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Degradation Studies and Stability Assessment of Terizidone using RP-HPLC Method

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Abstract: *In recent years the growing interest in drug stability problem has been observed. The stability of pharmaceutical products seems to play an important role from the economical point of view. The present study was undertaken with the primary objective to establish the inherent stability of Terizidone through stress studies under a variety of ICH-recommended test conditions and to determine the Terizidone degradation using validated HPLC method. Terizidone is an anti-tuberculosis drug used in the treatment of multi-drug resistant and extensively drug-resistant tuberculosis but present with polyneuropathic adverse effects in some patients. In present work, forced degradation study of Terizidone was carried out according to the ICH guideline Q1A (R_2). The drug was subjected to acid (0.1N HCL), alkaline (0.1N NaOH) and neutral hydrolysis conditions as well as an oxidative decomposition at room temperature. Photo stability and thermal study was also carried out.*

Keywords: Terizidone, drug stability, degradation studies, RP-HPLC method, validation

I. INTRODUCTION

Terizidoneis a broad spectrum antibiotic having molecular formula $C_{14}H_{14}N_4O_4$. It is a reserved class/second line anti-tubercular drug[1,2]with chemical name 4,4'-[1,4-phenylenebis(methylylidynenitrilo)]bis(isoxazolidin3-one)[3]. It is white to pale yellow powder, soluble in water, slightly soluble in methanol and propylene glycol. Terizidone contains two molecules of cycloserine attached to each other by one molecule of Teriphthaldehyde.

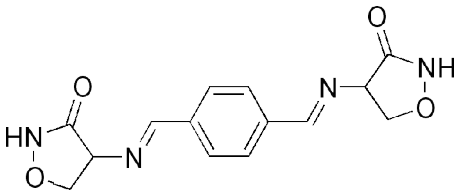

Figure: 1 Chemical Structure of Terizidone

It has an antibiotic activity against mycobacterium tuberculosis and *M. avium*for the treatment of tuberculosis, i.e. pulmonary and extra pulmonary[4].It is bacteriostatic and works by preventing the cell wall synthesis in bacteria that cause tuberculosis. The emergence of strains of Mycobacterium tuberculosis which is resistant to all the first line drugs (MDR-TB) is causing serious concern. Therefore, study of second line drugs has gained importance. Literature survey reveals methods reported are area under curve and first order derivative spectrophotometry[5], simple UV spectrophotometric method[6] and RP-HPLC method[7] for estimation of Terizidone.

II. MATERIALS AND METHODS

Chemicals: All the solvents & chemical reagents used were HPLC grade and were purchased from LobaChemiePvt. Ltd. Mumbai, India.Terizidone is available in market with brand name Tericox (Label claim: 250 mg) as capsules.

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Instrumentation:

High Performance Liquid Chromatography

The liquid chromatography system (Perkin Elmer) comprised of Jasco PU 1580 intelligent pump, an injector equipped with 20 ul sample loop and a UV/VIS Detector (Jasco UV 1575). Total chrome navigator software was used for data acquisition, reporting & analysis.

HPLC method development conditions C18 hypersil 4.6 (i.d.) x 250mm

The analytical column used for chromatographic separations was C_{18}hypersil (250 mm x4.6 mm i.d., particle size 5 µm). Mobile phase was pumped through the column at a flow rate of 1 ml/min, at room temperature. The analytical wavelength of the UV/VIS spectrophotometric detector was set at 200-400 nm (Figure 2- UV spectra for Terizidone). An accurately weighed quantity of 50mg Terizidone was dissolved in Water in 50ml volumetric flask and volume was made up to the mark, to get the concentration 1000µg/ml. The 1.0 ml portion of standard stock solution of Terizidone was diluted up to 100ml with water to get final concentration 10µg/ml. Samples of 10 µl of solution were injected. In addition, Whatman filter paper no. 45 & single use syringe was used.Terizidone exhibited absorption maxima at 260 nm.

Mobile phase preparation

The mobile phase composed of Acetonitrile: Water (70:30%v/v). The mobile phase was filtered through Whatman filter paper no.41& degassed by sonication for 30 min.The stability of Terizidone in mobile phase was investigated by keeping drug in mobile phase for 24hr. Turbidity was not found in the sample solution indicating the stability of terizidone in the selected mobile phase.

Preparation of standard solutions

An accurately weighed 50mg terizidone was dissolved in mobile phase in 50ml volumetric flask and volume was made up to the mark to form 1000µg/ml. The aliquot portion of standard stock solution of terizidone was further diluted with mobile phase to get series of concentration ranging from 10-50 µg/ml. The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained.

The 20 µl of the prepared solutions were directly injected into the column and the peak area of the various dilutions was recorded at 260nm.A graph was plotted as concentration of drug in µg/mlverses peak area to get standard calibration curve.

Preparation of sample solution of Terizidone

Accurately weighed quantity 10 mg of Terizidone dissolved in mobile phase and volume was made up to 100 ml mark to get standard stock solution having concentration 100µg/ml of Terizidone. It was degassed by sonication for 15 min. The stock standard solution was diluted further with mobile phase to get final concentration of about 50 µg/ml of Terizidone.

Method validation

The previously filtered and degassed (sonicated for 15 min.) mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. A 20 µL standard solution of drug mixture was injected in five replicates and the system suitability parameters were recorded.Accuracy of the method was ascertained on basis of recovery studies (Table 1) performed by standard addition method. These studies were carried out at three levels i.e. multiple level recovery studies.An accurately weighed quantity of pre-analysed Terizidone 50mg was taken in 50 ml volumetric flasks and to it 80%, 100%, and 120% of Terizidone pure drug and mobile phase was added and sonicated for 15 minutes, finally volume was made to mark with the mobile phase and filtered through Whatman filter paper No. 41, and required dilution were made.

Precision of the method was determined with 5 replicate sample solutions. The results were expressed as the % S.D. (Standard Deviation) & % R.S.D. (Relative Standard Deviation) of the series of measurements(Table-1). For studying the linearity and range, accurately weighed quantity of Terizidone drug equivalent to 80, 90, 100, 110, and 120 % was

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taken and dissolved in mobile phase, diluted appropriately with mobile phase to obtain a concentration in the range of 80% to 120% of the test concentration. The chromatograms of the resulting solutions were recorded. The plot showing linearity and range study for Terizidone is shown in the Figure 4.The study of ruggedness of the method was carried out under two different conditions i.e. by performing the assay with different analysts & on different days. System suitability studies (Table 2) were carried out on freshly prepared standard stock solutions of concentration 10 µg/ml to validate chromatographic parameters.

Sr. no.	Level of recovery	Weight of drug taken(mg)	% amount of drug found on pre-analysed basis	Amount of pure drug added (mg)	Peak area	% Recovery
1	80%	50.1	99.99	40.8	2433754	99.70
2	100%	50.2	99.01	52.0	2706253	99.84
3	120%	50.1	99.99	61.2	2981087	100.07
					Mean	99.87
					S.D.	0.1973
					R.S.D.	0.01976
					C.V.	0.197616

Table-1:Results of recovery study

Sr. no.	Peak Area	Retention Time	Capacity Factor	No. of Theoretical plate	Asymmetry
1	1357607	4.083	0.634	23716	0.00365
2	1357727	4.092	0.638	24336	0.00359
3	1357643	4.085	0.635	24964	0.00362
4	1357543	4.057	0.624	23104	0.00358
5	1357510	4.037	0.616	23839	0.00358
Mean	1357625.2	4.070	0.629	23991.8	0.003604
S.D.	88.092	0.0231	0.00064	140.8581	0.0009746
R.S.D.	0.000648	0.00567	0.000102	0.0058710	0.027042
C.V.	0.006488	0.5722	0.1011	0.5939	0.2704

Table -2 :Results for system suitability test

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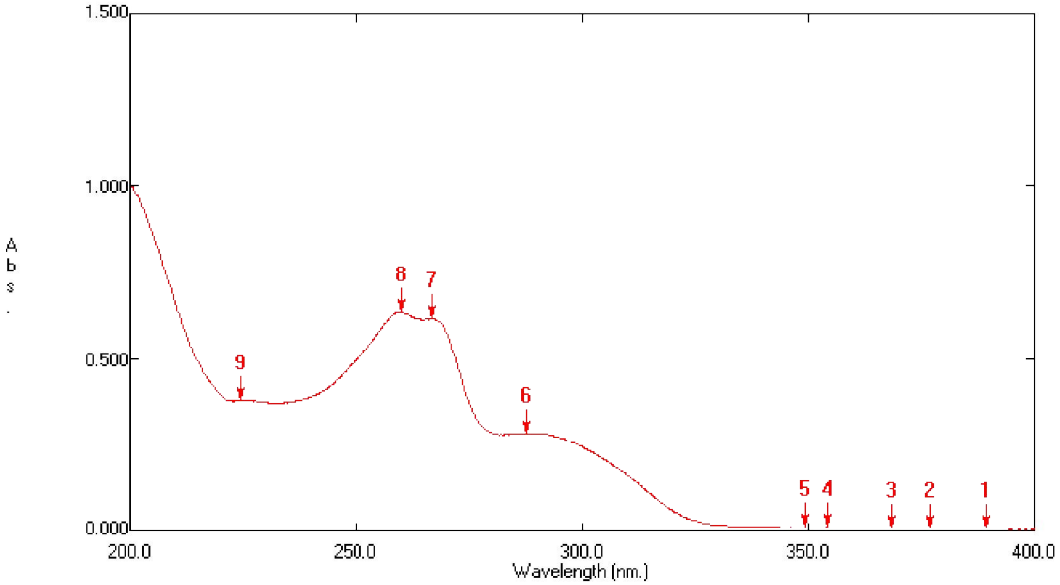

Figure 2: UV spectrum for Terizidone in water

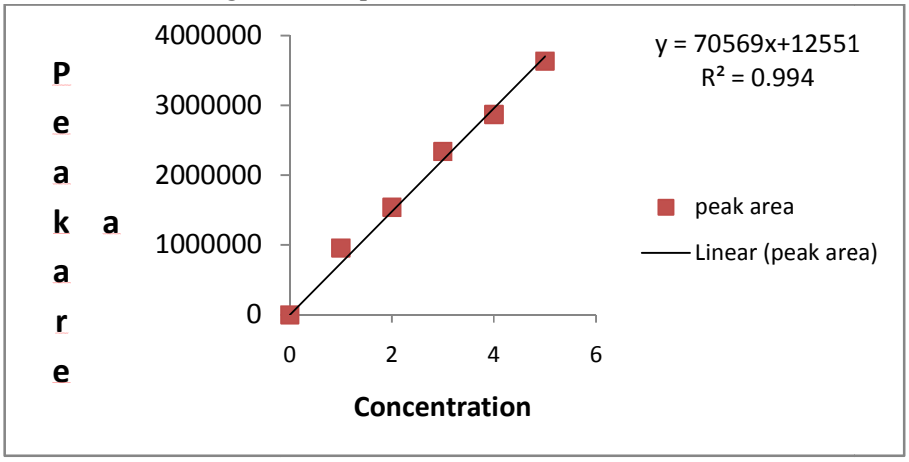

Figure 3: Standard calibration curve for determination of terizidone

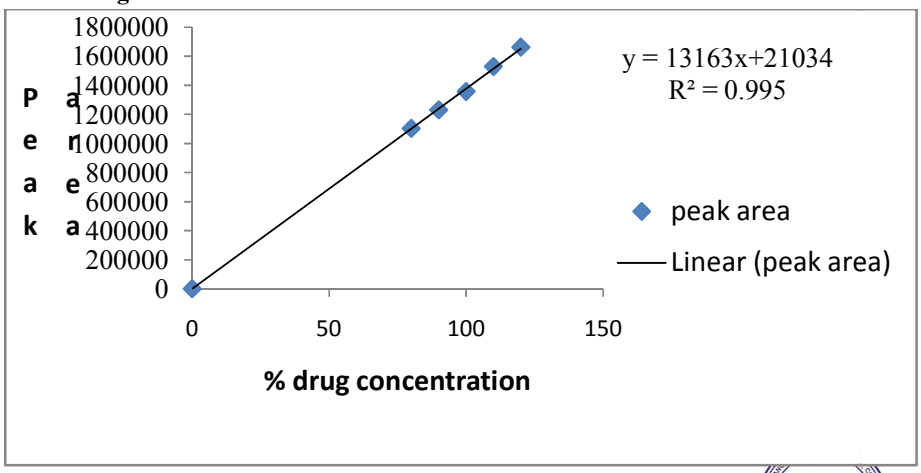

Figure 4: Plot of linearity and range for Terizidone by HPLC

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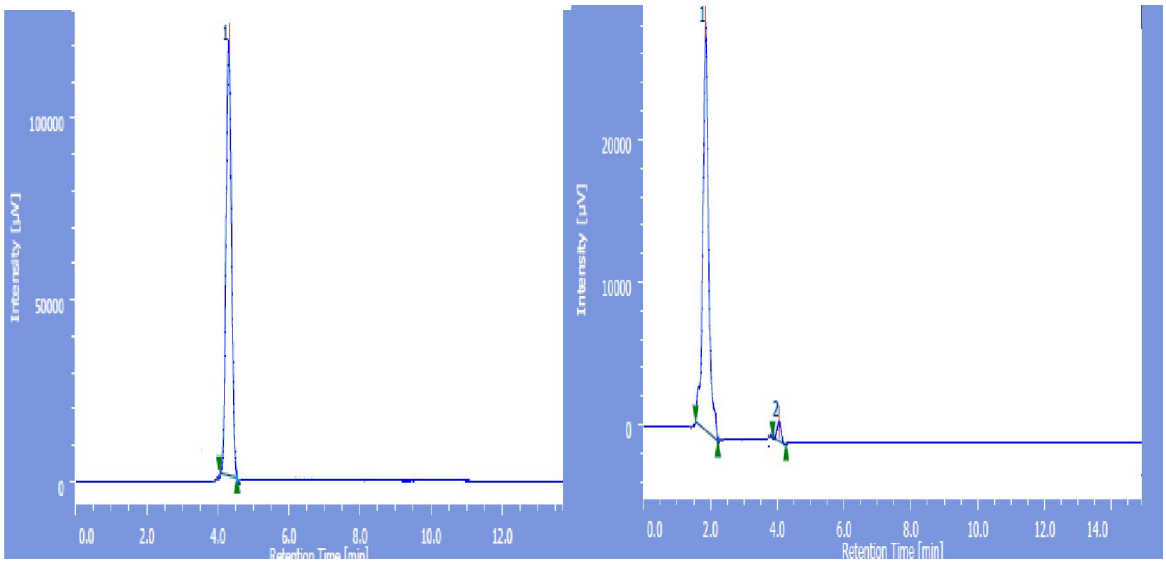

Figure 5:Chromatogram of Terizidone after acid hydrolysis(0hr) and (2hr) respectively

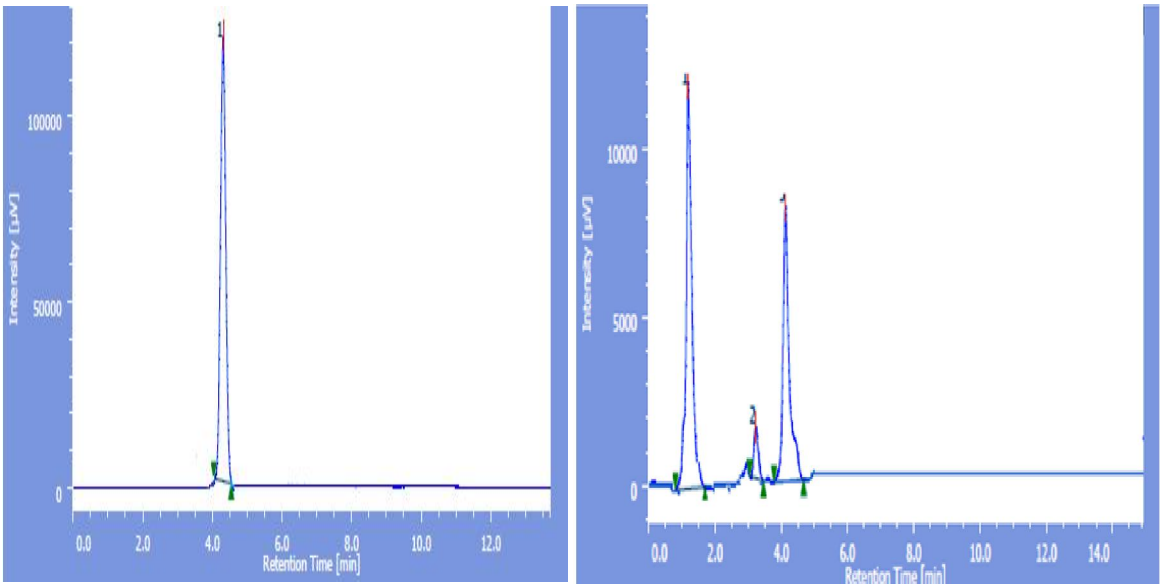

Figure 6: Chromatogram of Terizidone after Alkali hydrolysis (0 hr) and (2hr) respectively

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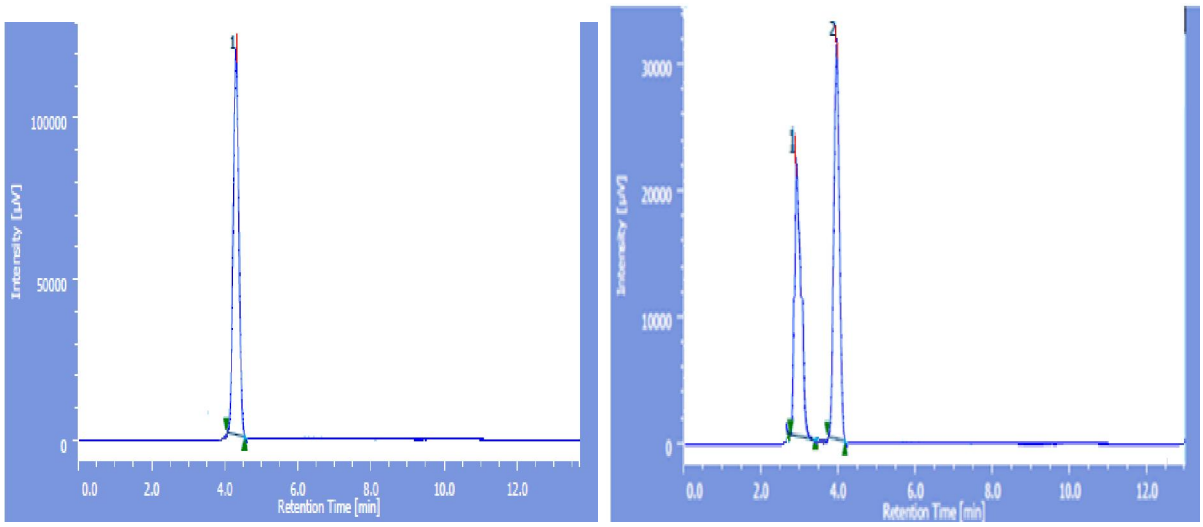

Figure 7: Chromatogram of Terizidone after Neutral hydrolysis (0hr) and (2hr) respectively

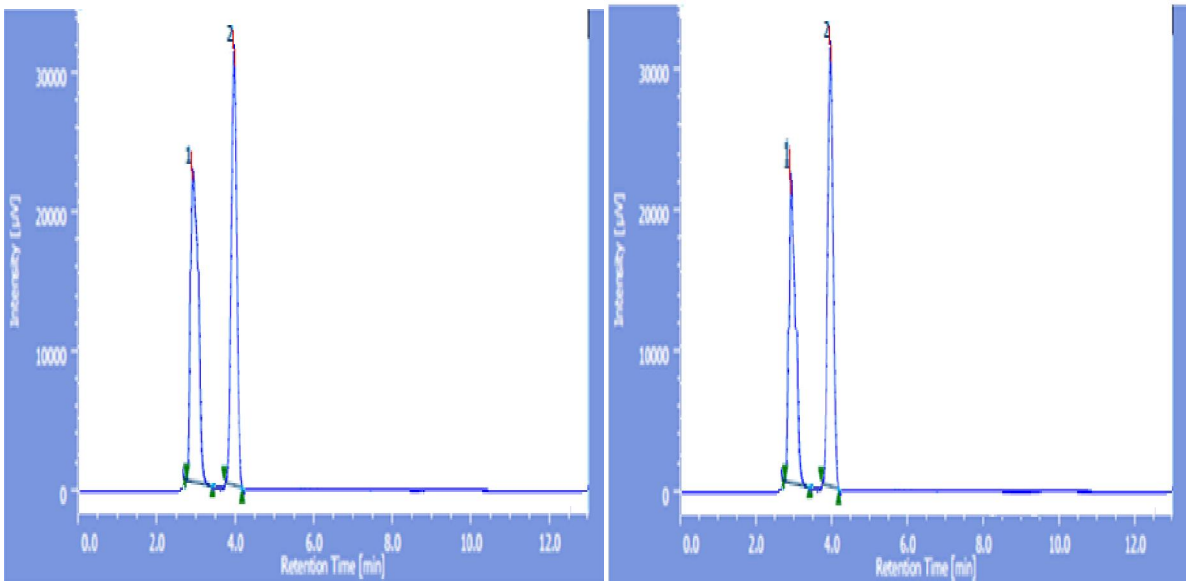

Figure 8: Chromatogram of Terizidone after Neutral hydrolysis(6hr) and (8hr) respectively

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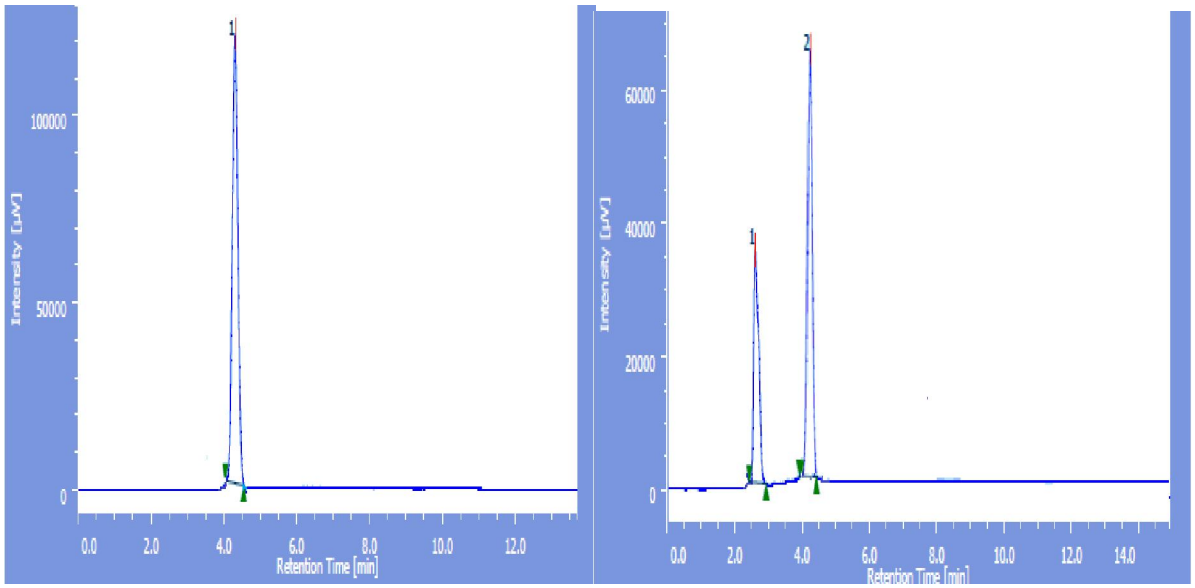

Figure 9:Chromatogram of Terizidone after peroxide study (0hr) and (1hr) respectively

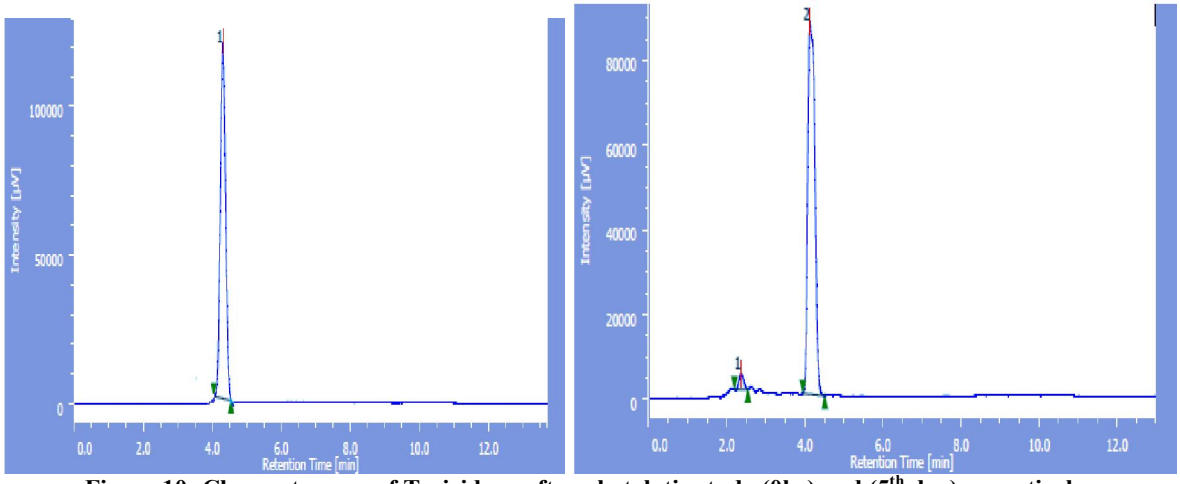

Figure 10: Chromatogram of Terizidone after photolytic study (0hr) and (5th day)respectively

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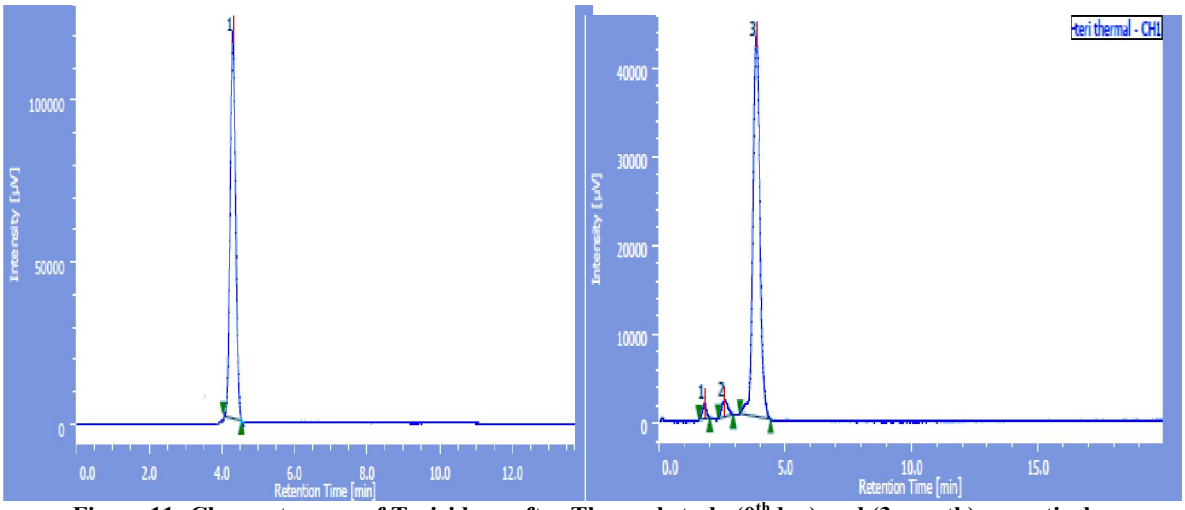

Figure 11: Chromatogram of Terizidone after Thermal study (0thday) and (3 month)respectively

III. RESULTS AND DISCUSSION

The present study deals with stability assessment and degradation studies of terizidone using under HPLC. The stability indicating assay method was established for the analysis of terizidone in presence of its degradation products by selecting a suitable detection wavelength and mobile phase. After scanning the solution of Terizidone at concentration 10µg/ml, the wavelength selected was 260 nm for further study.The quality and purity of terizidone was confirmed by melting point and the results of melting point areaccording to the specification.

The stability of terizidone in mobile phase was tested by keeping drug in mobile phase for 24hrs. No turbidity was found in the sample solution indicating that the terizidone was stable in mobile phase. The calibration curve isplotted in concentration range 10-50µg/ml of terizidone and it was found to be linear with the correlation coefficient of 0.994. To confirm the suitability of the method for its intended purpose, the method was validated in accordance with ICH guidelines[8-11], for system suitability, accuracy, precision, ruggedness, linearity and range.

System suitability was an integral part of method development and has been used to ensure adequate performance and reproducibility of the chromatographic system. Retention time (Rt), Capacity factor(K), asymmetry factor and number of theoretical plates (N) were evaluated for five replicate injections of Terizidone at a concentration 10µg/ml. The mean result for five replicates and the parameters obtained are shown in Table-3.

Sr. No.	Parameter	Mean
1	Peak Area	1357625
2	Retention time	4.070
3	Capacity Factor	0.629
4	Number of Theoretical plate	23991.8
5	Asymmetry	0.003604

Accuracy of the method was determined by performing the recovery experiments. Quantitative recoveries calculated from spiked samples at concentration levels 80%,100% and 120%. Terizidone content was analysed by proposed method. The result of recovery study for the given drug was found to be around 98-101%w/w with R.S.D.=0.01976 showing that the method was accurate and precise.

Intraday, inter-day and different analyst studies were performed. For intraday study, 10µg/ml concentration of Terizidone was analysed on different days. In different analyst study same concentration of drug was analysed by two persons. The % RSD values were determined andwere found to be less than 2% as per ICH guidelines.The intraday and

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inter-day study did not show much variation (R.S.D.=0.01528) and (R.S.D.=0.03426) respectively, by ICH and USP, signifies that the method is rugged.

The different analyst study showed the R.S.D.= 0.001579 signifies that the method is rugged and not shown variation when performed by two personals. The linearity of the proposed method was evaluated according to the ICH guidelines. Terizidone was found to be linear in the concentration range of 80% to 120% of test concentration. The linear regression equation for terizidone was found to be y=13163X +21034 with $R^2 \approx$ 0.995. Where X is the concentration, y is the observed response and $R^2$is the correlation coefficient. The results showed that a good linear correlation exists between the peak area and concentration of the analyte.

HPLC studies on terizidone under different stress conditions suggested the following degradation behaviour.

Acidic condition: The process was carried out by refluxing terizidone under studied water bath for 2 hrs with 0.1N HCL. After 2 hr 0.84% of drug was estimated. This study indicated that 99 % of the drug was degraded under acidic condition and hence terizidone was more prone to acid hydrolysis.

Basic condition : In basic condition, terizidone was dissolved in 0.1N NaOH and the reaction mixture was place at 40^0c for 2hrs in reflux. More degradation was observed after 2hr study leaving 7.91% of intact drug. This study indicated that 93% the drug was degraded under alkaline condition.

Neutral condition : Terizidone was refluxed under student water bath for 8hrs with water. HPLC study of the resulting solution show additional peaks in the chromatogram. This study indicating that terizidone is not stable in neutral condition.

The oxidative degradation of terizidone was associated with the formation of a major degradation product eluted at 2.5min. Terizidone was found to be more unstable in 3% H_2O_2 at room temperature. Almost 69.80 % of the drug was degraded after 1 hr. After 7 days only 65.46 % drug remained. This shows that almost 35 % drug was degraded within a week indicating Terizidone is less prone to peroxide degradation.

For photo stability study[12], Terizidone was spread on Petri plate and placed inside the photo stability chamber under UV light for 5 days. 82.54% drug remained after 5 days which shows that it is less prone to photolytic degradation.

For thermal study, the drug was spread on Petri plate and placed in an oven at 50^oC for 3months. After 3months, solution was prepared and injected in HPLC system. 60.09% drug remained which shows that the drug is comparatively less prone to thermal degradation.

IV. CONCLUSION

The present study was undertaken with the primary objective to establish the inherent stability of Terizidone through stress studies under a variety of ICH-recommended test conditions and to determine the Terizidone degradation using validated HPLC method.

The value obtained for the degradation products through HPLC studies helped us to confirm the presence of the degradants. Based on the HPLC studies, it can be concluded that Terizidone was found to be extensively degraded under Hydrolysis condition. The result of degradation studies undertaken according to the ICH guidelines revealed that the method is selective and stability-indicating.

Stability testing evaluates the effect of environmental factors on the quality of the drug substance or a formulated product which is utilized for predictions. Moreover, the data generated during the stability testing is an important requirement for regulatory approval of any drug or formulation.

In present work, forced degradation study of Terizidone was carried out according to the ICH guideline Q1A (R_2). The drug was subjected to acid (0.1N HCL), alkaline (0.1N NaOH) and neutral hydrolysis conditions as well as an oxidative decomposition at room temperature. Photostability and thermal study was also carried out. The solid drug was kept at 50^0C for 90 days in the oven for thermal study and 5 days in UV chamber for photostability study. The product formed in the different stress conditions were investigated by HPLC. The HPLC separates all degradation products formed under various stress conditions using a mobile phase, Acetonitrile: Water (70:30% v/v) at a flow rate of 1 ml/min, on an isocratic HPLC system containing UV-visible detector and Hypersil C18 column [4.6 x 250 mm (id)], the detection was carried out at 260 nm.

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Maximum degradation products were formed under the Hydrolytic condition while significant degradation was observed in photolytic conditions respectively. The drug was comparatively stable in neutral, oxidative and thermal conditions.

The Proposed stability indicating HPLC method can be successfully applied for determination of Terizidone in presence of degradation products during stability studies on the bulk in the routine quality control laboratories and would be helpful for the multiple generic manufacturers of the drug around the globe by saving them from unnecessary repetition of the same studies. The information presented here in could be very useful for quality monitoring of bulk samples and also employed to check the quality monitoring of bulk samples and also employed to check the quality of drug during stability studies.

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