CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1197068

Available online at: http://www.iajps.com Research Article

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF TERIZIDONE

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Abstract:

*A simple, rapid, sensitive, and linear stability indicating reverse phase HPLC method was developed for estimation of Terizidone. In this method separation was carried out on HiQSil C8 column (250mm*4.6 mm, 5µm) using Ammonium Acetate Buffer pH 3 (adjusted with glacial acetic acid) and methanol (60:40 v/v) as mobile phase at flow rate of 1 ml/min. The quantification was carried out at 264 nm. The retention time (t_R) of drug was 7.3± 0.10 min. The method was validated with respect to linearity, precision, assay, accuracy and robustness. The data of linear regression analysis indicated a good linear relationship over the range of 5-30 µg/ml concentrations with a correlation coefficient (r^2) of 0.9837. Terizidone was subjected to different stress testing conditions. The degradation products were well resolved from the drug under the tested conditions.*

Keywords: *Terizidone, High performance liquid chromatography, stability indicating method, validation.*

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*Please cite this article in press as Santosh V. Gandhi et al., **Development and Validation of a Stability Indicating RP-HPLC Method for the Determination of Terizidone**, Indo Am. J. P. Sci, 2018; 05(03).*

INTRODUCTION:

The molecular formula of Terizidone is $C_{14}H_{14}N_4O_4$. Terizidone IUPAC name is 4-[(E)-({4-[(1E)-[(3-oxo-1,2-oxazolidin-4-yl)imino]methyl]phenyl}methylidene)amino]-1,2-oxazolidin-3-one. Terizidone is not official in IP/BP/USP [1]. It has an antibiotic activity against mycobacterium tuberculosis and *M. avium* for the treatment of tuberculosis, i.e. pulmonary and extra pulmonary [2]. This drug comes under second line drugs that means it is used only when first line drugs are not able to show expected results [3]. The MIC of Terizidone for M. tuberculosis is 5-20 mg/ml [4]. The chemical structure of Terizidone is given in Fig.1

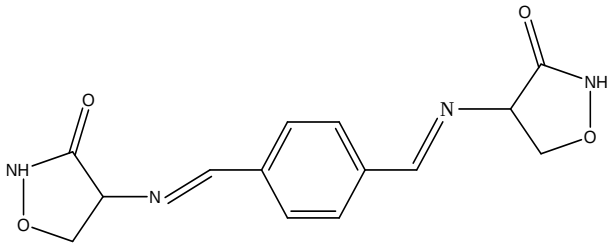

Fig. 1: Chemical Structure of Terizidone

Literature survey reveals methods reported are area under curve and first order derivative spectrophotometry [5] and simple UV spectrophotometric method [6] for estimation of Terizidone.

To the best of our knowledge no stability indicating RP-HPLC method has been reported for estimation of Terizidone. The present work describes a stability indicating RP-HPLC method in bulk and pharmaceutical dosage form (Tericox) according to the International conference on harmonization (ICH) guidelines [7-8].

MATERIALS AND METHODS:

Reagents and Solutions:

Methanol (HPLC Grade), Ammonium Acetate (AR Grade), Sodium hydroxide (NaOH) and Hydrochloric acid (HCl) and Hydrogen peroxide (H_2O_2) were obtained from Loba Chemie (India). HPLC grade water is collected at college using ELGA water purification system having model name Purelab UHQ-II. Glacial Acetic Acid (AR Grade) was obtained from SD Fine Chem Ltd. All chemicals were of analytical grade and used as received. Terizidone is available in market with brand name Tericox (Label claim: 250 mg) as capsules.

Instrumentation and Chromatographic Conditions:

HPLC system used was JASCO system equipped with model PU 2080 Plus pump, Rheodyne sample injection port (20 µl), JASCO PDA MD-2010 Plus detector and Borwin chromatography software (version 1.5) with HiQSil C8 (250mm*4.6 mm, 5µm) column. The optimized mobile phase is ammonium acetate buffer (pH 3): methanol (60:40 v/v). The pH of ammonium acetate buffer was adjusted by glacial acetic acid. The overall run time was 12 min and the flow rate was 1.0 ml/min. The quantification was carried out at 264 nm.

Preparation of Standard Stock Solution:

Terizidone stock solution was prepared by dissolving 10 mg of terizidone in 4 ml of DMSO and then volume made upto10 ml with methanol to get solution having concentration 1000 µg/ml. Further dilutions made with methanol. The representative chromatograph is given Fig. 2.

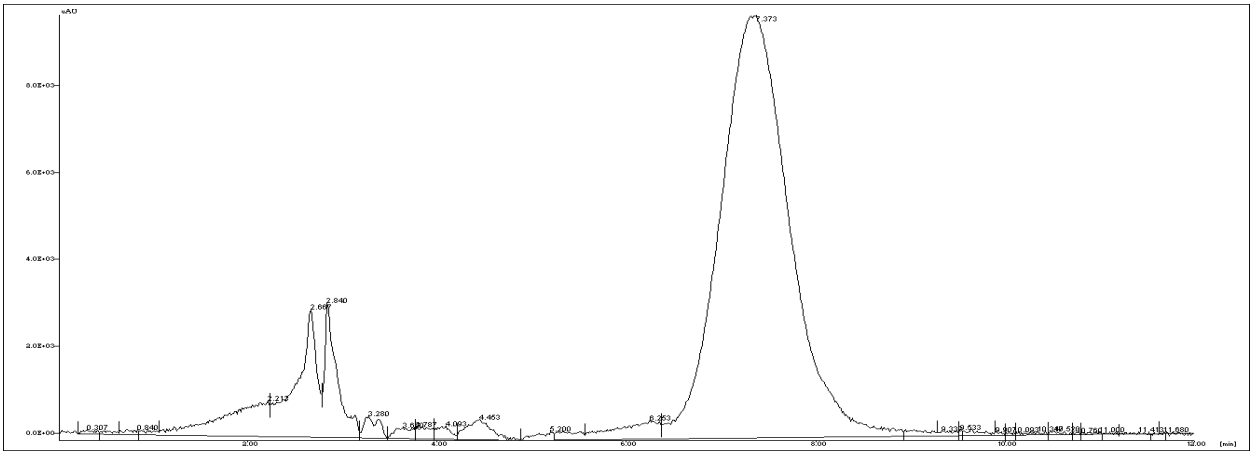

Fig. 2: Chromatogram of Terizidone (10 µg/ml)

Forced Degradation Studies [7]

Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like hydrolysis, oxidation, temperature, etc. Dry heat and photolytic degradation was carried out in the solid state.

Acidic

1 ml working standard solution of Terizidone (1000 µg/ml) was mixed with 1 ml of 0.5 N HCl and kept for 4 hours at room temperature. After exposure the volume was made up to 10 ml (100 µg/ml) with methanol. 2 ml of this solution further diluted to 10 ml (20 µg/ml) with mobile phase and then injected. The percent recovery of Terizidone was found 96.704 %. The representative chromatogram is shown in Fig.3.

Alkaline Degradation Studies:

1 ml working standard solution of Terizidone (1000 µg/ml) was mixed with 1 ml of 0.5 N NaOH and kept for 4 hours at room temperature, after exposure the volume was made up to 10 ml (100 µg/ml) with methanol. 2 ml of this solution further diluted to 10ml (20 µg/ml) with mobile phase and then it is injected. The percent recovery was found 18.67 %. The representative chromatogram is shown in Fig.4.

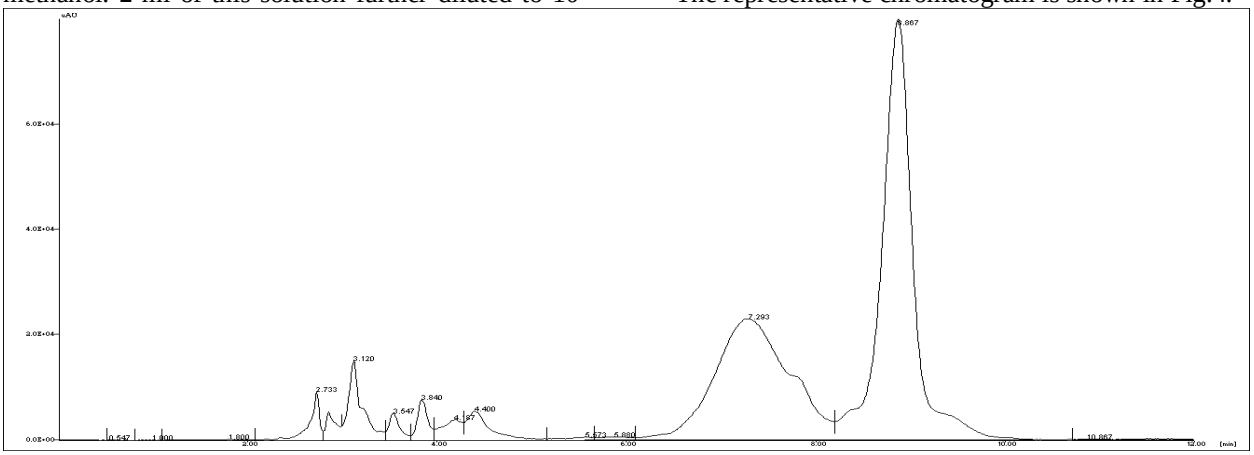

Fig. 3: Representative Chromatogram of Acid Degradation (20 µg/ml)

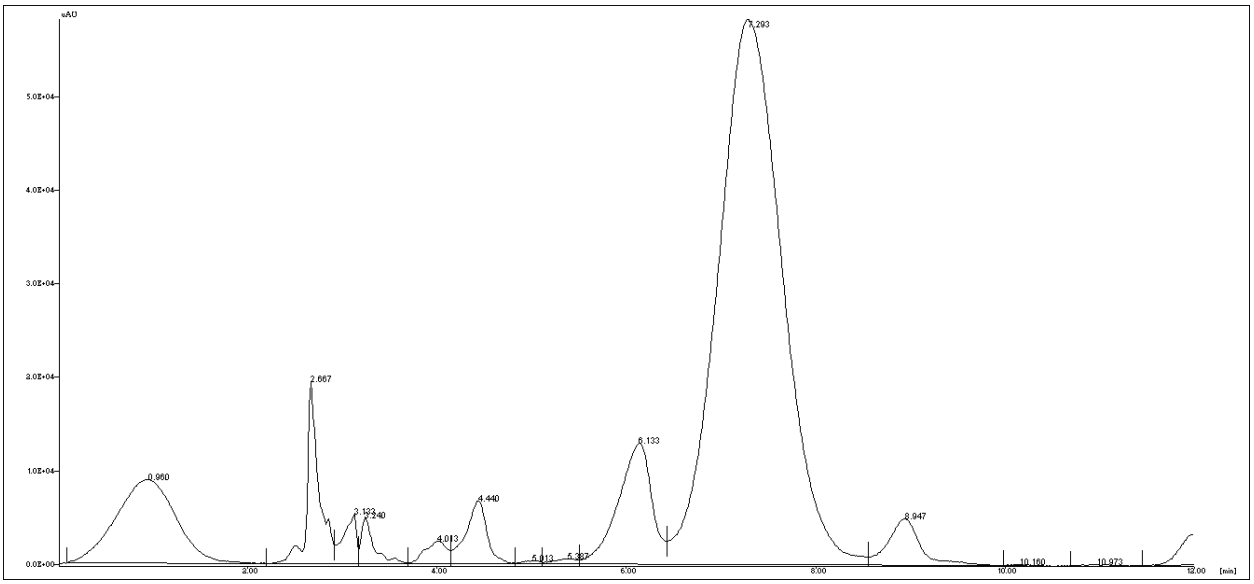

Fig. 4: Representative Chromatogram of Alkaline Degradation (20 µg/ml)

Oxidative Degradation Studies:

1 ml working standard solution of Terizidone (1000 µg/ml) was mixed with 1 ml of 10 % H_2O_2 solution and was kept for 4 hours at room temperature, after exposure the volume was made upto 10 ml (100 µg/ml) with methanol. 2 ml of this solution further diluted to 10 ml (20 µg/ml) with mobile phase and then injected. The percent recovery in the oxidative condition obtained was 62.32 %. The representative chromatogram is shown in Fig. 5.

Thermal Degradation Studies:

Dry heat study was performed by keeping drug sample in oven (80^0 C) for a period of 24 hours. A sample was withdrawn after 24 hours, dissolved in DMSO and methanol to get solution of 1000 µg/ml. 1 ml working standard solution of Terizidone (1000 µg/ml) was mixed with 1 ml of sample solution previously prepared. This solution was diluted to 10 ml (100 µg/ml) with methanol. 2 ml of this solution further diluted to 10 ml (20 µg/ml) with mobile phase and then injected as final concentration. Under dry heat degradation condition, percent recovery obtained for Terizidone was 99.26 % with no peak of degradant. The representative chromatogram is shown in Fig. 6.

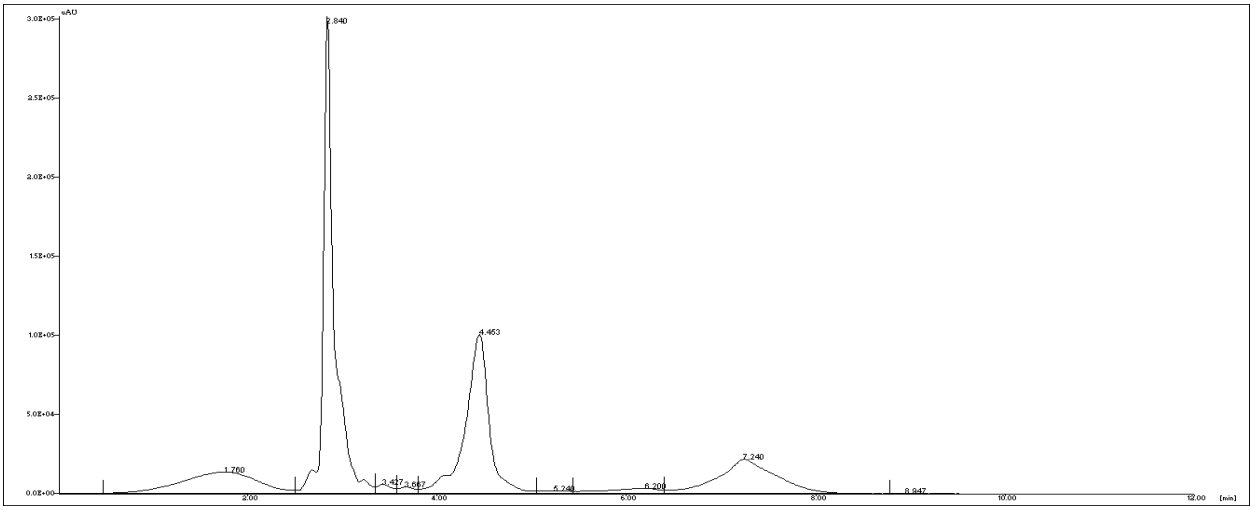

Fig. 5: Representative Chromatogram of Oxidative Degradation (20 µg/ml)

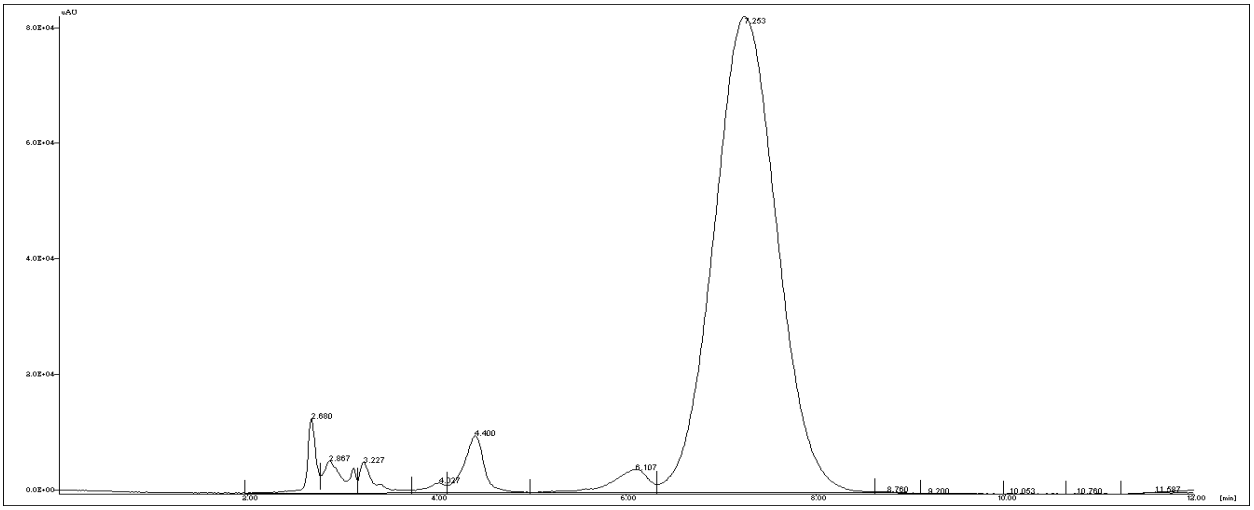

Fig. 6: Representative Chromatogram of Thermal Degradation (20 µg/ml)

Photolytic degradation studies:

The photo degradation study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/m^2 followed by exposure to cool white fluorescence light of NLT 1.2 million Lux-Hr. After exposure accurately weighed 10 mg of drug was transferred to 10 ml of volumetric flask containing DMSO and the volume was made up with methanol (1000 µg/ml). 1 ml working standard solution of Terizidone (1000 µg/ml) was mixed with 1 ml of sample solution previously prepared. This solution was diluted to 10 ml (100 µg/ml) with methanol. 2 ml of this solution further diluted to 10 ml (20 µg/ml) with mobile phase and then injected as final concentration. Average 98.97 % of Terizidone was recovered with no peak of degradant. The representative chromatogram is shown in Fig.7.

Neutral

1 ml working standard solution of Terizidone (1000 µg/ml) was mixed with 1 ml water, and was kept for 4 hours at room temperature, after exposure the volume was made upto 10 ml with methanol (100 µg/ml). 2 ml of this solution further diluted to 10 ml (20 µg/ml) with mobile phase and then injected. On neutral hydrolysis, Terizidone 98.30 % was recovered with no peak of degradant. The representative chromatogram is shown in Fig. 8.

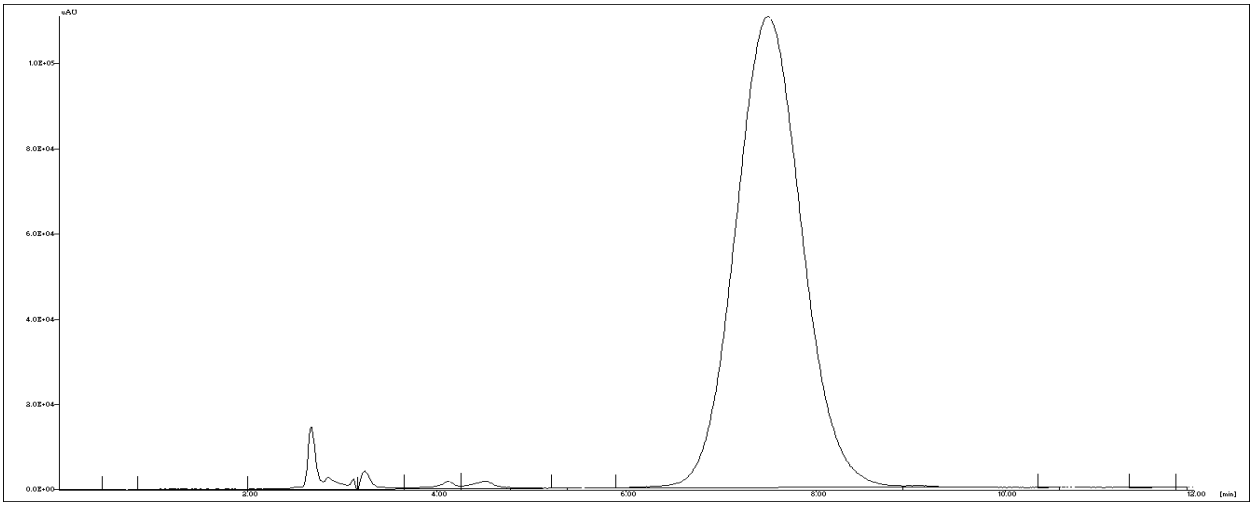

Fig. 7: Representative Chromatogram of Photolytic Degradation (20 µg/ml)

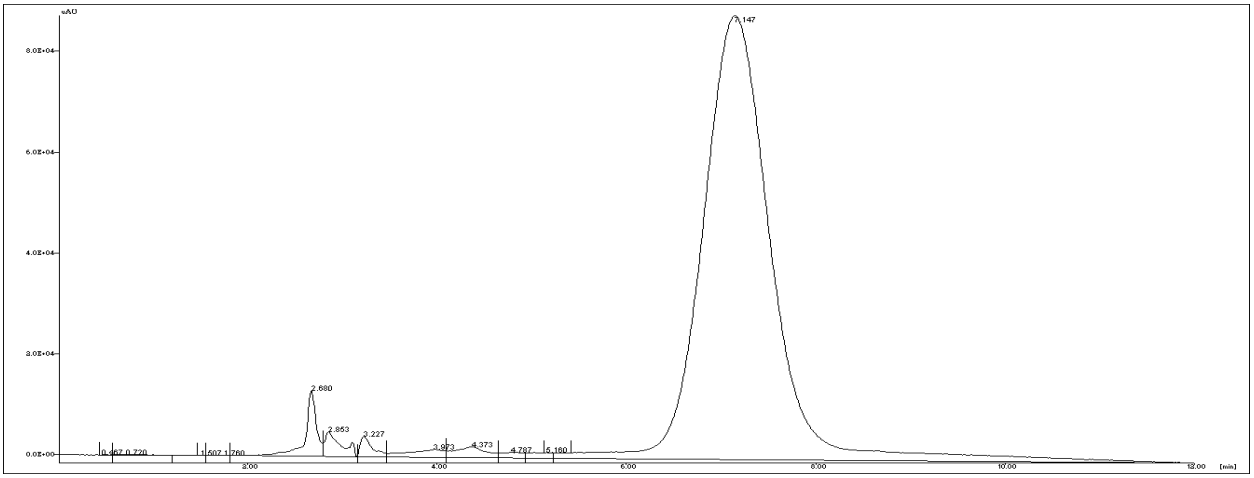

Fig. 8: Representative Chromatogram of Neutral Type of Degradation (20 µg/ml)

Method Validation [8]

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 991, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity:

From the standard stock solution (1000 µg/ml) of Terizidone, solution was prepared containing 100 µg/ml of Terizidone with methanol. This solution was further used to prepare range of solution containing six different concentrations. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 5-30 µg/ml, the equation of calibration curve was found to be y = 44685x + 110741. The peak area of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig. 9.

Precision

The precision of the method was demonstrated by intraday and interday variation studies. In the Intraday studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the interday variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intraday and interday variations are shown in Table 1.

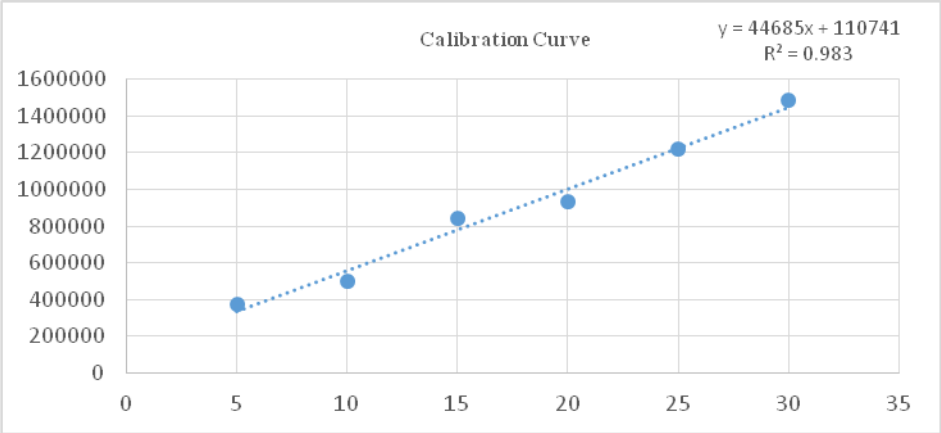

Fig. 9: Calibration Curve of Terizidone

Table 1: Precision of Terizidone

Concentration (µg/ml)	Intra-day Precision		Inter-day Precision	
	Average	% RSD	Average	% RSD
15	101.306	0.319	101.150	0.321
20	99.861	1.869	100.386	1.663
25	99.108	1.24	99.752	1.215

Assay:

Twenty capsules were emptied and the contents were finely powdered. Powder equivalent to 10 mg of Terizidone was accurately weighed and transferred into a 10 ml volumetric flask and volume was made up with DMSO and methanol as mentioned under section preparation of stock solution (1000 µg/ml). The volumetric flask was sonicated for 10 min to enable complete dissolution of Terizidone and the solution was filtered through 0.45 µm whatmann filter paper. From the filtrate further dilution was made with methanol to get 100 µg/ml solution. Finally this solution was further diluted with mobile phase to yield a concentration of 10 µg/ml and then it is injected. The procedure was repeated for six times. The results obtained for assay of Terizidone are shown in Table 2.

Accuracy:

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the capsule solution, at three different levels around 50, 100 and 150 %. Basic concentration of sample solution chosen was 10 µg/ml. Percentage recovery was determined from linearity equation. The results obtained are shown in Table 3.

Table 2: Assay of Terizidone

Drug	Peak Area (Avg.)	Amount Recovered (µg/ml)	% Recovery	Mean % Recovery	SD	% RSD
Terizidone	559821.7	10.049	100.49	101.14	0.661	0.653557
	559794.1	10.049	100.49			
	566360	10.196	101.96			
	560647.7	10.068	100.68			
	565077.4	10.167	101.67			
	564608.5	10.157	101.57			

Table 3: Accuracy of Terizidone

Level % of Accuracy	Sample (µg/ml)	Standard (µg/ml)	% Recovery	RSD
50	10	5	101.66	1.000
100	10	10	101.87	0.036
150	10	15	100.40	1.021

Sensitivity/Limit of Quantification (LOQ) and Limit of Detection (LOD):

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S where, σ = standard deviation of the y-intercept of linearity equations and S = slope of the calibration curve of the analyte. LOD was found to be 0.52 µg/ml. LOQ was found to be 1.58 µg/ml.

Robustness:

Robustness of the method was checked by carrying out the analysis under conditions during which mobile phase composition (± 2% Composition), quantification wavelength (± 2 nm), flow rate (± 0.05 ml/min) were altered and the effect on the area were noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

RESULTS AND DISCUSSION:

Terizidone has absorbance maxima at 264 nm. After few trials Ammonium acetate buffer (pH 3): methanol (60:40) was chosen as mobile phase. The flow rate is 1 ml/min. The calibration curve data show good linear relationship in the concentration range of 5-30 µg/ml. Recovery study is carried out at three different level 50%, 100% and 150% by adding pure drug to the previously analysed test sample. Percentage recovery for drug was determined by linearity equation method and found to be within acceptance criteria. The Precision and Accuracy was found to be good, which is evident by low standard deviation of values. The forced degradation study was carried out under various forced degradation conditions like acidic, basic, neutral hydrolysis, oxidation, dry heat and photolysis. The major degradation of drug was found under alkaline and oxidative condition. Summary of validation parameter and stability study was given in following Table no. 4 and 5 respectively.

Table 4: Summery of Validation Parameters

Sr.No.	Parameter	Terizidone
1	Linearity	y = 44685x + 110741
2	Range	5-30 µg/ml
3	Precision	%RSD
	Intraday	0.31-1.87
	Interday	0.32-1.67
4	Assay	101.14 %
5	Accuracy	% Recovery
	50%	101.66
	100%	101.86
	150%	100.40
6	LOD	0.52 µg/ml
7	LOQ	1.58 µg/ml.
8	Specificity	Specific
9	Robustness	Robust

Table 5: Summary of Forced Degradation Study

Stress condition/Duration	% Assay of active substance	% Degradation
Acidic/ 0.5 N HCl / at room temperature 4 hours	96.70	3.295
Alkaline/ 0.5 N NaOH / at room temperature 4 hours	18.67	81.324
Oxidative/ 10 % H_2O_2 / at room temperature 4 hours	62.32	37.678
Dry heat / 80ºC / 24 hours	99.26	0.742
UV/200 watt hours/square meter / for 24 hours	98.97	1.026
Neutral/ H_2O/ at room temperature 4 hours	98.30	1.697

CONCLUSION:

The developed method was found to be simple, sensitive, selective, accurate, and repeatable for analysis of Terizidone in bulk and pharmaceutical dosage form without any interference from the excipients. The results indicated the suitability of the method to study stability of Teizidone under various forced degradation conditions.

ACKNOWLEDGEMENT:

Authors are thankful to the Principal and Management of AISSMS College of Pharmacy, Pune for providing required facilities for research work.

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