Journal of Pharmaceutical Research International

33(39B): 273-282, 2021; Article no.JPRI.71722
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

RP-HPLC Method Development and Validation for Determination of Tigecycline in Bulk and Pharmaceutical Dosage form

K. Bhavyasri[1*], C. H. Mounika[1], Narmada Vallakeerthi[2] and M. Sumakanth[1]

[1]Department of Pharmaceutical analysis, RBVRR Women's College of Pharmacy. Barkathpura, Hyderabad-500027, India.
[2]Department of Pharmacy, University College of Technology, Osmania University, Hyderabad-500007, Telangana, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i39B32204
Editor(s):
(1) Dr. Thomas F. George, University of Missouri- St. Louis, USA.
Reviewers:
(1) Qasim Shahzad, Bahauddin Zakariya University, Pakistan.
(2) Zaid Mahdi Jaber Al-Obaidi, University of Alkafeel, Iraq.
Complete Peer review History: https://www.sdiarticle4.com/review-history/71722

Original Research Article

Received 25 May 2021
Accepted 01 August 2021
Published 03 August 2021

ABSTRACT

Aims: To develop and validate a new, simple, rapid, precise and accurate Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for the quantitative determination of Tigecycline in bulk and pharmaceutical dosage form.
Study Design:
Place and Duration of the Study: RBVRR women's college of pharmacy, Barkatpura, Hyderabad, between june 2019 and july 2020.
Methodology: The RP-HPLC method was developed on Sunsil C18 150 mm x 4.6mm x 5µ column using acetonitrile : water (pH maintained at 3.5 with acetic acid) [70:30] as mobile phase at flow rate 0.8 ml/min and UV detection at 250 nm.
Results: Tigecycline exhibited linearity over the concentration range of 5-40 µg/mL (R2 > 0.999). The analytical method showed good precision with % RSD below 2. The method showed suitable accuracy and robustness.
Conclusion: Validation of the developed method was done as per International Conference on Harmonization (ICH) Q2R1 guidelines.

**Corresponding author: E-mail: bhavya.khagga@gmail.com;*

Keywords: Reverse Phase High Performance Liquid Chromatography; Tigecycline; method development; Validation; International Conference on Harmonization (ICH) Q2R1.

1. INTRODUCTION

Tigecycline is the first drug clinically available under the class of Glycylcyclines which are a new class of antibiotics derived from tetracycline. Tigecycline is a new glycylcycline with broad spectrum antibiotic activity. It is chemically (4S,4aS,5aR,12aS)-9-[2-(tert-butylamino)acetamido]-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide [1].

Tigecycline inhibits protein translation in bacteria by binding to the 30S ribosomal subunit and interfering with the entry of amino-acyl tRNA molecules into the A site of the ribosome. This blocks incorporation of amino acid residues into elongating peptide chains, thereby preventing protein synthesis and eventually bacterial cell growth. Glycylcyclines appear to bind more effectively compared to tetracyclines. It has activity against a broad range of Gram-positive and Gram-negative bacteria, including tetracycline-resistant organisms. This tetracycline analogue overcomes tetracycline resistance by two mechanisms namely resistance mediated by acquired efflux pumps and ribosomal protection. It is used for the intravenous treatment of complicated skin and skin structure infections caused by susceptible organisms [2-3].

The present research work describes the development and validation of a simple, rapid, accurate and precise RP-HPLC [4-13] method for estimation of Tigecycline in bulk and pharmaceutical formulation.

2. MATERIALS AND METHODS

2.1 Instruments

Shimadzu HPLC (LC-20AD Multi-solvent delivery system, SPD-20A UV-Visible detector, LC solution software). Labman sonicator was used for sonication of the sample solution. Thermo scientific pH meter was used to measure pH. Vacuum pump filter was used for filtration of mobile phase solvents and they were provided by RBVRR women's college of pharmacy, barkatpura, Hyderabad, India.

2.2 Chemicals

Tigecycline pure drug was obtained as gift sample from Gland Pharma Hyderabad, India. Tigecycline formulation (TGKEM) was purchased from local drug store. HPLC grade water, methanol, acetonitrile and glacial acetic acid were purchased form SD Fine Chemicals, Mumbai, India.

3. CHROMATOGRAPHIC CONDITIONS

The isocratic mobile phase consisted of Acetonitrile: Water (pH adjusted to 3.5 with Acetic acid) [70:30], flowing through the column at constant flow rate 0.8 ml/min. Sunsil C18 column (150 mm x 4.6mm x 5µm) was used as the stationary phase. 250 nm was selected as the detection wavelength for UV-Visible detector.

4. PREPARATION OF TIGECYCLINE STANDARD SOLUTIONS FOR RP-HPLC METHOD

4.1 Preparation of Standard Stock Solution

Accurately 10 mg of Tigecycline standard drug was weighed and transferred into a 10 mL volumetric flask. The volume was made up to the mark using the mobile phase resulting in 1 mg/mL concentration primary stock solution. From this, 1mL was pipetted out and transferred into a 10 mL volumetric flask and diluted to obtain 100 µg/mL secondary stock solution.

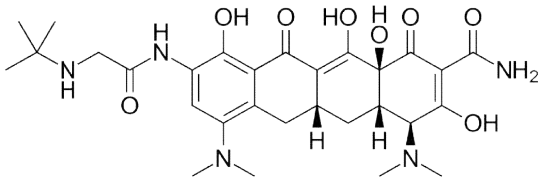

Fig. 1. Chemical structure of tigecycline

4.2 Preparation of Working Standard Solutions

From the secondary stock solution 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 & 4.0 mL aliquots were transferred into series of 10 mL volumetric flasks and further diluted with mobile phase to obtain 5, 10, 15, 20, 25, 30, 35 and 40µg/mL working standard solution.

4.3 Determination of λmax

The absorption spectrum for Tigecycline was recorded by scanning 10µg/ml working standard solution using UV-Visible spectrophotometer in the range of 200-400nm. λmax was found to be 250nm. Fig. 2 shows the spectrum of Tigecycline.

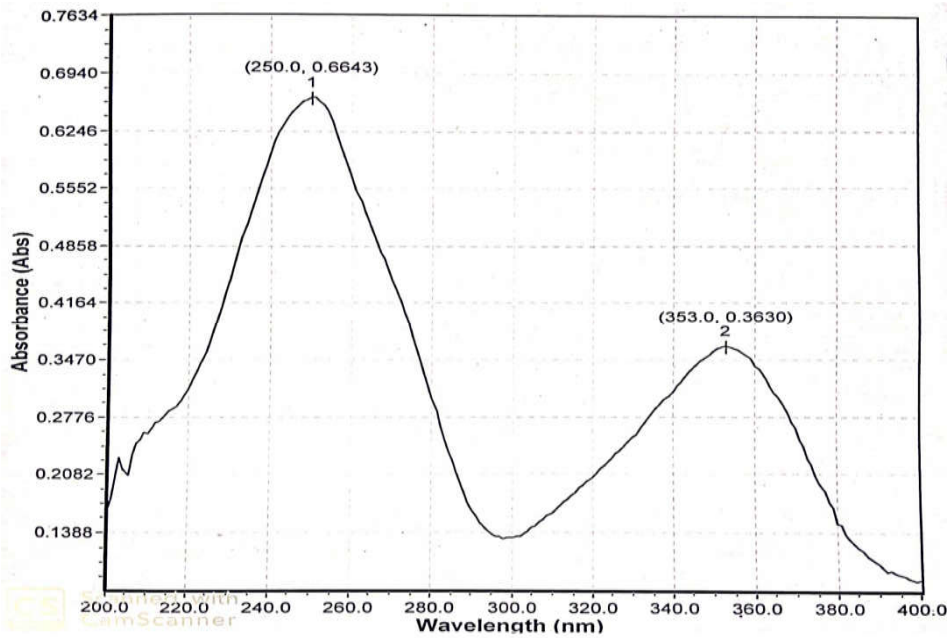

Fig. 2. UV Spectrum for determination of Tigecycline λmax

5. RESULTS AND DISCUSSION

5.1 RP-HPLC Method Development

Based on the drug solubility and pKa value the following chromatographic conditions have been selected to initiate the method development trials for determination of Tigecycline.

Trial 1:

Chromatographic conditions:

Column	:	Sunsil C18 150 mm x 4.6mm x 5µ
Mobile Phase	:	Methanol: Water [70:30]
Flow rate	:	0.8 ml/min
Detection	:	UV-Visible Spectrophotometer at 250 nm
Temperature	:	25°C
Injection Volume	:	10 µL
Run time	:	20 minutes
Pump Mode	:	Isocratic

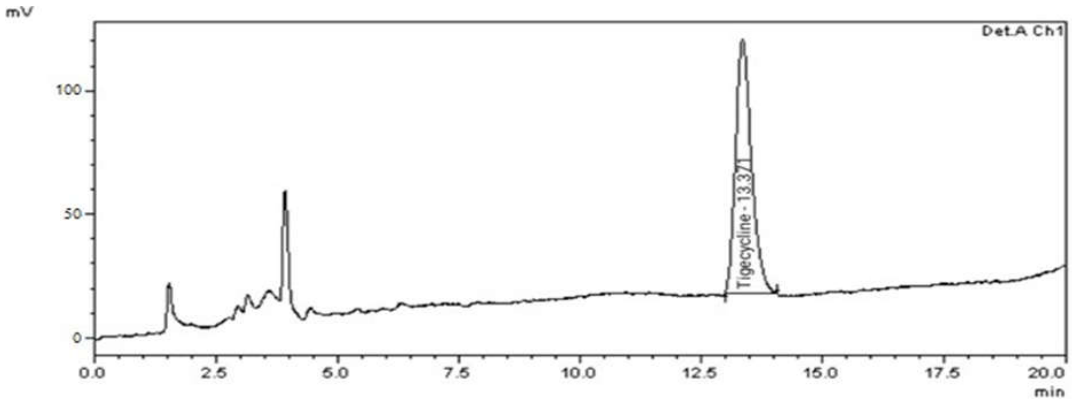

Fig. 3. Chromatogram of Trial – 1

Table 1. Trial- 1 Chromatogram Data

Ret. Time	Peak Area	Theoretical Plate count	Tailing Factor
13.371 mins	781779	3051.953	1.299

Inference: Improper baseline and bad peak shape were observed.

Trial 2:
Chromatographic conditions:

Column : Sunsil C18 150 mm x 4.6mm x 5µ
Mobile Phase : Acetonitrile: Water [50:50]
Flow rate : 0.8 ml/min
Detector : UV-Visible Spectrophotometer 250 nm
Temperature : 25°C
Injection Volume : 10 µL
Run time : 20 minutes
Pump Mode : Isocratic

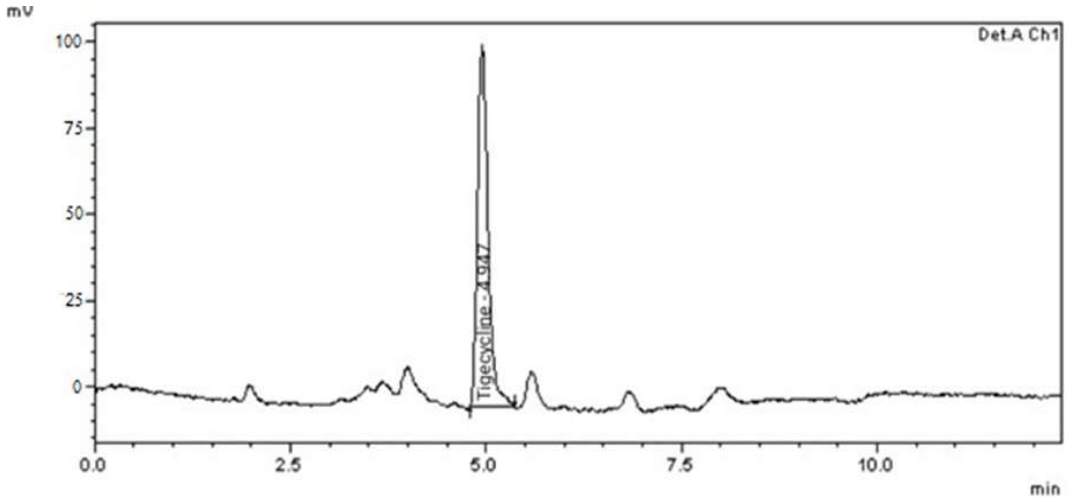

Fig. 4. Chromatogram of Trial – 2

Table 2. Trial- 2 Chromatogram data

Ret. Time	Peak Area	Theoretical Plate count	Tailing Factor
4.947 mins	701269	3375.749	1.346

Inference: Better peak shape compared to the initial trial but improper baseline and delayed retention time was observed.

Trial 3:
Chromatographic conditions:

Column	:	Sunsil C18 150 mm x 4.6mm x 5µ
Mobile Phase	:	Acetonitrile: Water [70:30]
Flow rate	:	0.8 ml/min
Detector	:	UV-Visible Spectrophotometer 250 nm
Temperature	:	25°C
Injection Volume	:	10 µL
Run time	:	20 minutes
Pump Mode	:	Isocratic

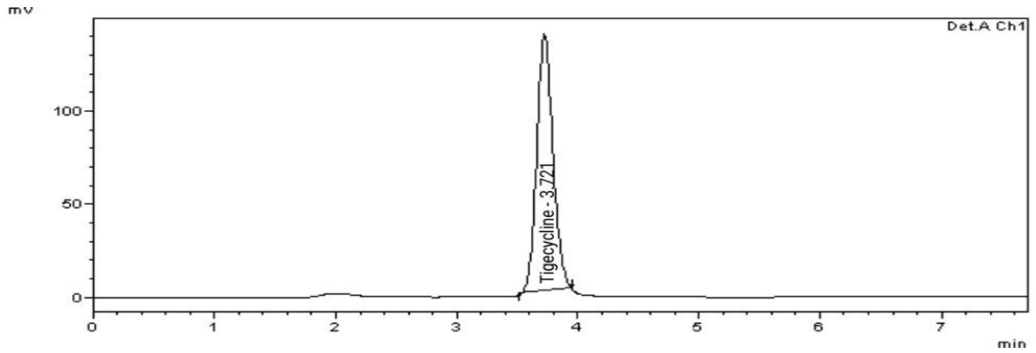

Fig. 5. Chromatogram of Trial – 3

Table 3. Trial- 3 Chromatogram data

Ret. Time	Peak Area	Theoretical Plate count	Tailing Factor
3.721 mins	941127	4326.174	1.243

Inference: Baseline was straight and also good peak shape was observed. Considering this, further method optimization was done.

6. METHOD OPTIMIZATION:

Optimized Chromatographic conditions:

Column	:	Sunsil C18 150 mm x 4.6mm x 5µ
Mobile Phase	:	Acetonitrile: Water (pH adjusted to 3.5 with Acetic acid) [70:30]
Flow rate	:	0.8 ml/min
Detector	:	UV-Visible Spectrophotometer 250 nm
Temperature	:	25°C
Injection Volume	:	10 µL
Run time	:	20 minutes
Pump Mode	:	Isocratic

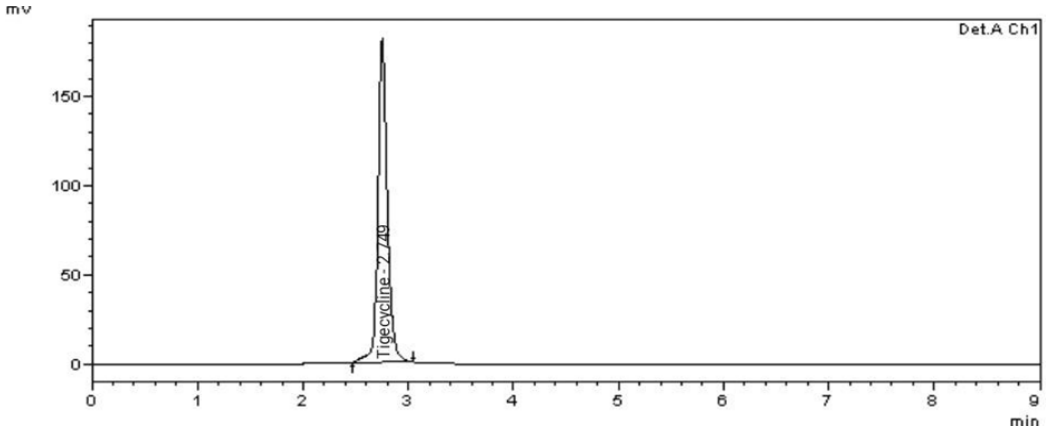

Fig. 6. Chromatogram of Optimized Trial

Table 4. Optimized trial chromatogram data

Ret. Time	Peak Area	Theoretical Plate count	Tailing Factor
2.749 mins	1121869	4191.541	1.351

Inference: Good peak shape and Rt were observed. Also system suitability parameters plate count and tailing factor were within the limits.

7. METHOD VALIDATION:

The developed method was validated according to ICH Guideline Q2 (R1). The following parameters were evaluated:

Specificity: It is the ability to assess the analyte unequivocally in the presence of other components which may be expected to be present. A blank (only diluent without drug) was injected into HPLC. No peaks were observed.

Linearity: Linearity was performed by injecting Tigecycline working standard solutions in the range of 5 to 40µg/ml in HPLC and response was recorded. Calibration curve was obtained by plotting concentration against respective peak area values. R^2 value was determined which was found to be 0.9995.

Precision: Precision was assessed by injecting six replicates of 10µg/ml Tigecycline standard solution, on the same day, and under the same experimental conditions. Peak area of six replicates of standard solution was obtained from chromatograms. % RSD was determined.

Accuracy: The accuracy of the proposed method was assessed by recovery studies. Tigecycline standard solution was spiked to sample solution at three concentration levels (50 %, 100 % & 150 %). Three replicates of each concentration level were prepared. These solutions were injected in HPLC and response was recorded. % Recovery was determined.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ were calculated according to ICH guidelines, where the factors 3.3 (for LOD) & 10 (for LOQ) were multiplied by the ratio of standard deviation (σ) and the slope obtained from calibration curve.

LOD (Detection Limit) = 3.3 σ/ Slope
LOQ (Quantitation Limit) = 10 σ/Slope

Table 5. Linearity data of Tigecycline RP-HPLC Method

Concentration	Peak Area
5 µg/ml	538764
10 µg/ml	1125843
15 µg/ml	1634598
20 µg/ml	2191785
25 µg/ml	2723598
30 µg/ml	3320164
35 µg/ml	3938749
40 µg/ml	4461695

Robustness: Robustness of the method was determined by injecting three replicates of 10 µg/ml Tigecycline standard solution by varying

the flow rate in chromatographic conditions at 0.8 ± 0.1ml/min and pH 3.5 ± 0.1. Chromatograms were obtained and % RSD was calculated.

System Suitability Test: Tigecycline working standard solution was prepared as per the procedure and five replicates were injected into the HPLC system. The system suitability parameters were evaluated from the obtained chromatograms by calculating the % RSD of Rt, peak areas, tailing factor and theoretical plates, which were found to be within range.

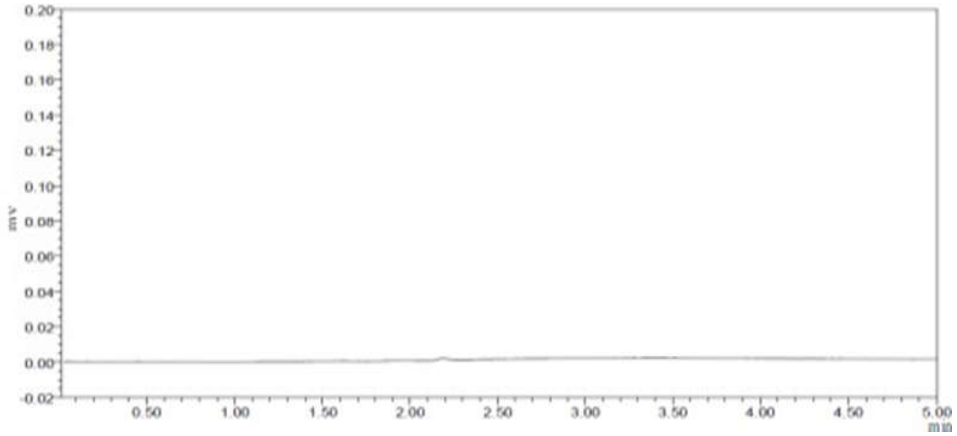

Fig. 7. Blank chromatogram

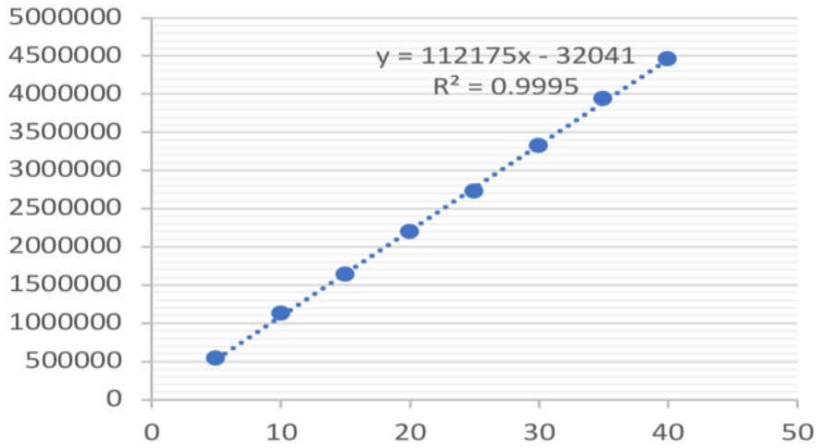

Fig. 8. Calibration curve of Tigecycline RP-HPLC Method

Table 6. Precision data of Tigecycline RP-HPLC Method

Injections	Peak Area	
	Intra Day Precision	Inter Day Precision
1	1125218	1082637
2	1094654	1134523
3	1121027	1112271
4	1098719	1086945
5	1089425	1079948
6	1109263	1094639
Mean	1106384.3	1098493.8
Standard Deviation	14569.6	19285.2
% RSD	1.31%	1.75%

Table 7. Accuracy Data of Tigecycline RP-HPLC Method

% Level	Sample	Standard Spiked	% Recovery	Mean % Recovery
			99.2	
			98.4	
50	5 µg/ml	2.5 µg/ml	100.4	99.3
			100.2	
			97.2	
100	5 µg/ml	5 µg/ml	98.6	98.6
			99.2	
			100.4	
150	5 µg/ml	7.5 µg/ml	98.6	99.4

Table 8. LOD & LOQ of Tigecycline RP-HPLC Method

Parameter	Value
LOD	0.1527 µg/ml
LOQ	0.4635 µg/ml

Table 9. Robustness Data of Tigecycline RP-HPLC method at 0.8 ± 0.1ml/min flow rate

Flow rate	Peak Area	Mean	S.D	% RSD
	998742			
	1013467			
0.8+0.1 ml/min	1026937	1013048.66	11514.36	1.136
	994395			
	1001724			
0.8-0.1 ml/min	1007398	1001172.33	5322.76	0.531

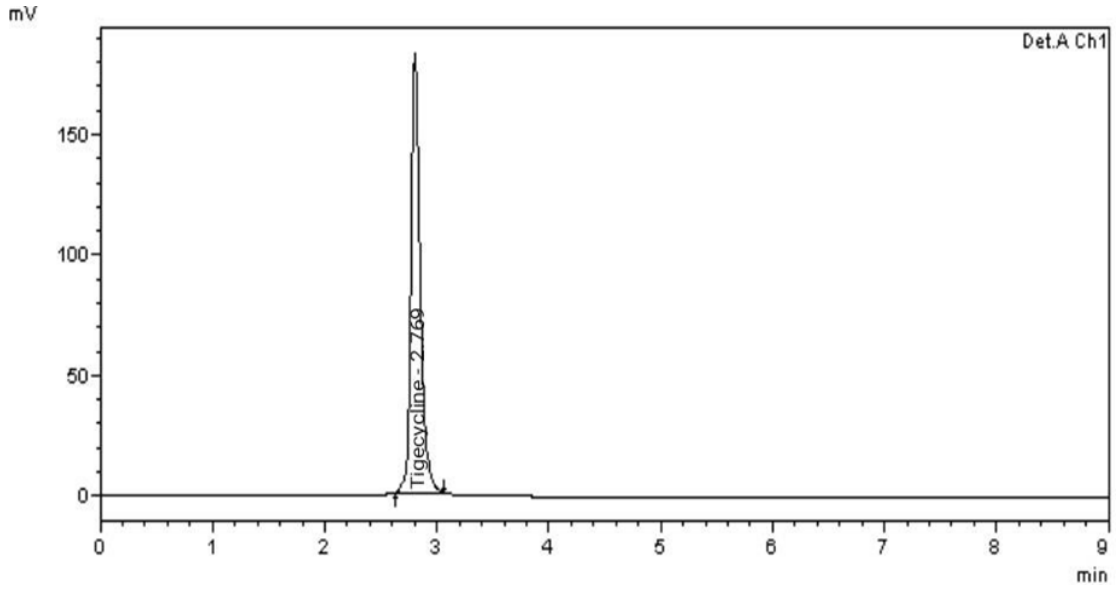

Fig. 9. Chromatogram of Tigecycline Sample Solution

%Assay= Peak area of sample/ Peak area of Standard x Concentration of Standard (µg/ml)/ Concentration of sample (µg/ml) x 100. %Assay = 1080610/1086330 x 10/10 x 100. %Assay = 99.47 %

Table 10. Robustness Data of Tigecycline RP-HPLC Method at pH 3.5 ± 0.1

Flow rate	Peak Area	Mean	S.D	% RSD
	996731			
	1014263			
pH 3.5 +0.1	992497	1001163.66	9422.52	0.941
	995134			
	997241			
pH 3.5-0.1	1011597	1001324	7314.85	0.730

7.1 ASSAY

Preparation of Tigecycline Sample Solution for RP-HPLC Method: Tigecycline lyophilized powder formulation equivalent to 10 mg was transferred into a 10 mL volumetric flask. It was dissolved in sufficient amount of mobile phase and sonicated. Then using mobile phase the volume was made up to the mark. The solution was filtered and from this 0.1 ml was pipetted out into a 10 mL volumetric flask and diluted using mobile phase. This solution was injected into HPLC and chromatogram was obtained. % Assay was calculated using the peak area.

8. CONCLUSION

A new, simple, rapid, precise and accurate RP-HPLC method was developed and validated as per the ICH guidelines. All the validation parameters were found to be within the limits. Therefore this method can be used for routine quality control tests of Tigecycline in bulk drug and pharmaceutical formulation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

Authors are thankful to Gland Pharma, Hyderabad for providing Tigecycline pure drug as a gift sample and management of RBVRR Women's College of Pharmacy for providing facilities to carry out this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Da Silva LM, Salgado HR. Tigecycline: A review of properties, applications, and analytical methods. Ther Drug Monit. 2010;32(3):282-288.
2. Chauhan AB, Patel DB. Area under the curve spectrophotometric method for determination of tigecycline in pharmaceutical formulation. J. Pharm. Sci. Biosci. Res. 2012;2(2):88-91.
3. Silva LM, Almeida AE, Salgado HR. Thermal analysis and validation of UV and visible spectrophotometric methods for the determination of new antibiotic Tigecycline in pharmaceutical product. Adv. Anal Chem. 2012;2:10-15.
4. Suneetha A, Priyanka GH, Sujitha J. Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Tigecycline in Bulk and its Parenteral Dosage Forms. WJPPS. 2017;6(8):1096-1107.
5. Kurien. J, Jayasekhar. P. Stability Indicating HPLC Determination of Tigecycline in Pharmaceutical Dosage Forms. IJPRS. 2013;2(4):164-171.
6. Da Silva LM, Salgado HR. Validation of a stability-indicating RP-LC method for the determination of Tigecycline in lyophilized powder. J. Chromatogr. Sci. 2013;51(2): 192-199.
7. Hua Xi, Wei Li. Determination of Tigecycline for injection with HPLC. Anhui Medical and Pharmaceutical Journal. 2009;9
8. Li C, Sutherland CA, Nightingale CH, Nicolau DP. Quantitation of tigecycline, a novel glycyclcycline, by liquid chromatography. J. Chromatogr. B. 2004; 811(2):225-229.
9. Zorpas KM, Valsami GN, Vryonis EV, Skoutelis AT, Archontaki HA. Robust and sensitive high-performance liquid chromatographic-UV detection technique

for the determination of tigecycline in rabbit plasma. J.AOAC Int. 2011;94(3): 847-856.

10. Ji AJ, Saunders JP, Amorusi P, Wadgaonkar ND, O'Leary K, Leal M, et al. A sensitive human bone assay for quantitation of tigecycline using LC/MS/MS. J. Pharm. Biomed. Anal. 2008; 48(3):866-875.
11. Mei S, Luo X, Li X, Li Q, Huo J, Yang L, et al. Development and validation of an LC□MS/MS method for the determination of Tigecycline in human plasma and cerebrospinal fluid and its application to a pharmacokinetic study. Biomed. Chromatogr. 2016;30(12):1992-2002.
12. Wang L, Hu X, Zhu H, Zhang X, Wang C, Han Z. Development and Validation of an LC–MS/MS Method for Determination of Tigecycline and its Epimer in Human Plasma and Its Application in a Pharmacokinetic Study. Acta Chromatogr. 2016;28(2):239-253.
13. Munyeza CF, Shobo A, Baijnath S, Bratkowska D, Naiker S, Bester LA, et al. Development and validation of a liquid chromatography□tandem mass spectrometry (LC□MS/MS) method for the quantification of tigecycline in rat brain tissues. Biomed. Chromatogr. 2016;30(6): 837-845.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/71722