DEVELOPMENT AND VALIDATION OF TERIZIDONE BY RP HPLC.

Khushbu Makwana[1*], Kartik B. Vyas[2], Janakkumar R. Shukla[3]

[1-3]Department of Chemistry,Sheth L. H. Science College, Mansa, Gujarat University,Ahmedabad, India

ABSTRACTS

Terizidone is the well-known psychoactive drug of class benzodiazepam. Terizidone is a bacteriostatic second-line agent for MDR-TB used internationally, but is not currently approved in the United States. It is a combination of two molecules of CS. The MOA includes competitively inhibiting l-alanine racemase and d-alanine ligase to inhibit cell wall synthesis. Known AEs are considered to be fewer than with CS, but still include seizures, dizziness, slurred speech, tremors, insomnia, confusion, depression, and suicidal tendencies. A simple, precise, accurate, reproducible RP-HPLC method was developed and validated for the simultaneous estimation ofTerizidone in bulk and in pharmaceutical dosage forms. Chromatographic separation was carried out on a Thermo-hypersil C8 column (250mm×4.6mm i.d,5µm) utilizing a mobile phase consisting of acetonitrile and 0.01M ammonium phosphate buffer (pH adjusted to 3.0 with ortho phosphoric acid) in the ratio of 55:45 v/v at a flow rate of 1ml/min with UV detection at 242nm. The retention times of Terizidone were 2.23 min. The developed method was validated in terms of selectivity, sensitivity, accuracy, precision, linearity, specificity, limit of detection and limit of quantification. The linear range was found to be 20-82µg/ml.

Keywords:Terizidone, Linearity, selectivity, sensitivity, Reverse Phase HPLC, validation, benzodiazepam.

1.1 Introduction

Terizidone have been majorly prescribed in many parts of the world; first as anxiolytics and then as hypnotics **[1]**. They are extensively indicated for various CNS disorders such as anxiolytics (chlordiazepoxide and diazepam), anticonvulsants (clonazepam, and clobazam), muscle relaxants, anesthesia (midazolam) and insomnia, for some motor disorders and in psychoses (olanzapine and clozapine) **[2]**. The benzodiazepines were classified as short-acting, intermediate acting and long-acting depending upon their duration of action **[3]**. The BDZs exert their effect by binding to the central benzodiazepine receptors which are located at the post and presynaptic membranes. However, certain side effects are associated with the short- and long-term use of benzodiazepines which includes confusion, drowsiness, amnesia, and ataxia **[4]**.Benzodiazepines (BZDs) represents one of the important and highly explored class of seven-membered aromatic heterocycles containing two ring nitrogen that are critical for numerous applications in the pharmaceutical industry and organic synthesis of complex molecules **[5]**. Due to their diverse spectrum of biological activities, they were considered as "privileged structures" in medicinal chemistry. Further, they are key synthons for the synthesis of various fused ring compounds **[6,7]**. As such, further development of BZDs has gained significant attention of organic and medicinal chemists in a quest to discover new and efficacious benzodiazepine based therapeutic agents **[8]**.

From the structure activity studied, compounds with high potency and expanded spectrum of activity, improved absorption, and distribution properties were synthesized and biologically evaluated in various disease areas. Benzodiazepine derivatives were reported to possess various pharmacological activities such as antimicrobial, anticancer, anti-anxiolytic, antidepressant, anticonvulsant, antitubercular, anti-inflammatory, analgesic, antihistaminic and anti-anxiety activities **[9-11]**. Various benzodiazepine-based compounds have different groups or substituents attached to their core structural motif at positions 1, 2, 5, or 7 respectively **[12]**. These different side groups affect the binding properties of molecules with the relevant target proteins or receptors (such as BET) and hence modulate their pharmacological properties, the potency of biological response and the pharmacokinetic profile **[13]**.

In presence study, we have reported method for development and validation of Terizidone by well-known RP-HPLC method.This study aims to develop a precise, rapid, accurate, and sensitive reversed-phase HPLC method to analyze Terizidone in pharmaceutical dosage and bulk drug forms. Peak asymmetry and calibration curves were employed to achieve an extraordinary retention time. The linearity graph of five various concentrations in the range of (20, 35, 50, 65, 82 mg/mL) has been run for accurate measurement of Terizidone in tablets mentioned in Fig. 1. for the analysis of Terizidone. Area (mV) found are 50, 75, 100, 125, 150.

1.2 Experimental Part

1.2.1 Apparatus and Software

Chromatography was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with Shimadzu LC-20AT pump and Shimadzu SPD20AV absorbance detector. Samples were injected through a Rheodyne 7725 injector valve with fixed loop at 20 µl. Data acquisition and integration was performed using Spinchrome software (Spincho biotech, Vadodara).The chromatographic elution of analyte was obtained by using Thermo-hypersil C8 column (250mm×4.6mm i.d,5µm).

1.2.2 Reagents and Chemicals

Terizidone was provided as gift sample from Samir Tech Pvt. Ltd. Vadodara, India. HPLC grade Acetonitrile and methyl alcohol, Dipotassium monohydrogen phosphate AR grade was purchased from Samir Tech Pvt. Ltd. Vadodara, India. The pharmaceutical samples used in the present study include Terizidone 5% ointment.

1.2.3 Chromatographic Conditions

The mobile phase comprised of Acetonitrile: Dipotassium monohydrogen phosphate buffer pH 3.2 in the proportion of 55:45. Resulting solution was degassed by ultrasonication for 10minutes.

1.2.4 Preparation of Standard Solution of Terizidone [14]

Stock solution of (1000 µg/ml) was prepared by accurately weighing 10 mg of Terizidone in 10 ml volumetric flask. The drug was dissolved in Acetonitrile and the solution was diluted to volume. Further dilutions were made from this stock solution and the injection volume was kept 20 µL. A calibration curve was plotted between concentrations against their respective area for Terizidone. From the calibration curve, it was found that linearity range is between 20- 82ug/ml.

1.3 Analysis of Marketed Formulation

Extraction Procedure:

An amount equivalent to 10mg [0.2g for **Ointment [15]**(5%), 0.5g for **Gel** (2%), and 0.46ml for **Injection** (2%), 0.1g equivalent to four sprays for **Aerosol** (10%)] was taken and dissolved in 10 ml of ACN to get 1000ug/ml of stock concentration. The stock solution was sonicated for 10 minutes and was filtered through Whatman filter paper. From the stock solution 0.6ml was taken in 10 ml volumetric flask. The volume was made up to the mark with ACN to get solution of 60ug/ml. The solution was finally filtered through 0.2um syringe filter was injected into HPLC.Transdermal patch(5%). An amount equivalent to 10 mg(0.2g) was taken in 10 ml of Dipotassium monohydrogen phosphate buffer 10 mM of pH3.0 and was magnetically stirred for 2 hours. The solution was then sonicated for 15 minutes and was then filtered through Whatman filter paper. From this stock solution 0.6 ml was taken in 10 ml volumetric flask. The volume was made up to the mark with ACN to get solution of 60ug/ml. The solution was finally filtered through 0.2um syringe filter was injected into HPLC

1.4 Result and Discussion

1.4.1 Optimization of Chromatographic Conditions

To optimize the chromatographic conditions, the effect of chromatographic variables such as composition of mobile phase, ratio of mobile phase and flow rate werestudied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, and theoretical plates were calculated. Finally, a simple and inexpensive method was developed by using a combination of Acetonitrile andDipotassium monohydrogen phosphate buffer in ratio 55:45. Optimized chromatographic conditions are listed in **Table 1**

Table 1

METHOD PARAMETER	OPTIMIZED VALUE
COLUMN	Thermo-hypersil C8 column (250mm×4.6mm i.d,5µm)
MOBILE PHASE	Acetonitrile and 0.01M ammonium phosphate buffer (pH adjusted to 3.0 with ortho phosphoric acid) in the ratio of 55:45 v/v
FLOW RATE	1 ml/min
RETENTION TIME tR (MINUTES)	3.86 min.
DETECTION WAVELENGTH(nm)	242
TEMPERATURE	Ambient
INJECTION VOLUME	20uL
TAILING FACTOR	1.3±0.018
THEORETICAL PLATES(N)	11935±88.34

1.4.2 Method Validation [16].

1.4.2.1 Linearity

Linearity Equation Y = 1.622X + 18.24

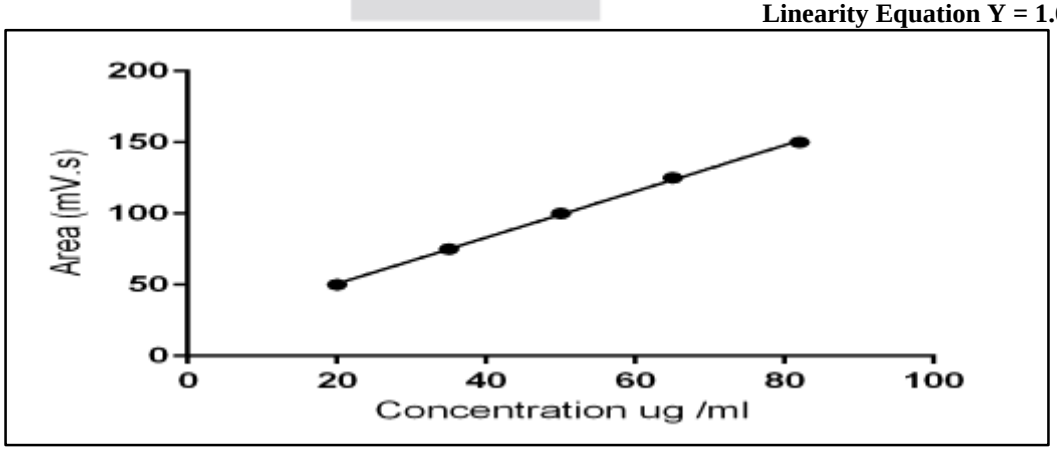

Figure-1 Calibration curve of Terizidone.

The calibration curve was constructed by plotting concentrations of Terizidone versus peak areas, and the regression equations were calculated. The linearity of the method was investigated by using concentrations in the range20-82 µg/ml.

Retention time for Terizidone was found to be3.863 min. The linear regression equation is **Y = 1.622X + 18.24 (R^2=0.9993)**. Theplotobtainedfromlinearregressionis given in **Figure 1.**

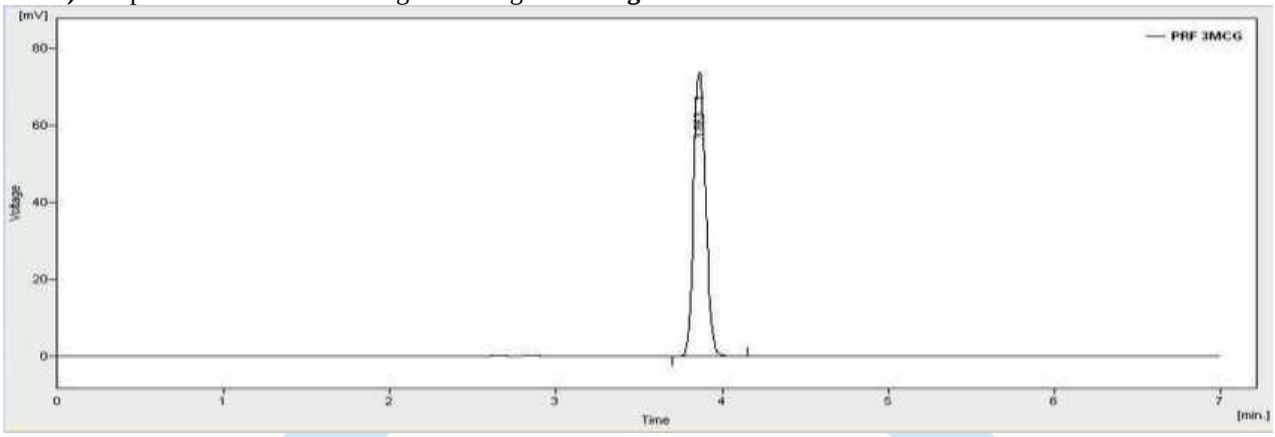

Figure 2: Chromatogram of Terizidone showing linearity in range 20-83ug/ml at tR3.863±0.01

1.4.2.2Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively, where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve**[16].**The LOD and the LOQ for HPLC were found to be 1.52ug/ml and 4.62ug/ml.

1.4.2.3Precision

The precision of the proposed method was assessed as intraday and interday precision. Three replicate injections of specific standard at various time intervals on the same day were injected into system for intraday precision and were repeated on three different days for Interday precision. The % RSD (Relative Standard Deviation) of the results was calculated

Table 2: Intraday precision ofTerizidone

CONC. (µg/ml)	MEAN AREA (mV.s)	SD	%RSD
40	35.33	0.310	0.87
60	50.70	0.460	0.90
80	68.90	0.452	0.63

Table 3: Interday precision ofTerizidone

CONC. (µg/ml)	MEAN AREA (mV.s)	SD	%RSD
40	35.20	0.355	0.97
60	50.60	0.615	1.22
80	68.65	0.654	0.90

1.4.2.4 Accuracy

Accuracy of the method was studied using standard addition method at three different levels (80, 100, and 120%) by recovery experiments. Known amounts of standard solutions containing Terizidone (45, 62,75ug/ml) were added to one of the marketed formulations of concentration 60 ug/ml to reach 80%, 100% and 120% levels. Percentage Recovery was the mean of three determinations at each standard addition level

Table 4: Accuracy data of Terizidone

% SPIKING	CONC TEST(ug/ml)	CONC ADDED (ug/ml)	CONC RECOVERED (ug/ml)	% RECOVERY ± STANDARD DEVIATION
80	60	45	45.1	100.1±0.55
100	60	62	61.9	99.8±0.42
120	30	75	74.9	99.9±0.42

1.4.2.5 Analysis of Marketed Formulation [16].

When the Terizidone marketed formulation was analyzed by these proposed HPLC method, sharp peaks was obtained at tR3.86 minutes, when scanned at 263nm. The amount of the label claim measured is given in table 6, all the formulations are within the limits (95%-105%), for patch the limits are (90%-110%)

Table 5: Assay results of marketed formulation

Sr.no	Formulation	% Assay
1	Ointment	102.1
2	Gel	99.1

3	Injection	100.1
4	Aerosol	99.6
5	Patch	95

1.5 Conclusion

A simple, accurate, unique, and particular RP-HPLC technique has been developed and demonstrated to estimate the pill dosage forms of Terizidone. The practical consequences advanced on this novel technique assertively concluded that this analytical technique turned into simple, unique, linear, particular, correct, and robust. The statistical implications of the evaluation manifestly confirmed that the system followed by the advanced HPLC advanced technique possessed fine precision and accuracy.

References

[1] C.N. Kaufmann, A.P. Spira, C.A. Depp, R. Mojtabai, Long-term use of benzodiazepines and nonbenzodiazepine hypnotics, 1999–2014, Psychiatric Services 69 (2018) 235–238.

[2] C. Paton, Benzodiazepines and disinhibition: a review, Psychiatric Bulletin 26 (2002) 460–462.

[3] E. Batlle, E. Lizano, M. Viñas, M.D. Pujol, Chapter 5: 1,4-benzodiazepines and new derivatives: description, analysis, and organic synthesis, Med. Chem. (2019) 63–90.

[4] L. Goodman, A. Gilman, The pharmacological basis of therapeutics, Anesthesia Analgesia 20 (1941) 232–300.

[5] L.H. Sternbach, The benzodiazepine story, J. Med. Chem. 22 (1979) 0022–2623.

[6] A. Walser, R. Ian Fryer, "Dihydro-1, 4-benzodiazepinones and thiones." chemistry of heterocyclic compounds: bicyclic diazepines: diazepines with an additional, Ring 50 (1991) 631–848.

[7] M. Qadir, Recent structure activity relationship studies of 1,4-benzodiazepines, Open J. Chem. 8 (2015) 08–12.

[8] N.C. Bernardy, M.J. Friedman, Psychopharmacological strategies in the management of posttraumatic stress disorder (PTSD): what have we learned? Curr. Psychiatry Rep. 17 (2015) 20.

[9] C. Barbui, A. Cipriani, V. Patel, J.L. Ayuso-Mateos, M.V. Ommeren, Efficacy of antidepressants and benzodiazepines in minor depression: systematic review and meta-analysis, Br. J. Psychiatry 198 (2011) 11–16.

[10] S.E. File, The history of benzodiazepine dependence: A review of animal studies, Neuro. Sci. Bio. Behav. Rev. 14 (1990) 135–146.

[11] N. Sawada, H. Uchida, T. Suzuki, K. Watanabe, T. Kikuchi, T. Handa, Persistence and compliance to antidepressant treatment in patients with depression: A chart review, BMC Psychiatry 9 (2009) 1–10.

[12] J.Y. Wick, The history of benzodiazepines, Consultant Pharmacist. 28 (2013) 538–548.

[13] F. López-Muñoz, C. Álamo, P. García-García, The discovery of chlordiazepoxide and the clinical introduction of benzodiazepines: Half a century of anxiolytic drugs, J. of Anxiety Disord. 25 (2011) 554–562.

[14] Narendra M Gowekar, Shailesh J Wadher. Development and Validation of HPLC Method for Simultaneous Determination of Lidocaine and Prilocaine in Tropical Formulation. Asian J Pharm Clin Res, Vol 10, Issue 10, 2017, 179-182. Published 22 June 2017.

[15] B. Karthik Kumar, V.S. ThiruvengadaRajan, N. Tanveer Begum. Analytical Method Development and Validation of Lidocaine in Ointment Formulation by U.V Spectrophotometric method. Int J Pharm Pharm Sci, Vol 4, Issue 2, 610-614. Published 29 Jan 2012.

[16] Narendra M Gowekar, Shailesh J Wadher. Development and Validation of HPLC Method for Simultaneous Determination of Lidocaine and Prilocaine in Tropical Formulation. Asian J Pharm Clin Res, Vol 10, Issue 10, 2017, 179-182. Published 22 June 2017.