Development And Validation of Stability Indicating HPTLC Method For Estimation of Terizidone

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Abstract- *A simple, sensitive and accurate stability indicating HPTLC method has been developed and validated for estimation of Terizidone in bulk and pharmaceutical dosage form. The chromatograph was developed by spotting drug on precoated silica gel 60 F_{254} aluminum plates using n-Hexane: Chloroform (5:5 v/v) as mobile phase. The retention factor (R_f) was found to be 0.25 ± 1.5. The detection of band was carried at 264 nm. The drug was subjected to different stress conditions like acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis. The method was successfully validated according to ICH guidelines Q2 (R1). The data of linear regression analysis indicated a good linear relationship over the concentration range of 200-1200 ng/band with correlation coefficient 0.94. The method found to be accurate as results of the recovery studies are close to 100 %. The developed method was found to be simple, sensitive, selective, accurate and repeatable and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.*

Keywords- High performance thin layer chromatography (HPTLC), Terizidone, Stability indicating, Validation.

I. INTRODUCTION

Terizidone will be further abbreviated as TERI. TERI IUPAC name is 4-[(E)-({4-[(1E)-[(3-oxo-methylidene)amino]-1,2-oxazolidin-3-one.TERI is not official in any pharmacopoeia [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]. Literature survey reveals methods reported are area under curve, first order derivative spectrophotometry [4], simple UV spectrophotometric method [5] and stability indicating HPLC for estimation of TERI [6]. To the best of our knowledge no stability indicating HPTLC method has been reported for estimation of TERI. The present work describes a stability indicating HPTLC method in bulk and pharmaceutical dosage form (Tericox) according to the International conference on harmonization (ICH) guidelines [7-8]. The chemical structure of TERI is given in Fig no.1

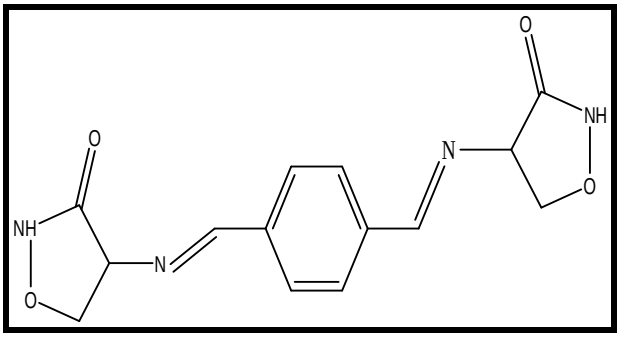

Fig no. 1-Structure of Terizidone

II. MATERIALS AND METHODS

Reagents and chemicals

The marketed formulation Tericox labeled to contain TERI 250 mg was procured form local market. Methanol (AR grade), n- Hexane (AR grade), Chloroform (AR grade), DMSO (AR grade), were purchased from S. D. Fine Chemical Laboratories, Mumbai. Hydrochloric acid (HCl), hydrogen peroxide (H_2O_2) and sodium hydroxide (NaOH) were purchased from LOBA Chemie, Mumbai. All chemicals were of analytical grade and used as received.

Chromatographic condition:

Chromatographic separation of drug was performed on aluminum plates precoated with silica gel 60 F_{254}, (10 cm × 10 cm with 250 µm layer thickness). Sample was applied on the plate as a band of 4 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of n-Hexane: Chloroform (5:5 v/v). 10 cm × 10 cm CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 10 min saturation conditions

and 10 ml of mobile phase was used per run, migration distance was 80 mm. Densitometric scanning was performed using Camag TLC scanner at 264 nm, operated by winCATS software, slit dimensions were 3.00 x 0.45 mm and Deuterium lamp was used as a radiation source.

Selection of Detection Wavelength

From the standard stock solution (1000 µg/ml) further dilutions were made using methanol and scanned over the range of 200-400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 264 nm. Representative UV spectrum of TERI is shown in Fig no. 2.

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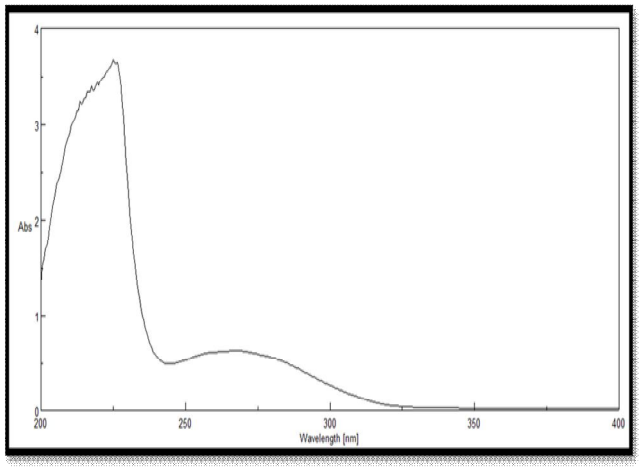

Fig no. 2- UV-VIS Spectra of TERI (10 µg/ml)

Preparation of Standard stock solution

TERI stock solution was prepared by dissolving 10 mg of TERI in 4 ml of DMSO and then volume made upto 10 ml with methanol to get solution having concentration 1000 µg/ml. From the standard stock solution, working standard solution was prepared containing 100 µg/ml of TERI. 4 µl of the resultant solution was applied on TLC plate to get concentration of 400ng/band. Representative densitogram of TERI (400ng/band) is shown in Fig no. 3.

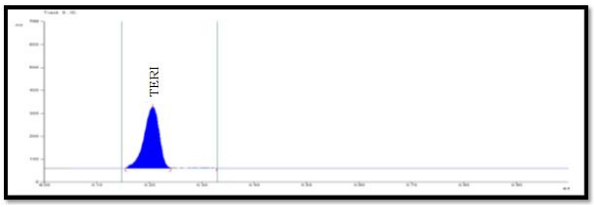

Fig no. 3- Representative Densitogram of Terizidone (400 ng/band)

Preparation of sample solution

A tablet containing 250 mg of TERI (Tericox) was weighed and powdered. Powder equivalent to 10 mg of drug was transferred to 10 ml volumetric flask and volume was made up with 4 ml DMSO and methanol to get concentration (1000 µg/ml) and was sonicated for 10 min. Solution was filtered and 1 ml further diluted to 100 ml. 4 µl of the resultant solution was applied on TLC plate to get concentration of 400 ng/band.

III. STRESS DEGRADATION STUDIES

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 acidic, alkaline, hydrolysis, and oxidation. Dry heat and photolytic degradation were carried out in the solid state. All studies were carried out at concentration level of 1000 ng/band.

Alkaline Degradation

To 1 ml stock solution of TERI (1000 µg/ml), 1 ml of 0.5 N NaOH was added. The above solution was kept for 4 hours at room temperature. The volume was made up to 10 ml with methanol. 10 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 20.275 % of TERI was recovered with one peak of degradation having Rf value 0.19. Representative densitogram is shown in Fig no. 4.

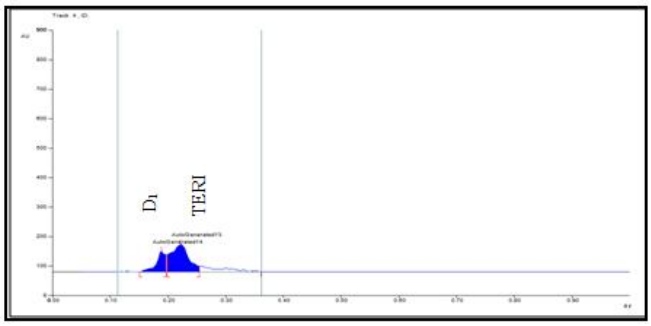

Fig no.4- Representative Densitogram of base induced degradation of Terizidone 1000ng/band

Acid Degradation

To 1 ml stock solution of TERI (1000 µg/ml), 1ml of 0.5 N HCl was added. The above solution was kept for 4 hour at room temperature. The volume was made upto 10 ml with methanol. 10 µl of the resultant solution was then applied at TLC plate and densitogram was developed. 71.739%TERI was recovered with no peak of degradant. Representative densitogram is shown in Fig no.5.

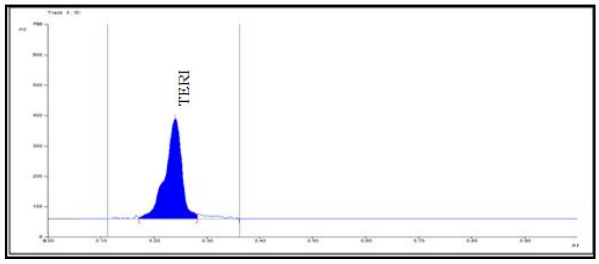

Fig no. 5- Representative Densitogram of Terizidone after acid degradation

Neutral Type of Degradation

To 1 ml of stock solution of TERI (1000 µg/ml), 1ml of distilled water was added. The above solution was kept for 4 hours at room temperature. The volume was made upto 10 ml with methanol. 10 µl of the resultant solution was then applied at TLC plate and densitogram was developed. 97.689% of TERI was recovered with no peak of degradant. Representative densitogram is shown in Fig no.6.

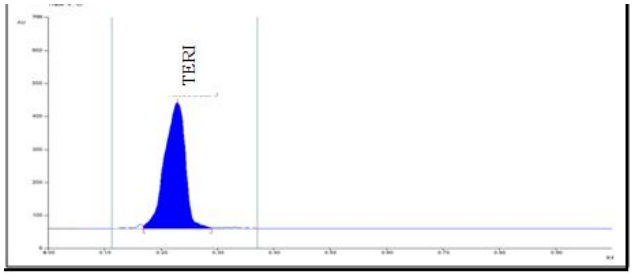

Fig no. 6- Representative Densitogram of Terizidone after Neutral Type of degradation

Degradation under oxidative condition

To 1 ml stock solution of TERI (1000 µg/ml), 1 ml of 10 % H_2O_2 was added. The above solution was kept for 4 hour at room temperature. The volume was made upto 10 ml with methanol. 10 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 89.187 % of TERI was recovered with no peak of degradant. Representative densitogram is shown in Fig no. 7.

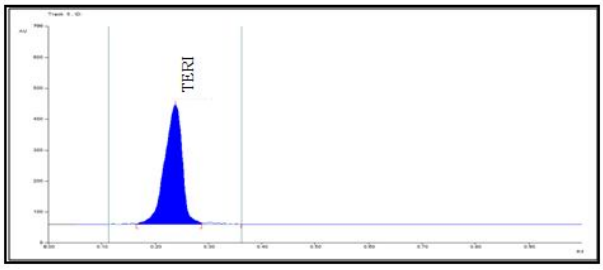

Fig no.7- Representative Densitogram of Terizidone after Oxidation degradation

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (80^0 C) for a period of 24 hours. Sample was withdrawn, dissolved in methanol and diluted to get 1000 µg/ml. 1 ml from this solution was mixed with 1 ml working standard solution of Terizidone (1000 µg/ml) previously prepared. This solution was diluted to 10 ml (100 µg/ml) with methanol. 10 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 103.285 % TERI was recovered with no peak of degradant. Representative densitogram is shown in Fig no.8.

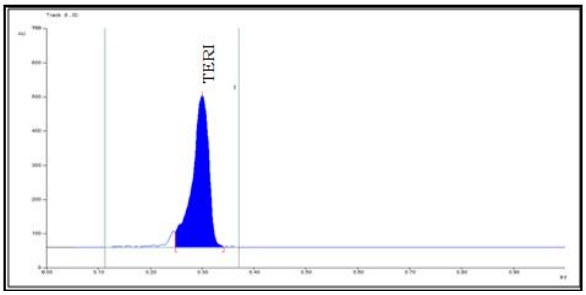

Fig no. 8- Representative Densitogram of Terizidone after Thermal degradation

Photolytic degradation

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. After exposure, sample was withdrawn, dissolved in methanol and diluted to get 1000 µg/ml.1 ml from this solution was mixed with 1 ml working standard solution of Terizidone (1000 µg/ml) previously prepared. This solution was diluted to 10 ml (100 µg/ml) with methanol. 10 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 98.585 % of TERI was recovered with no peak of degradant. Representative densitogram is shown in Fig no.9.

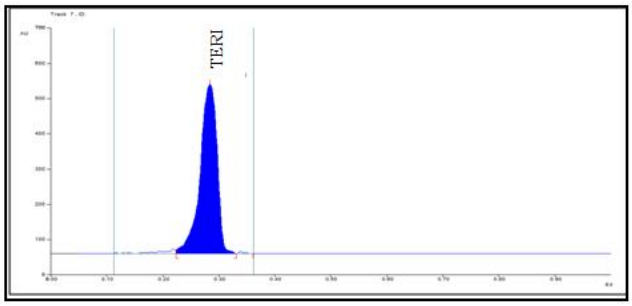

Fig no.9- Representative Densitogram of Terizidone after Photolytic degradation

IV. VALIDATION OF ANALYTICAL METHOD

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.998, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity

From the 100 µg/ml solution of TERI, Six replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analyzing six concentrations over the concentration range of 200-1200 ng/band for TERI. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve shown in Fig.5

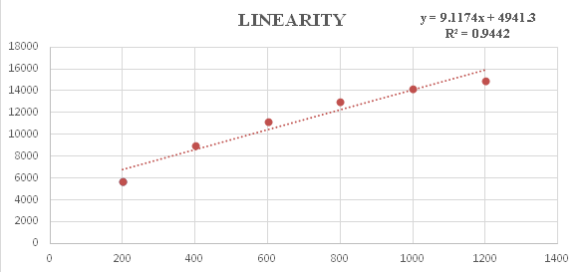

Fig no.10-Calibration curve of Terizidone

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies 3 replicates of 3 concentrations were analyzed on the same day and percentage RSD were calculated. For the inter dayvariation studies, 3 replicates of 3 concentrations were analyzed on 3 consecutive days and percentage RSD were calculated. For intraday precision and inter-day precision results obtained are shown in Table 1.

Table 1: Intraday and Interday variation studies data for Terizidone

Concentration (µg/ml)	Intra-day Precision			Inter-day Precision		
	Average area	% Recovery	% R.S.D	Average area	% Recovery	% R.S.D
400	8549.1	98.926	1.381	8619.1	100.846	1.229
	8536.3	98.575		8536.3	98.575	
	8628.8	101.112		8549.1	98.926	
800	10408.5	99.941	1.416	10471.6	101.094	1.842
	10317.8	98.283		10317.8	98.283	
	10471.6	101.094		10508.5	101.769	
1200	12279.3	100.604	1.575	12318.6	101.143	1.173
	12339.9	101.435		12353.6	101.623	
	12117.4	98.385		12189.9	99.379	

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

From the linearity data the limit of detection and quantitation was calculated, using the formula LOD = 3.3 σ/ S and LOQ = 10 σ/ S where σ is standard deviation of the y intercept of linearity equation and S is slope of the calibration curve of the analyte. The LOD and LOQ were found to be 33.38 ng/ band and 101.153 ng/band, respectively.

Assay

Tericox (250 mg) tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was applied and area was recorded. Basic concentration of sample chosen was 400ng/band from tablet solution. Concentration and % recovery was determined from linear equation. Assay results obtained are shown in Table 2.

Table 2: Assay of marketed formulation

Drug	Peak Area	Amount Recovered (µg/ml)	%Recovery	± %RSD
Terizidone	8619	403.372	100.843	0.995
	8535.1	394.169	98.542	
	8549.5	395.749	98.937	
	8575.7	398.622	99.656	
	8584	399.533	99.883	
	8625.5	404.084	101.021	

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the tablet solution, at three different levels 50, 100 and 150 %.Basic concentration of sample chosen was 400 ng/band. % recovery was determined from linearity equation. The results obtained are shown in Table 3.

Table 3: Accuracy Studies of Terizidone

Level	Amount of sample taken (ng/band)	Amount standard spiked (ng/band)	Area	% Recovery	±% RSD
50%	400	200	11246.1	98.8	1.196
			10666.8	101.007	
			10650.6	100.710	
100%	400	400	11977.6	100.992	1.325
			12916.4	98.371	
			12033.4	99.975	
150%	400	600	14046.4	99.865	0.660
			13989.9	99.245	
			13627	98.556	

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which Detection wavelength, Time was changed from spotting to development and development to scanning and the effect on the area was noted. It was found that method is robust.

V. RESULTS AND DISCUSSION

Method development: It was observed that the drug showed considerable absorbance at 264 nm. After few trials, n-Hexane: Chloroform (5:5 v/v) was chosen as mobile phase with saturation time 10 min, which gave good resolution and acceptable peak parameters. The densitometric analysis of drug was carried out at 264 nm. The R_f value was found to be 0.25 ± 0.10.The results indicated the suitability of the method to study the stability of TERI under various forced degradation conditions like acidic, basic, hydrolysis, oxidation, dry heat and photolysis. The method was found to be accurate, specific, Precise and robust.

Table 4: Summary of validation parameters

Sr.No.	Parameter	Terizidone
1	Linearity	y = 9.1174x + 4941.3
2	Range	200 – 1200 ng / band
3	Precision	%RSD
	Intraday	1.38 – 1.58
	Interday	1.17 – 1.85
4	Assay	0.995%
5	Accuracy	% Recovery
	50%	100.17
	100%	99.78
	150%	99.22
6	LOD	33.38 ng/ band
7	LOQ	101.153 ng/band
8	Specificity	Specific
9	Robustness	Robust

VI. CONCLUSION

A simple, precise, accurate, reproducible and stability-indicating HPTLC method without interference from the excipients or from degradation products has been developed and validated for the determination of TERI as bulk drug and in tablet dosage form. The developed method can be used for quantitative analysis of TERI in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

VII. ACKNOWLEDGEMENT

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