International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.9, pp 457-464, 2016

Estimation of Terizidone in Bulk and Capsule Dosage form by Area Under Curve and First Order Derivative Spectrophotometry

Hemant K. Jain*, Rahul R. Mane

Department of Quality Assurance Techniques, Sinhgad College of Pharmacy, Vadgaon (Bk.), Pune- 411041, Maharashtra, India

Abstract : Two simple, precise, rapid, accurate and economical spectrophotometric methods have been developed for the estimation of terizidone in bulk and capsule dosage form. First method was based on measurement of area under curve for spectrum in a wavelength range between 268-278 nm and second method involved first order derivative spectrophotometry at a wavelength 297 nm. The working standards and sample solutions of terizidone were prepared in 0.1 N NaOH. These methods have been validated as per ICH guidelines. The linearity for first method was found in the concentration range of 4-12 µg/ml and the value of correlation coefficient (R^2) was found to be 0.9994. Linearity of second method was found in 4-12 µg/ml concentration and the value of correlation coefficient (R^2) was found to be 0.9914. The % assay values obtained by both methods were found within acceptance limits. Percent R.S.D. for precision study by both methods were also found to be satisfactory indicated reproducibility of the methods. The values of % R.S.D. were found to be satisfactory suggested both methods were precise. The accuracy of both methods was assessed by recovery studies and % recovery values were found within acceptance criteria. Thus, proposed methods can be applied for routine analysis of terizidone.

Keywords : Terizidone, UV-Spectrophotometry, Area under Curve, First order derivative, Method validation.

1. Introduction

Terizidone is chemically 4, 4′-[*p*-Phenylenebis (methylene amino)] bis (isoxazolidin-3-one) (figure no. 1).[1, 2] It acts as an anti-tubercular drug. It has an antibiotic activity against mycobacterium tuberculosis and *M. avium* for the treatment of tuberculosis, i.e. pulmonary and extra pulmonary.[3] It is classified as a second-line drug and only used when first line drugs cannot show expected results.[4] Terizidone is obtained by combining two molecules of cycloserine and one molecule of terephtalaldehyde which is a broad spectrum antibiotic that improved the disadvantages associated with cycloserine.[5, 6]

Literature survey reveals that there is one UV-Spectrophotometric method has been reported for determination of terizidone at absorption maxima [7] and no area under curve (AUC) and 1st order derivative method has been reported for routine laboratory analysis. Therefore, the objective of this work was to develop simple, precise, accurate and economical UV-spectrometric methods for estimation of terizidone in capsule dosage form.

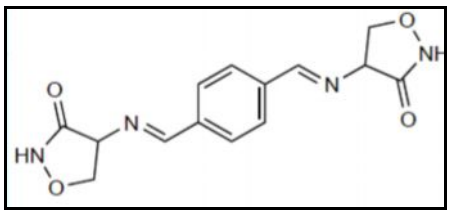

Figure 1: Structure of terizidone

2. Experimental:

2.1. Apparatus and Instruments

Shimadzu UV-1800 UV- Visible spectrophotometer with two matched quartz cells and UV probe software was used for the work. Shimadzu ATY 224 single pan electronic balance, Biosystems ultrasonic cleaning bath sonicator and calibrated volumetric glassware's (Borosil) were used in this study.

2.2. Chemicals and Reagents

Terizidone pure drug was obtained from Lupin Ltd., Pune as a gift sample. Commercially available capsules of 250 mg strength were purchased from the local pharmacy. AR grade of NaOH was obtained from Pallav chemicals and solvents Pvt. Ltd., Mumbai. Double distilled water was prepared in-house using Easy Still 2000, Infusil India Pvt. Ltd., Mumbai.

2.3. Preparation of Standard Stock Solution

Standard stock solution of terizidone was prepared by transferring, accurately weighed 100 mg of terizidone to 100 ml volumetric flask containing 50 ml 0.1N NaOH. The drug was dissolved properly and volume was made up to mark with 0.1N NaOH to make a concentration 1000 µg/ml. This solution was further diluted with 0.1N NaOH to get different concentrations between 4-12 µg/ml. [8, 9]

2.4. Area under Curve (Method A):

This method involves calculation of integrated value of absorbance with respect to wavelength in indicated range. Area calculation processing item calculates the area bounded by the curve and horizontal axis. Here horizontal axis represents baseline. [10, 11]

$$Area\,calculation\,(\alpha + \beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$$

Whereas,
α is area of portion bounded by curve data and a straight line connecting the start and end point,
β is area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis.
λ_1 and λ_2 are wavelengths representing start and end point of curve region.

In this study area under curve was integrated between wavelength ranges from 268-278 nm (figure 2). The calibration curve was prepared between concentrations and their respective area.

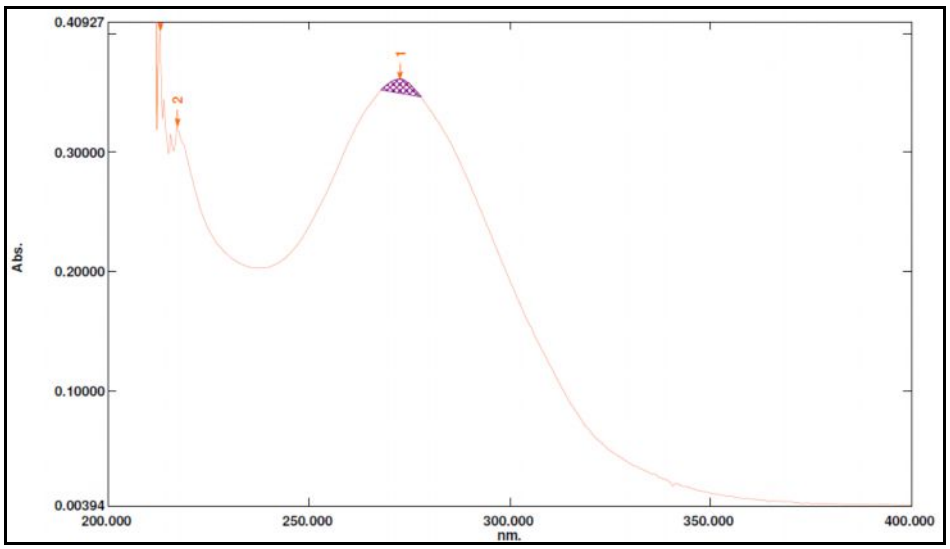

Figure 2: Area under curve of terizidone

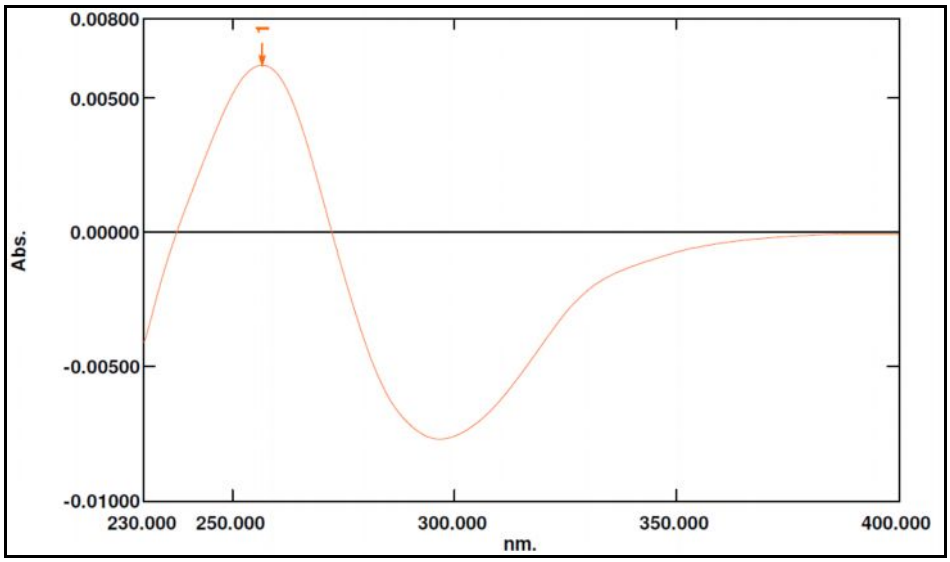

Figure 3: First order derivative spectra of terizidone

2.5. First Order Derivative Spectroscopy (Method B)

Solutions of terizidone were scanned in the spectrum mode from 400-200 nm. The first order derivative spectrum (figure 3) was obtained by data processing mode from this spectrum. [12] Derivative spectrum of all working standards was obtained in the range 4-12 µg/ml. The calibration curve was performed between concentration and dA/dλ. [13, 14]

2.6. Assay of Capsule formulation

Twenty capsules of terizidone were accurately weighed and average weight of a capsule was calculated. The capsule powder equivalent to 100 mg of terizidone was accurately weighed and transferred to a 100 ml of volumetric flask and diluted up to mark with 0.1 N NaOH. [15, 16] This solution was filtered through whatmann's filter paper (no. 41) and the first few ml of filtrate was discarded. The solution was further diluted in concentration range 4-12 µg/ml. Results of analysis of capsule by both methods are shown in table 1.

Table 1: Assay of marketed formulation of terizidone

Method	Label claim	% Label claim estimated (Mean ± S.D.)*	% R.S.D.
A	250 mg	99.12 ± 0.014	1.586
B	250 mg	98.95 ± 0.009	1.487

*Average of three determinations; ± Standard Deviation

2.7. Method validation

The proposed methods were validated according to ICH Q2 (R1) guidelines. [17-19] Linearity, Accuracy, Precision, LOD and LOQ, Robustness were performed.

2.8. Linearity

Working standards were suitably prepared between 4-12 µg/ml. Linearity was determined by plotting the curve between concentrations and corresponding values of area in method A. Similarly, Linearity was determined by plotting the curve between concentrations and corresponding values and dA/dλ in method B. Calibration curves of terizidone by method A and B are shown in figure 4 and 5 respectively. The optical characteristics of terizidone are shown in table 2.

Table 2: Optical characteristics of terizidone

Sr. no.	Parameters	Method A	Method B
1	Wavelength/wavelength range (nm)	268-278	297
2	Concentration range for linearity	4-12	4-14
3	Correlation coefficient (R2)	0.9994	0.9914
4	Slope (m)	0.0651	0.0014
5	Intercept	0.0258	0.00004

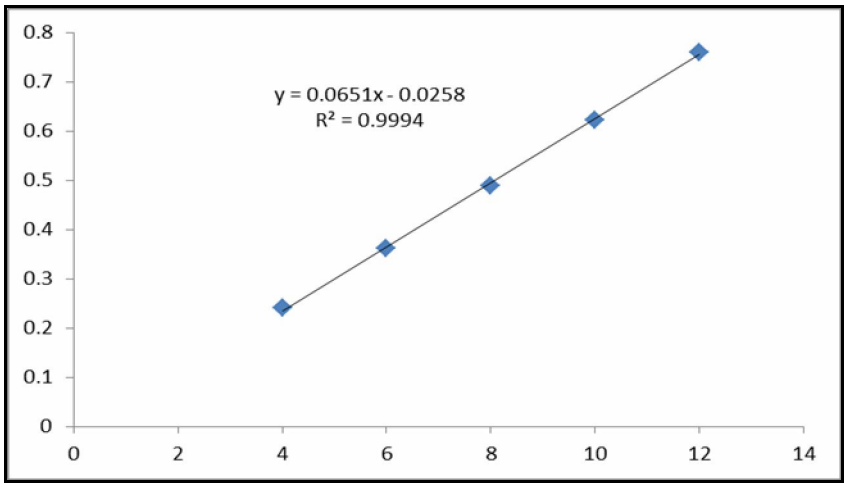

Figure 4: Calibration curve of terizidone by AUC (Method A)

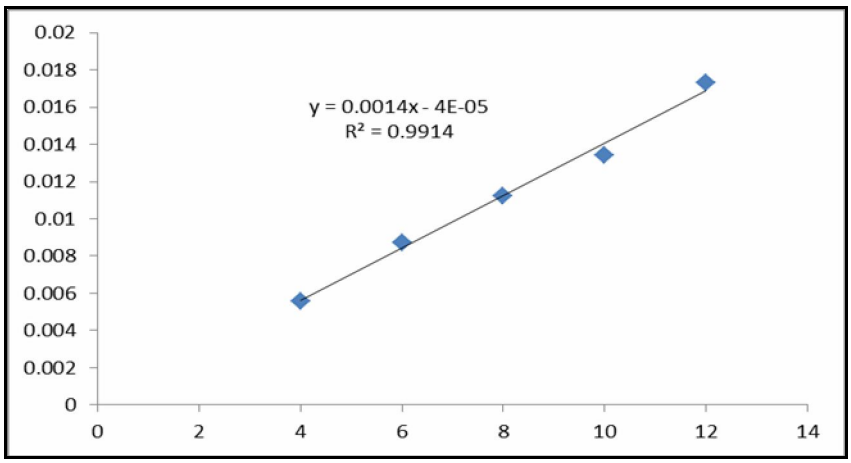

Figure 5: Calibration curve of terizidone by first order derivative (Method B)

2.9. Accuracy

The accuracy of the methods was determined by calculating % recovery of the drug by standard addition method. [20] Percent recovery of terizidone was determined at three different levels 80%, 100% and 120% of the target concentration in triplicate for both methods. The results of accuracy study are shown in table 3.

Table 3: Results of Recovery studies

Levels (%)	Standard drug added in sample sol^n (µg/ml)		% Recovery ± S.D.*		% R.S.D.	
	Method A	Method B	Method A	Method B	Method A	Method B
80	6.4	6.4	98.79 ± 1.075	98.85 ± 1.120	1.345	0.921
100	8	8	99.26 ± 1.034	98.74 ± 0.980	1.041	1.317
120	9.6	9.6	99.30 ± 0.903	99.12 ± 1.021	0.988	1.278

*Average of three determinations; ± Standard Deviation

2.10. Precision

The intraday and interday precision studies were carried out with three concentrations of terizidone with three replicates. The values of % relative standard deviation were calculated. The methods were precise and % RSD values were within acceptable limit (Table 4).

Table 4: Results of Precision

Sr. no.	Intraday precision		Interday precision	
	SD*	% RSD	SD*	% RSD
Method A	0.940	1.012	0.853	0.984
Method B	1.347	1.486	1.210	1.320

*(n=3)

2.11. Robustness

Robustness study was carried out by change in wavelength for determination of robustness of methods and the respective absorbance was recorded. The result of robustness study is presented in table 5.

Table 5: Results of Robustness

Change in Wavelength	Amount (µg/ml)	%R.S.D.*	
		Method A	Method B
272 nm	8	0.813	0.958
273 nm	8	0.725	1.290

*(n=3)

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

Five sets of the concentrations (4-12 µg/ml) were prepared and measured the values of area under curve and dA/dλ was determined its absorbance. Calibration curves were plotted for each set using both methods. The standard deviation of the Y-intercept and average slope of the calibration curve was used to calculate LOD and LOQ using following formula.

$$LOD = 3.3 \times \frac{\sigma}{\text{Average of slope}(S)} \qquad LOQ = 10 \times \frac{\sigma}{\text{Average of slope}(S)}$$

Where, SD is standard deviation of y-intercept of the calibration curves; S is the mean slope of five calibration curves. The LOD and LOQ determination is given the table 6.

Table 6: Results of LOD and LOQ

Methods	LOD (µg/ml)*	LOQ (µg/ml)*
Method A	1.78	5.40
Method B	1.81	4.90

*obtained by y-intercept of calibration method

3. Results and Discussion:

The values of correlation coefficients obtained by both methods (Table 2) demonstrated the good relationship between response and concentrations. Therefore, the developed methods were linear in concentration range of 4-12 µg/ml of drug. Percent estimation values in assay study (Table 1) of commercial tablets were found within acceptance criteria. Accuracy of proposed methods was ascertained by recovery studies. Percent recovery for terizidone and values of R.S.D. obtained by both methods (Table 3) were found satisfactorily indicating the accuracy of both the methods. Percent R.S.D. for Intraday and Interday precision was found to be 01.012 and 0.984 for Method I and 1.456 and 0.320 for Method II (Table 4). This study indicates good precision. The values of percent R.S.D. in robustness study (Table 5) was found to be within acceptance criteria which showed the reliability of both methods. The low values of LOD and LOQ indicated that the methods are sensitive (Table 6).

Table 7: Summary of Validation Parameters

Parameter		Method A	Method B
Wavelength		273nm	273nm
Linearity range		4-12µg/ml	4-12µg/ml
Correlation coefficient (*R2*)		0.9994	0.9914
% Assay		99.12	98.95
Accuracy (% mean recovery)		98.79-99.30	98.79-99.12
Precision (%RSD)	Intraday	1.012	1.486
	Interday	0.984	1.320
LOD (µg/ml)		1.78	5.40
LOQ (µg/ml)		1.81	4.90
Robustness	272nm	0.813	0.958
	274 nm	0.725	1.290

4. Conclusion:

It can be concluded from validation results that the proposed methods were simple, sensitive, accurate, precise, robust and economical for the determination of terizidone in capsules. Thus both methods can be applied for routine estimation of terizidone in bulk and capsule dosage form.

5. Acknowledgement:

Authors are thankful to Lupin Ltd., Pune for proving terizidone as a gift sample and Principal, Sinhgad College of Pharmacy, Pune for providing the necessary facilities. Authors are also thankful to Mr. Amit Sonawane for his support during this project.

6. References:

1. Pattar V., Nandibewoor S., Electrochemical studies for the determination of an antibiotic drug, dcycloserine, in pharmaceutical and human biological samples. Journal of Taibah University for Science, 2016, 10, 92–99.
2. Giorgis G., Oliaro A., Boaro D., Ardizzi A., Barberis S., Massaglia G., Tolerability to terizidone (TZ) in the treatment of pulmonary tuberculosis in dialyzed patients. Minerva Med, 1991, 82(7), 477-481.
3. Hwang T., Wares D., Jafarov A., Jakubowiak W., Nunn P., Keshavjee S., Safety of cycloserine and terizidone for the treatment of drug-resistant tuberculosis: a meta-analysis. Int J Tuberc Lung Dis, 2013, 17(10), 1257-1266.
4. Zitkova L., Tousek J., Pharmacokinetics of cycloserine and terizidone, Chemotherapy, 1974, 20, 18–28.
5. Musson D., Maglietto S., Hwang S., Gravellese D., Bayne W., Simultaneous quantification of cycloserine and its Prodrug Acetylacetonyl cycloserine in plasma and urine by High-Performance Liquid Chromatography using ultraviolet absorbance and fluorescence after post-column derivatization. Journal of Chromatography, 1987, 414, 121-129.
6. Ojeda C., Rojas F., Review recent developments in derivative ultraviolet/visible absorption spectrophotometry, Analytica Chimica Acta, 2004, 518, 1–24.
7. Khairnar S., Nagras M., Sonawane A., Development and validation of UV spectrophotometric method for the estimation of terizidone in bulk and pharmaceutical dosage form, Inventi Rapid: Pharm Analysis & Quality Assurance, 2016, 3, 1-4.
8. Rizwan S., Sastry V., Imad Q., Stability indicating method development and validation of Bosentan in bulk drug and formulation by RP-HPLC method, International Journal of PharmTech Research, 2015, 8(4), 569-579.
9. Yadav P., Majee C., Method development and validation for quantification of Propranolol HCl in pharmaceutical dosage form by RP-UPLC, International Journal of PharmTech Research, 2014-2015, 7(1), 197-203.
10. Jain H., Patel K., Development and validation of UV spectrophotometric area under curve method for estimation of Loratadine in bulk and tablet dosage form, American Journal of PharmTech Research, 2013, 3(4), 492-499.
11. Jain H., Gupta R., Agrawal R., Simultaneous estimation of Isoniazid and Pyridoxine Hydrochloride in tablets by spectrophotometric method, Indian Drugs, 2004, 41(3), 153-155.
12. Dudhe P., Kamble M., Komerwar A., Sonawane A., Van S., Development and validation of first order derivative method for Metronidazole in bulk and tablet using UV Visible spectroscopy, International Journal of ChemTech Research, 2016, 9(4),140-144.
13. Kumar R., Subramanian N., Role of UV-Visible Spectrophotometry in the determination of Lincomycin Hydrochloride in bulk and tablet dosage forms, International Journal of PharmTech Research, 2015, 8(7), 175-179.
14. Rangari N., Puranik P., Chaudhari S., Spectrophotometric determination of Losatan Potassium in pure form and in tablet dosage form, International Journal of PharmTech Research, 2015, 8(1), 142-145.
15. Goud N., Achaiah G., Sivaramakrishna V., Mayuri P., Development and validation of RP-LC method for Lisinopril Dihydrate in bulk and its pharmaceutical formulations, International Journal of PharmTech Research, 2015, 8(3), 448-452.

16. Gorle A., Shinde J., Development and validation of stability indicating assay method of Ofloxacin in bulk and pharmaceutical dosage form by RP-HPLC, International Journal of PharmTech Research, 2016, 9(4), 289-298.
17. ICH, Q2 (R1), Validation of analytical procedure: text and methodology International conference on Harmonization, Geneva, 2005.
18. Mishra P., Upadhyay S., Tripathi A., Saraf S., Stability indicating HPLC-UV method for simultaneous estimation of Pantoprazole, Domperidone and Drotaverine, International Journal of PharmTech Research, 2015,8(5), 912-923.
19. Sherje A., Londhe V., Stability Indicating HPLC method for determination of Paliperidone in bulk, International Journal of PharmTech Research, 2015, 8(8), 157-163.
20. Sinaga S., Intan M., Silalahi J., Protein analysis of Canned Legumes by using visible spectrophotometry and Kjeldahl method, International Journal of PharmTech Research, 2015, 8(6), 258-264.

*****