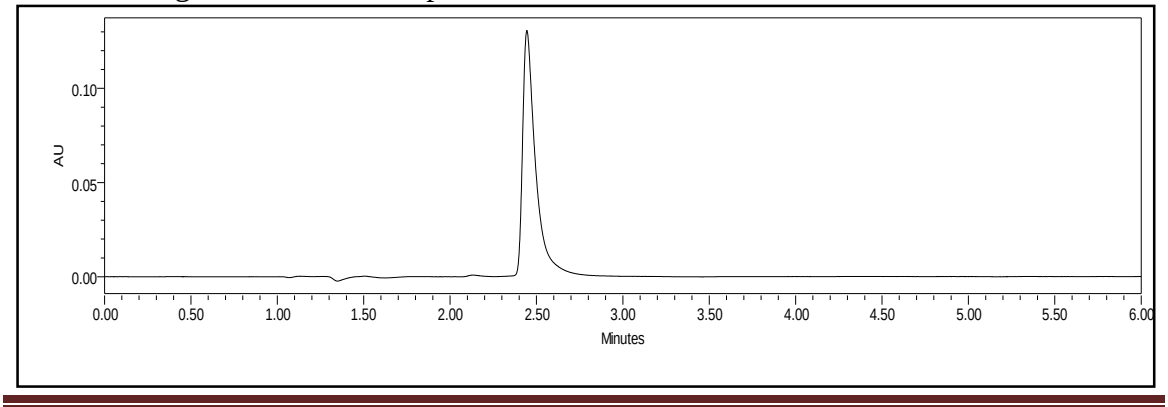
Weigh accurately 50.17 mg of terazosin hydrochloride dihydrate standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 501.7 µg/ml of Terazosin hydrochloride dihydrate.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.17 µg/ml of Terazosin hydrochloride dihydrate.

Table-13: Sequence for flow rate robustness study

No.	Description	Injection Replicate	Chromatographic Parameter
1	Blank	1	Flow rate: 0.24 mL/min
2	Standard Preparation	5	
3	Test Preparation	2	
4	Bracketing Standard	1	
5	Blank	1	Flow rate: 0.26 mL/min
6	Standard Preparation	5	
7	Test Preparation	2	
8	Bracketing Standard	1	

Chromatogram of robustness parameter flow rate 0.24 ml/min



Chromatogram of robustness parameter flow rate 0.26 ml/min

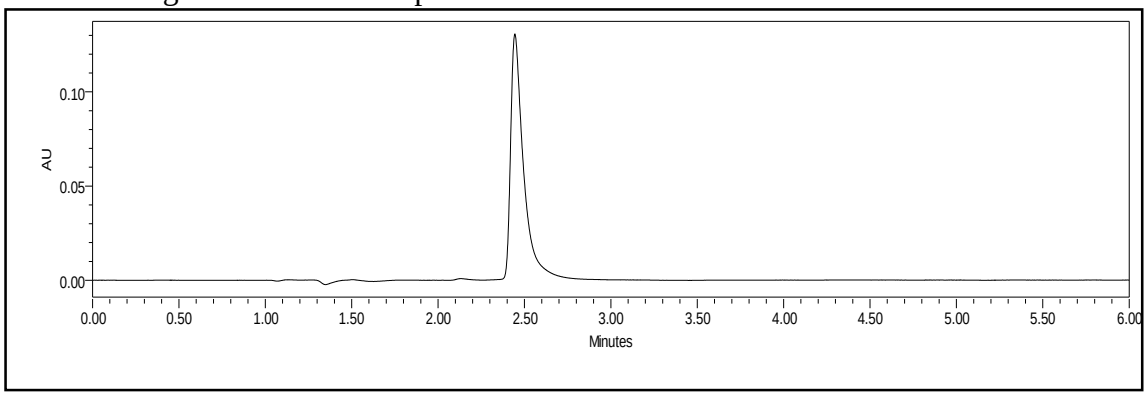


Table-14: Summary for flow change parameter of robustness study

At 0.24 mL/min flow rate		**At 0.26 mL/min flow rate**	
Data for standard preparation		*Data for standard preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	691175	1	651782
2	692335	2	652117
3	690287	3	652530
4	694634	4	654943
5	691023	5	643235
Mean	691891	**Mean**	650921
Std.dev.	1700.01	**Std.dev.**	4472.46
%RSD	0.25	**%RSD**	0.69
Data for Test preparation		*Data for Test preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	691285	1	657264
2	691029	2	657563
Mean	691157	**Mean**	657414
Standard wt. (mg)	50.21	Standard wt. (mg)	50.21
Test wt. (mg)	50.17	Test wt. (mg)	50.17
%Assay	99.39	%Assay	100.49

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{100} x \frac{5}{50} x \frac{\mathbf{100}}{W_2} x \frac{50}{5} xP$$

Where,

A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

P = Potency of Working Standard (%).

5.5.2 Change in mobile phase composition:

In this experiment the test samples were analyzed at the mobile phase proportion of (Buffer:Acetonitrile) 82:18 and 78:22 v/v each and the results were observed in terms of assay value.

Blank preparation:

Diluent was used as blank.

Standard preparation:

Refer standard preparation under flow rate parameter of robustness study

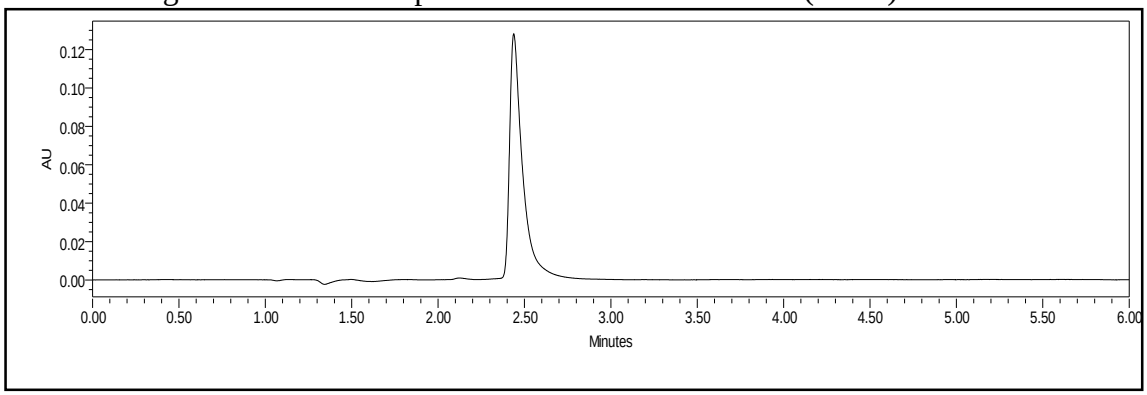
Test preparation:

Refer test preparation under flow rate parameter of robustness study.

Table-15: Sequence for change in composition robustness study

No.	Description	Injection Replicate	Chromatographic Parameter
1	Blank	1	Buffer:Acetonitrile (82:18)
2	Standard Preparation	5	
	Test Preparation	2	
4	Bracketing Standard	1	
5	Blank	1	Buffer:Acetonitrile (78:22)
6	Standard Preparation	5	
7	Test Preparation	2	
8	Bracketing Standard	1	

Chromatogram of robustness parameter Buffer:Acetonitrile (72:18)



Chromatogram of robustness parameter Buffer:Acetonitrile (78:22)

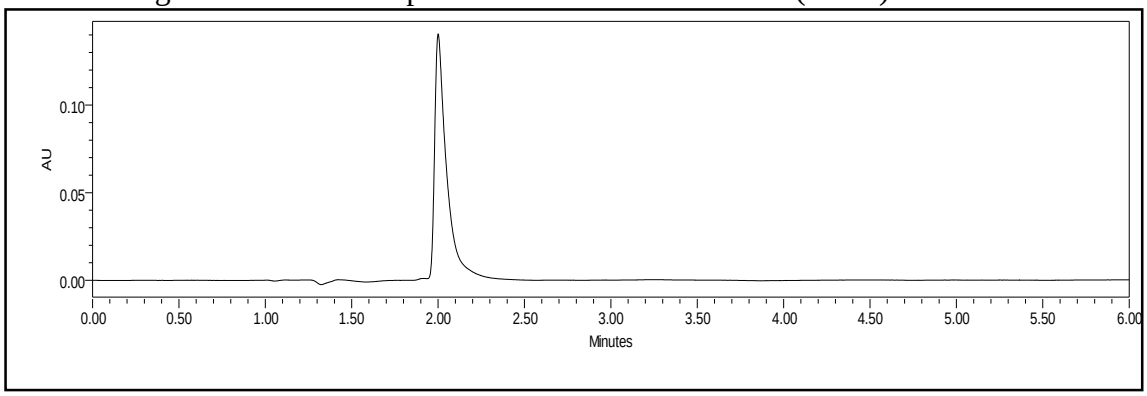


Table-16: Summary for change mobile phase composition.

Buffer:Acetonitrile (82:18)		**Buffer:Acetonitrile (78:22)**	
Data for standard preparation		*Data for standard preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	688174	1	663343
2	688385	2	662187
3	686431	3	669285
4	687902	4	663544
5	685229	5	662519
Mean	687224	**Mean**	664176
Std.dev.	1352.68	**Std.dev.**	2911.05
%RSD	0.20	**%RSD**	0.44
Data for Test preparation		*Data for Test preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	689243	1	663574
2	687154	2	663028
Mean	688199	**Mean**	663301
Standard wt. (mg)	50.21	Standard wt. (mg)	50.21
Test wt. (mg)	50.17	Test wt. (mg)	50.17
%Assay	99.64	%Assay	99.37

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{100} x \frac{5}{50} x \frac{\mathbf{100}}{W_2} x \frac{50}{5} xP$$

Where,

A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

P = Potency of Working Standard (%).

5.5.3 Robust Parameter: Change column lot

In this parameter, column used in analytical method was changed to different lot. Sample was assayed by changing the lot of column.

Blank preparation:

Diluent was used as blank.

Standard preparation:

Refer standard preparation under flow rate parameter of robustness study

Test preparation:

Refer standard preparation under flow rate parameter of robustness study.

Table-17: Sequence for change in composition

No.	Description	Injection Replicate	Chromatographic Parameter
1	Blank	1	Change column lot
2	Standard Preparation	5	
3	Test Preparation	2	
4	Bracketing Standard	1	

Chromatogram of robustness parameter column change

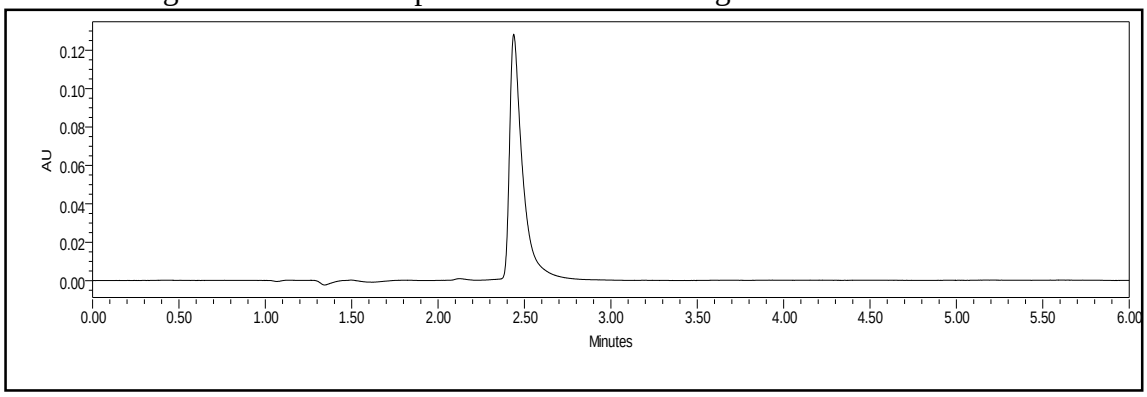


Table-18: Summary for column change parameter of robustness study

Column lot change	
Data for standard preparation	
Replicate	**Area**
1	671226
2	672802
3	672114
4	672556
5	672301
Mean	672199.8
Std.dev.	603.2024535
%RSD	0.09
Data for Test preparation	
Replicate	**Area**
1	672253
2	672865
Mean	672559
Standard wt. (mg)	50.21
Test wt. (mg)	50.17
%Assay	99.55

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{100} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} xP$$

Where,

A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

P = Potency of Working Standard (%).

Table-19: Summary of robustness study

Summary of Robustness Study				
Robust Condition	**% Assay**	**Retention time (min.)**	**System Suitability**	
			Theoretical Plates	**Asymmetry**
Flow Change 0.23 ml/min	99.39	2.47	5750	1.19
Flow Change 0.27 ml/min	100.49	2.42	4274	1.15
MP Proportion Change A:B= 82:18	99.64	2.43	4359	1.20
MP Proportion Change A:B= 78:22	99.37	2.10	6284	1.15
Column Lot Change	99.55	2.45	5011	1.16

The data and the chromatogram given above suggest that there is no considerable influence of the change in flow rate, mobile phase composition and column lot change on the result of the analysis by this method or on chromatographic suitability of this method. Hence, it can be conclude from this experiment that the method is highly robust.

5.6 Solution stability study:

Solution stability period for the solutions of standard preparation and test preparation was evaluated. The solutions were stored at 5° C and ambient temperature without protection against light and tested at interval of 6, 12 and 24 hrs. The responses for the aged solution were evaluated using a freshly prepared standard solution..

Table-20: Summary of solution stability

Time intervals	**Absolute difference in assay for standard solution %**		**Absolute difference in assay for sample solution %**	
	At 5°C	**At room temperature**	**At 5°C**	**At room temperature**
After 6 hours	0.08	0.13	0.80	0.74
After 12 hours	0.28	0.50	1.06	1.22
After 24 hours	1.92	2.22	2.42	2.74

Result of solution stability shows that standard solution is stable up to 24 hrs when stored at 5C and up to 12 hours when stored at room temperature. Sample solution of terazosin is stable to 12 hours when stored at 5C and room temperature.

5.7 System suitability study:

A system suitability test for the chromatographic system was performed before each validation experiment. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same. Only

after the system suitability results were in acceptance criteria the experiments were precede further.

The Theoretical plates should be more than 2000, Asymmetry should be less than 2.0 and % RSD should be less than 2.0. As the data suggest the system suitability was within the criteria in each validation experiment. Hence the system was found suitable to perform the validation experiment which confirms the reliability of the data generated during the method validation.

6. CONCLUSION:

The surveillance and outcome obtained from each validation experiment including specificity, linearity and range, LOD and LOQ, precision, accuracy, robustness, solution stability and system suitability lies well inside the acceptance criteria. Since, all the results are with-in the limit, the developed Analytical method is considered as validated and suitable for anticipated use.

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