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Novel RP-HPTLC and UV-AUC Methods for the Simple, Economical and Rapid Determination of Terizidone in bulk and Capsules

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ABSTRACT: Terizidone was examined in both bulk and capsule dosage forms utilizing newly designed and validated HPTLC (high-performance thin-layer chromatography) and UV-spectroscopic area under curve (UV-AUC) techniques that are easy to use, quick, and accurate. As the stationary phase, aluminium sheets (10 × 10 cm) pre-coated with silica gel 60 RP-18 F254S (E. Merck) were used in the research. The mixture that made up the mobile phase included acetonitrile, water, and acetic acid in a 2:2.5:0.5 (v/v/v) ratio.RP-HPTLC was used to quantify terizidone, with UV detection set at 262 nm. The RP-HPTLC technique was used to quantify at concentrations ranging from 200 to 1200 ng/band. Terizidone produced a compact and distinct band with a retardation factor (Rf) of 0.55 ± 0.02 using the HPTLC technique. The RP-HPTLC method calibration data from linear regression analysis showed a solid linear association with the regression coefficient; r^2 = 0.9978. The area was calculated at a wavelength of 258.50 to 267.50 nm using the UV-AUC technique, which was established with a 5% v/v sodium lauryl sulfate (SLS) solution as the solvent. The UV-AUC investigation revealed a correlation coefficient of r^2 = 0.9994. Terizidone a fine linear relationship was shown by the established UV-AUC method in the 3–18 µg/mL concentration range.The designed procedures were verified for precision, robustness, sensitivity, accuracy, and ruggedness in accordance with the stated requirements of the International Conference on Harmonization (ICH).The established techniques were found to be accurate, sensitive, robust, and exact based on statistical analysis results. They could also be successfully used in the analysis of terizidone in pharmaceutical formulations and in bulk.

KEYWORDS: Terizidone; HPTLC; UV-AUC

I. INTRODUCTION

Although the tuberculosis-causing bacterium, Mycobacterium tuberculosis, can infect various body parts, pulmonary TB is the most contagious subtype [1]. Terizidone is a second-line antitubercular drug used in the treatment of multidrug-resistant tuberculosis (MDR-TB)[2].Terizidone works against bacteria by inhibiting D-alanine ligase and L-alanine racemase, which prevents the synthesis of peptidoglycan used to construct bacterial cell walls[3].Two molecules of d-cyclosenine and one molecule of terphthalic di-aldehyde form the Schiff's base of terizidone. In the treatment of tuberculosis, terizidone is just as effective as cycloserine, and patients often tolerate it better [4]. The molecular weight of this drug is 302.29 g/mol, and its formula is $C_{14}H_{14}N_4O_4$. It is chemically known as 4,4′-[1,4-Phenylenebis(methylidynenitrilo)]bis-3-isoxazolidinone (Figure no. 1) [5].

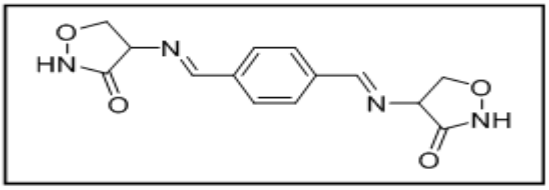

Figure No. 1: Chemical Structure of Terizidone

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The review of the literature found uncommon, diverse, and highly critical reviews for effective analytical determinations of terizidone.Terizidone was measured in pharmaceutical dosage form and in bulk using the UV spectrophotometric method, which was developed and validated [6]. AUC and first-order derivative spectrophotometry are used to estimate the dose of terizidone in bulk and capsule form [7]. Pyridoxine and terizidone simultaneous detection and quantification in pharmaceutical formulations utilizing an approved RP-HPLC technique [8]. Utilizing the HPLC-UV method to analyze terizidone in plasma and applying it to the pharmacokinetic analysis of patients with drug-resistant tuberculosis [9].RP HPLC methods for the development and validation of terizidone [10]. Chromatographic and associated techniques for terizidone impurity profiling, the first-line antitubercular drug [11]. Method development and validation for terizidone determination using RP-HPLC stability indicating [12]. Development and validation of HPTLC and LC MS-MS methods for terizidone quantification in pharmaceutical dosage forms [13].Developing and validating a stability-indicating HPTLC technique to estimate terizidone[14]. An application of a hyphenated chromatographic method and forced degradation investigation to two new terizidone degradation products [15].

Terizidone is practically insoluble in water; therefore, a hydrotropic agent was utilized to achieve complete drug dissolution, enabling its analysis using UV-AUC spectroscopy and RP-HPTLC.The objective of this study was to develop and validate RP-HPTLC and UV-AUC spectroscopy methods for the estimation of terizidone in pharmaceutical dosage forms. The methods were validated in accordance with ICH guidelines.[16]

II. EXPERIMENTAL

2.1 Drug and Chemicals

A gift sample of terizidone standard was acquired from Macleods Pharmaceuticals Ltd. (Gujarat, India), methanol (HPLC grade), sodium lauryl sulfate (SLS), acetic acid, and acetonitrile (ACN) used during the study were procured from (Merck Fine Chemicals, India).Throughout the entire experiment, chromatography and spectroscopy were conducted using double-distilled water.

2.2 Equipment and experimental condition

2.2.1 For HPTLC analysis

The Camag TLC system (Muttenz, Switzerland) consists of a Camag binary trough chamber (20 x 10 cm), a Camag Linomat 5 automatic sample applicator, a 100µL Hamilton hype, a Camag TLC Scanner 3, and Camag winCATS software (version 1.3.0). During the study, an ultrasonicator made in India by ENERTECH Electronics Pvt. Limited was employed. Chromatographic analysis was performed using aluminum-backed pre-coated silica gel 60 RP-18 $F_{254}S$ (10 X 10 cm) and HPTLC plates with a 200 µm thickness (E. Merck, Mumbai, India). Before utilization, an oven set to 100°C was used to pre-wash and dry the HPTLC plates. WinCATS software was installed on a Camag TLC Scanner 3 (Camag, Muttenz, Switzerland) in order to perform densitometric detection.With a steady nitrogen gas flow, the Linomate 5 (Camag) applicator was used to apply drug standards and samples to the HPTLC plates. A 10µL sample was positioned 6 mm from the plate's edge.A binary trough glass chamber (20 x 10 cm) (Camag, Muttenz, Switzerland) was used to developthe plates. The mobile phase was a 5 mL volume. Prior to use, the components of the mobile phase were combined, and the vapors were allowed to fill the development chamber for 25 minutes at room temperature (25°C ± 2).The ascending technique was used to develop the plate up to an 8 cm migration distance, and an air drier was employed to dry it. A fixed 6 mm × 0.45 mm (micro) slit and a 20 mm scanning speed were used. Using a deuterium lamp that continually produces a UV spectrum between 200 and 400 nm, densitometric scanning was performed at 262 nm in absorbance–reflectance mode.The fixed measurement of the slit was 6 mm.

2.2.2For UV-AUC analysis

For all spectroscopic measurements, a Shimadzu 2450 UV-Visible Spectrophotometer equipped with UV Probe 2.21 software was used, along with two identical 10-mm quartz cells and a 1 nm spectral bandwidth. We increased the solubility of terizidone by using 5% v/v sodium lauryl sulfate (SLS) as a hydrotropic and wetting agent. It was also used as the solvent for the terizidone UV spectrophotometric measurement. SLS surfactant properties enabled us to fully dissolve the drug in double-distilled water. SLS is commonly used to aid in the dissolution of medications due to its well-known wetting, spreading, and surfactant qualities.

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2.2 Preparation of stock standard

2.3.1 For HPTLC analysis

A SHIMADZU AUX-120 analytical balance was used to weigh 10 mg of terizidone to produce a 1000 µg/mL stock standard solution. After that,it was diluted with 5% SLS in a 10 mL calibrated flask, and 5% SLS was used to adjust the volume, and it was sonicated for an additional 10 minutes using an ultrasonicator made in India by ENERTECH Electronics Pvt. Ltd.A suitable volume of 0.2–1.2 µL of terizidone was applied to the HPTLC to attain 200, 400, 600, 800, 1000, and 1200 ng of concentration per band, respectively.

2.3.2 For UV-AUC analysis

In water, terizidone is essentially insoluble; 5% v/v sodium lauryl sulfate (SLS) was used as a solvent to fully dissolve the medication in water and develop its spectrum properties.SLS is a hydrotropic substance that makes very slightly soluble terizidone more readily soluble in water.As stated in Section 2.3.1,10 mg of terizidone were precisely weighed to prepare a stock standard solution.then dissolved in 25 mL of 5% SLS, sonicated in an ultrasonicator for 15 minutes to eliminate any foaming that might have developed, and then 5% SLS was added gradually to get the volume up to 100 µg/mL for the standard solution used in the UV-AUC measurement.To avoid foam formation and any potential mistakes in the analysis caused by foam and trapped air, dilutions were prepared using a very gradual addition of 5% SLS v/v.A terizidone solution (10 µg/mL) was analysed within the 400-200 nm UV range following proper dilution.

2.3.3 Sample Preparation

Twenty terizidone capsules were weighed in accordance with Section 2.3.1 and ground into a powder in a glass mortar. 10 mg powdered terizidone was added to a 10 mL volumetric flask, followed by the addition of 5 mL of 5% SLS solution. The same solvent was used to dilute the liquid to volume following 20 minutes of sonication.The resulting solution was filtered using a 0.45 µm filter (Millifilter, Milford, MA, USA).

For HPTLC conclusions for the drug capsule assay, 6 µL of the filtrate was utilized for HPTLC plates.

For UV-spectroscopic analysis, 10 mg of terizidone powder and 50 ml of 5% SLS were added to a 100 ml volumetric flask. For 20 minutes, the flask was gently sonicated, and then the same solvent was used to dilute it to volume.The ultimate concentration of 6µl/ml terizidone was obtained by gently filtering the resultant solution through a 0.45µm filter.After transferring a volume of filtrate equal to 10µl of solution into a 10 ml volumetric flask, the volume was labelled with 5% SLS. After applying the suggested technique to the resultant solution, the concentration of terizidone was ascertained.

III. RESULTS AND DISCUSSION

Following established protocols, the linearity, accuracy, specificity, robustness, precision, selectivity, sensitivity, and ruggedness of the optimized HPTLC and UV-spectroscopic-AUC techniques were confirmed.

3.1 Development of Optimum Mobile Phase

Various mobile phase concentrations were tested to achieve excellent resolution and repeatable peaks.To get the best results for the required parameters, the mobile phase was ACN-Water-Acetic acid (2:2.5:0.5 v/v/v).For the maximum sensitivity, 262 nm was determined to be the ideal wavelength. After 25 minutes of room temperature saturation of the compartment by the mobile phase, a distinct and well-resolved peak for terizidone was found at an Rf of 0.55 ± 0.02.

3.2 Linearity and calibration curve

During the HPTLC method, linearity was examined. Terizidone was used as a stock standard at 1000 µg/mL, and HPTLC plates were spot-spotted with a constant volume of 0.2–1.2 to obtain terizidone at 200, 400, 600, 800, 1000, and 1200 ng/band, in that proportion. Following the guidelines and using the proper chromatographic conditions, the plate was prepared and scanned.To generate a calibration curve, the peak area for each band was plotted against the drug concentration.For HPTLC, the result of the linear regression analysis is $y = 8.2315x + 1238.6$ with regression coefficients ($r^2 = 0.9978$).This is typically seen as evidence that the fit is appropriate.Results are shown in**Figure no. 3.**

By producing working standards from a stock standard solution of 100 µg/mL of terizidone, solutions with 3–18 µg/mL of terizidone can be produced, the linearity of the UV-AUC approach was confirmed. The UV 400–200 nm range was then used to scan these solutions against a blank solution that included 5% SLS. Terizidone maximum absorbance (λmax) was measured at 262 nm. The area under the curve (AUC) was measured at wavelengths of 258.50 and 267.50 nm. Figure no. 2. It was discovered that the AUC vs.

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concentration calibration curve was linear between 3-18 µg/mL. The UV-AUC linear regression equation was found to be y = 0.0118x + 0.0042 with a regression coefficient of r^2 = 0.9994.

3.3 Precision

When evaluating precision in analytical method validation, repeatability and intermediate precision are essential because they show that a laboratory operator can consistently obtain the same results when analysing a batch of material at different times using the same developed method, tools, and reagents.Linear regression data was used to assess the intraday and interday precisions of the calibration curve for the HPTLC and UV-AUC investigations; Terizidone was assessed at 6, 9, and 12 µg/mL for the UV-AUC test and 600, 800, and 1000 ng/band for HPTLC analysis.A percentage relative standard deviation (%RSD) is used to represent the accuracy of the established UV-AUC and HPTLC methods. As indicated in Table No. 1, the findings showed the highest level of accuracy for both analyses and data.

Table no.1: Terizidone precision studies for HPTLC and UV-Zero order UV-AUC analysis.

Analysis	Concentration (ng/band)	Intraday		Inter-day	
		Amount found (ng/band)	%RSD	Amount found (ng/band)	%RSD
HPTLC	600	611.53	0.11	611.20	0.12
	800	814.94	0.19	805.15	0.32
	1000	1003.71	0.64	1007.38	0.44
UV-AUC	6	5.92	0.73	5.91	0.55
	9	8.92	0.18	8.91	0.32
	12	11.78	0.28	11.86	0.60

3.4 Limits of detection and quantification

The detection limits (LOD) and limit of quantification (LOQ) for both methods were calculated using the absolute deviation of the responses and the slopes of the resulting calibration curves (n = 3), in compliance with the International Conference for Harmonization guidelines Q2(R1). (ICH, 1997; amendment added in 2005).Terizidone solutions of 200, 250, 300, 350, and 400 ng/band were applied to HPTLC plates to validate the HPTLC procedure and ascertain the LOD and LOQ.The lower-end values of 2.0, 2.5, 3.0, 3.5, and 4.0 µg/mL from the linearity range were utilized to determine the LOD and LOQ for the UV-AUC investigation. The limits of detection (LOD) and quantification (LOQ) were calculated using the formula LOD = 3.3 × N/B and LOQ = 10 × N/B. In this formula, "B" stands for the calibration curve's slope, and as a noise measure, N" is the standard deviation of the drug's peak regions (n = 3). Consequently, the lowest concentrations at which the analyte may be consistently identified and measured were determined to be 0.17 and 0.51 µg for UV-AUC analysis and 18.33 and 55.55 ng for HPTLC, respectively.

3.5 Robustness

For robustness investigation, six duplicates were conducted at a concentration of 600 ng/band. This study investigated the impact of four parameters: saturation time, development distance, volume, and mobile phase composition. A slight modification in the mobile phase composition was examined to assess its impact on the results. Chromatograms were produced using various ratios of ACN, water, and acetic acid (2.5:2.5:0.5, 2:3:0.5, v/v). The saturation duration (25 ±5 min, i.e., 20 and 30), the mobile phase concentration (5 ±1 mL, i.e., 4 and 6), and the development distance were maintained at 8 ± 0.5 cm, ranging between 7.5 cm and 8.5 cm, and were also altered. The time intervals between terizidone application and plate development, as well as between plate development and scanning, were changed to 10, 20, or 30 minutes.

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Table no. 2: Robustness for Terizidone RP-HPTLC Analysis [n=3]

Parameters	**%RSD**
Mobile phase composition	
ACN: Water: Acetic acid (2.5:2.5:0.5 v/v/v)	0.46
ACN: Water: Acetic acid (2:3:0.5 v/v/v)	0.68
Mobile phase volume (ml)	
4	0.66
6	0.55
Development distance (cm)	
7.5	0.27
8.5	0.90
Duration of saturation (min)	
20	0.41
30	0.28

3.6 Accuracy

Performing the recovery research in three stages allowed for the determination of the accuracy of the approach. The pre-analysed, known concentration of the drug standard terizidone solution was mixed with three different concentrations of the standard drug: 80%, 100%, and 120% of the solution to perform recovery studies. The drug's recovery rates at various extra concentrations range from 98.69% to 99.60% for HPTLC, 99.43% to 100.56% for UV-Zero order, and 99.81% to 100.05% for UV AUC analysis, as shown in Table no. 3.

Table no. 3: Terizidone recovery studies for UV-AUC and HPTLC analysis.

Drug	Initial amount applied	Amount over spotted	% Recovery	% RSD
HPTLC	600 ng/band	480	98.72	0.62
		600	99.60	0.22
		720	98.69	0.94
UV-AUC	6µg/mL	4.8	100.05	1.02
		6	99.81	0.82
		7.2	100.05	1.18

3.7 Ruggedness

The HPTLC method's robustness was assessed at 600 ng/band, and the drug UV-AUC was assessed at 6 µg/mL. When two separate analysts conducted the analysis in the identical experimental and environmental settings, the methods were determined to be robust.

Table no. 4: Overview of HPTLC and UV-AUC analysis regression, validation, and capsule formulation assay parameters

Parameters	**HPTLC**	**UV-AUC**
Linearity rang	200-1200 ng/band	3-18 (µg/ml)
correlation coefficient	0.9978	0.9994
Analysis of bulk material (% amount found)	101.03	99.53
Capsule analysis (% amount found)	100.96	99.53
Ruggedness (% amount found)		
Analyst I	101.30	99.29
Analyst II	101.36	99.76

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Accuracy	98.69-99.60	99.81-100.05
Precision		
Repeatability	101.34	99.65
Intraday	0.11-0.64	0.18-0.73
Inter-day	0.12-0.44	0.32-0.60
Sensitivity (µg)		
Limit of Detection	18.33	0.17
Limit of Quantification	55.55	0.51
Robustness	Robust	Robust

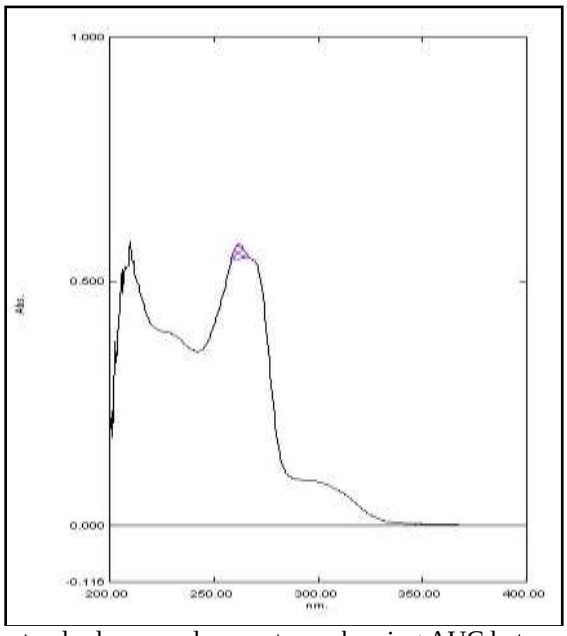

Figure no. 2: Terizidone standard zero-order spectrum showing AUC between 258.50and 267.50 nm.

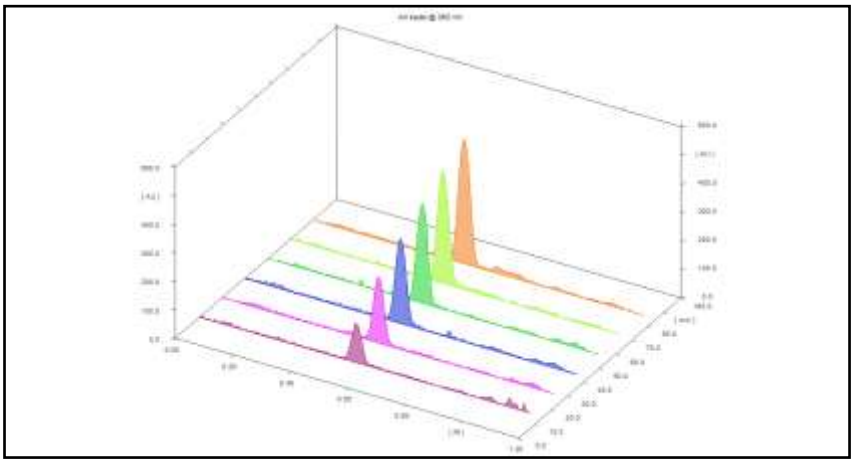

Figure no. 3: Terizidone linearity studies: Using ACN: Water: Acetic acid (2:2.5:0.5 v/v/v) as amobile phase during HPTLC.

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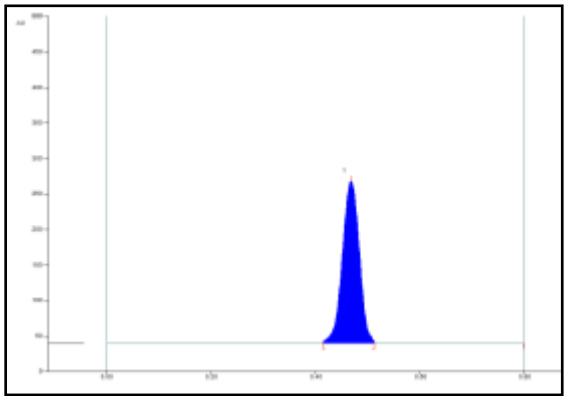

Figure no. 4: Terizidone standard HPTLC chromatogram in ACN: Water: Acetic acid (2:2.5:0.5 v/v/v) as the mobile phase.

IV. CONCLUSION

A novel HPTLC technique was invented to accurately determine terizidone in bulk as well as customized mixtures. The method's validity was established using the ICH recommendations, and it was found to possess the following attributes: affordability, simplicity, accuracy, linearity, and robustness.

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