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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP- HPLC METHOD FOR ESTIMATION OF TIGECYCLINE IN BULK AND ITS PARENTERAL DOSAGE FORMS

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ABSTRACT

The present method describes the development of a validated RP-HPLC method for determination of Tigecycline. Separation was carried out on a Kromasil ODS C-18 column (150×4.6mm, 5µ) using Buffer: Acetonitrile 83: 17 as mobile phase at a flow rate of 1.2 ml/min. UV detection was performed at 247nm. The method was validated with respect to specificity, selectivity, linearity, accuracy, precision and robustness. The assay method was found to be linear in the range of 80 to 120 µg/ml with a correlation coefficient of 0.9999. The percentage recovery of active pharmaceutical ingredient from parenteral dosage form ranged from 99.5 to 100.2%. The results showed that the developed RP-HPLC method is suitable for determination of Tigecycline in bulk as well as stability samples of pharmaceutical dosage forms containing various excipients.

KEYWORDS: High performance liquid chromatography, Tigecycline, Validation, Peak Area.

1. INTRODUCTION

Tigecycline is an antibiotic used to treat a number of bacterial infections.[1,2] It is a glycylcycline that is administered intravenously. It was developed in response to the growing rate of antibiotic resistance in bacteria such as Staphylococcus aureus, Acinetobacter baumannii, and E. coli. As a tetracycline derivative antibiotic, its structural modifications has expanded its therapeutic activity to include Gram-positive and Gram-negative organisms,

including those of multi-drug resistance. Tigecycline is marketed by Pfizer under the brand name Tygacil[3]. It was given a U.S. FDA [4] fast-track approval and was approved on June 17, 2005. Literature review was carried out to enumerate the reported analytical methods for the selected drug Tigecycline in Biological fluids & parenteral dosage forms. There are two methods reported so far using complexing agents for the estimation of drug in lyophilised powder form.

MATERIALS AND METHODS

Instrumentation

The present investigation was aimed to develop a liquid chromatographic method for quantitative estimation of Tigecycline using waters 2695 Separations module series HPLC[5,6,] instrument on a KromasilC_{18} column (150 mm x 4.6 mm, 5µ). The Instrument is equipped with variable wavelength UV-VISIBLE detector[7, 8]. Data was analysed by using Empower2 software. Shimadzu UV-Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

Preparation of Standard stock solution

Accurately weighed quantity of 50 mg Tigecycline was transferred to a 100 ml volumetric flask, dissolved in 60 ml of diluent, sonicated for 15 min and the volume was made up to the mark with diluent.

Preparation of working standard solutions

Working standard solutions of Tigecycline were prepared by diluting 4 ml, 4.5 ml, 5 ml, 5.5 ml and 6 ml of stock solution with the diluent in 25ml volumetric flask to get the concentrations of 80 µg/ml, 90 µg/ml, 100 µg/ml, 110µg/ml and 120 µg/ml of Tigecycline.

Preparation of Sample solution

For the preparation of sample solution 5 vials were taken and add 5ml of diluent to each vial and transfer the contents into 500 ml volumetric flask .Rinse each vial thrice with 5ml of diluent and transfer the contents into the flask .The contents of the flask were sonicated for about 20 min for complete solubility of the drug and the volume was made up to 500 ml with diluent. Then the mixture was filtered through 0.45µ membrane filter. It was further diluted and 10 µL was then injected six times into the column.

Method Development & Optimisation of Chromatographic Parameters

Selection of wave length: Sensitivity of an HPLC[9&12-15] method that uses UV detection depends up on the proper selection of wavelength. An ideal wavelength is one that gives good response for all the components to be detected. UV spectra of Tigecycline Figure-1 in the mobile phase was recorded and detection wave length i.e. 247 nm was selected for further analysis.

Optimized Chromatographic Conditions

Stationary phase	: Kromasil ODS C-18 column (150×4.6mm, 5µ)
Mobile phase	: Buffer: Acetonitrile = 83: 17
Flow-rate	: 1.2 ml/min
Injection volume	: 10µL
Detection wavelength	: 247nm
Temperature	: Ambient temperature
Run-time	: 14min

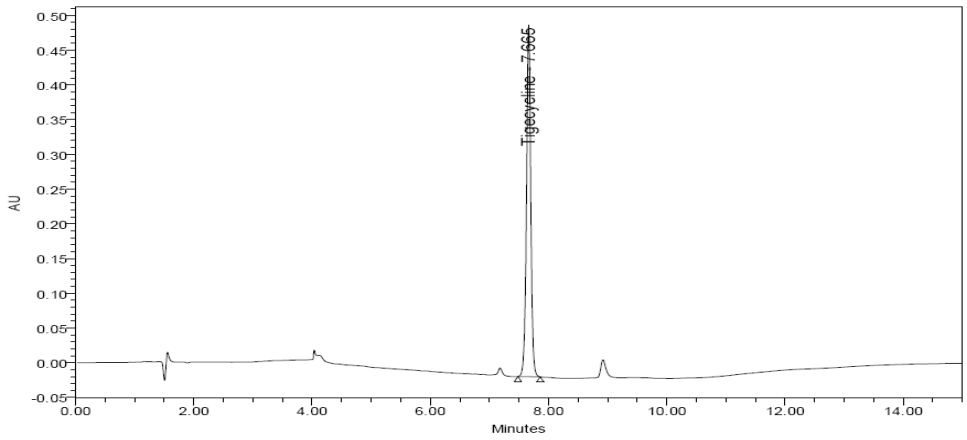

Figure 1: Chromatogram of Tigecycline under optimised conditions.

RESULTS AND DISCUSSIONS

Preliminary development trials have been performed under isocratic conditions with mixtures of solvents like acetonitrile, methanol and water, with buffers in different combinations and the method was opted under trail 5, which is incorporated.

Chromatographic conditions were optimized to obtain, an adequate separation of eluted compounds. Mobile phase and flow rate selection were based on peak parameters (Height, Theoretical plates, Tailing or Symmetry factor), run time and resolution.

After the optimization of method, estimation of Tigecycline in parenteral dosage form by the developed RP-HPLC method was carried out. The standard and sample solutions were prepared and the chromatograms were recorded in Figure2&3. The assay procedure was performed and percentage of Tigecycline in formulation was calculated and results are shown in Table 1.

Table 1: Summary of Assay Results By Hplc.

S.No	Drug name	Label claim	Amount found*	% Assay*
1.	Tigecycline	50 mg	50.05 mg	100.5%

*Average of Six determinations

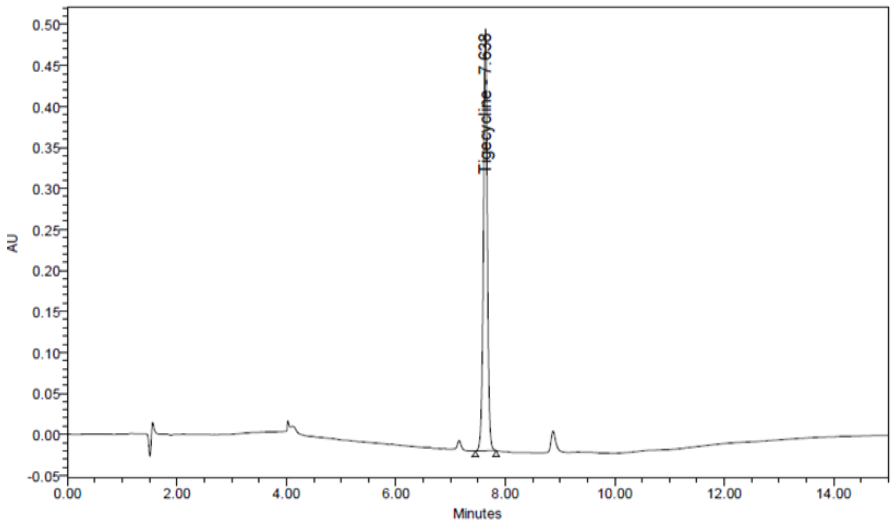

Figure 2: Standard Chromatogram of Tigecycline.

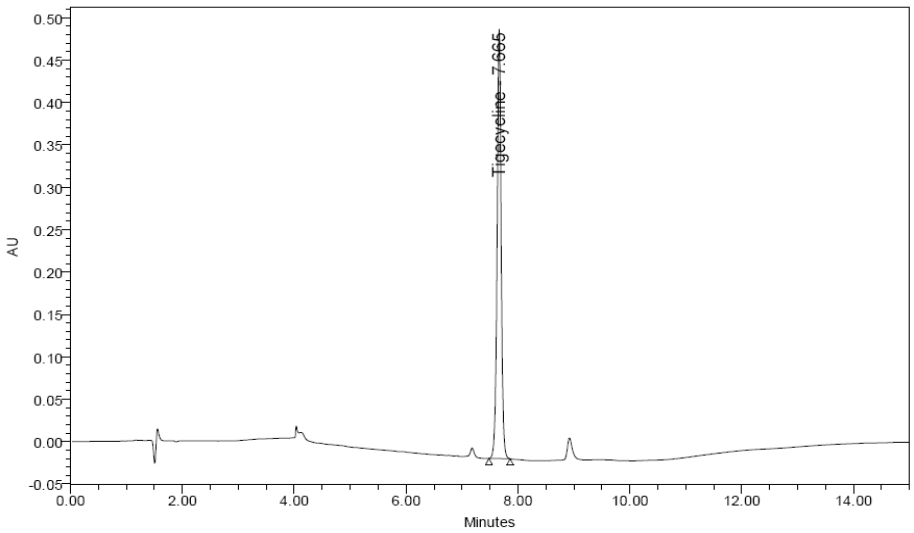

Figure 3: Test Chromatogram of Tigecycline.

Validation

After the method development, the method is validated in terms of parameters like linearity, LOD, LOQ, precision, accuracy, robustness, specificity and system suitability parameters as per ICH guidelines.[10, 11]

System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*R*T), number of theoretical plates (*N*), tailing factor (*T*), and peak asymmetry (*A*S), resolution (RS) was evaluated for six replicate injections of the drug. The system suitability test was performed using six replicate injections of standard.

Table 2: System Suitability Parameters.

Parameters	Tigecycline	Acceptance Criteria
USP Tailing	0.99	NMT 2.0
No. of theoretical plates	57616	NLT 15000
% RSD of Peak area	0.12	NMT 2.0
Retention time (min)	7.63	--

Specificity

The ability of the analytical method to measure the analyte free from interference due to other components. Specificity was determined by comparing test results obtained from analysis of sample solution containing ingredients with that of test results those obtained from standard drug. Chromatograms for standard &samples were recorded Figure 4 & 5 and they represent no interference.

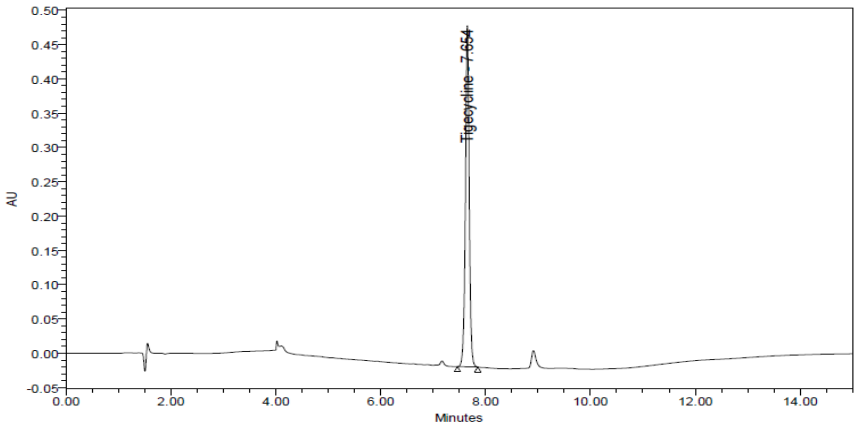

Figure 4: Standard Chromatogram of Tigecycline for Specificity.

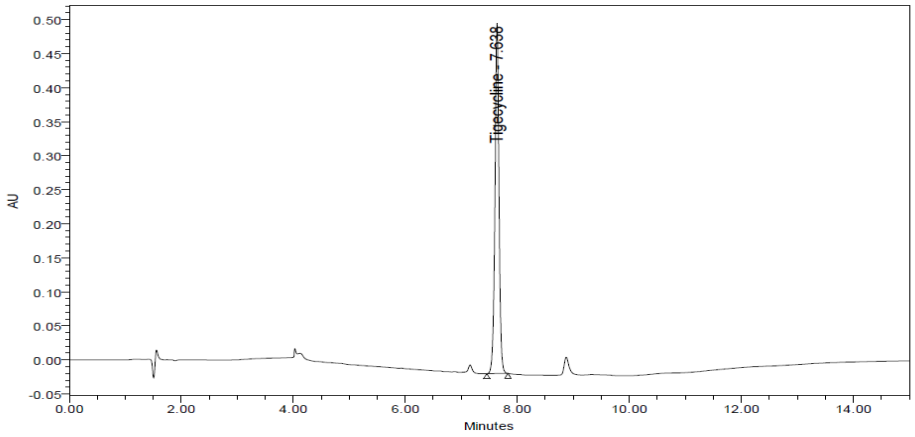

Figure 5: Sample Chromatogram of Tegicycline for Specificity.

3.1.3. LINEARITY

Linearity was performed by preparing standard solutions of Tigecycline at different concentration levels ranging from 80-120µg/ml. Ten microliters of each solution was injected into the HPLC system. The peak responses were measured at 247 nm and the corresponding chromatograms were recorded. The calibration curve was linear in the range of 80% - 120% for Tigecycline. The slope, intercept and correlation coefficient (r^2) were determined and shown in Tab-3 which are found to be within limits. Calibration curve for Tigecycline was shown in the Figure 6.

Table 3: Analytical performance parameters for Linearity.

S.No	Linearity level	Tigecycline	
		Concentration	Peak area
1.	Level-1	80	2038339
2.	Level-2	90	2300639
3.	Level-3	100	2564201
4.	Level-4	110	2814949
5.	Level-5	120	3066269
Linearity range (µg/ml)		80% - 120%	
Slope		25583x	
Correlation co-efficient (r^2)		0.999	

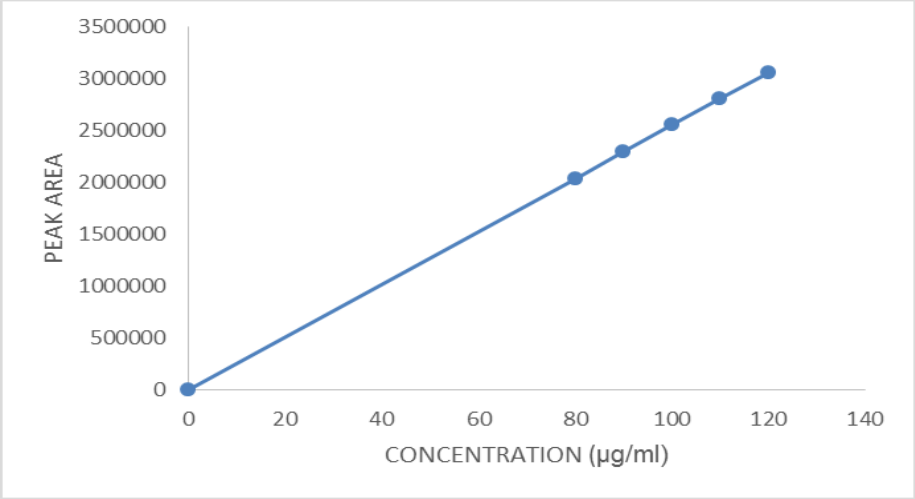

Fig 6: Calibration graph of Tigecycline.

3.1.4. ACCURACY

The sample solutions were analyzed in triplicate at 80%, 100%, 120% level by HPLC and resulting chromatograms were recorded. The percentage recovery was calculated and the results were presented in Tab.4.The results obtained were found to be within the limits which indicate that the method was accurate. The accuracy studies showed that the % recovery of the Tigecycline in the range 99.74-101.5%.

3.1.5. PRECISION

The precision of the method was demonstrated by System and Method precision studies. System precision studies were performed by injecting six (6) repeated injections of 100% concentration of standard solution. Peak area and %RSD were calculated and reported in Tab.5. Method precision studies were done by injecting six (6) repeated injections of 100% concentration of sample solution. Peak area and %RSD were calculated and reported in Tab.6. The % RSD values were less than 1, hence the method was found to be more precise.

Table 4: System Precision results for Tigecycline.

Sample	Conc. (in ppm)	Injection No.	Peak Areas	Mean	SD	%RSD (Acceptance criteria ≤ 2.0%)
Tigecycline	100	1 2 3 4 5 6	2568833 2564201 2538615 2545252 2540602 2564831	2553722	13664.98	0.53%

Table 5: Method Precision results for Tigecycline.

Sample	Conc. (in ppm)	Injection No.	Peak Areas	Mean	SD	%RSD (Acceptance criteria ≤ 2.0%)
Tigecycline	100	1 2 3 4 5 6	2502295 2494924 2492449 2503304 2510022 2513804	2502800	8281.65	0.33%

3.1.6. SENSITIVITY

The Sensitivity of measurement of Tigecycline by use of the proposed method was estimated in terms of the Limit of Detection (LOD) and the Limit of Quantitation (LOQ).

Limit of detection (LOD) and Limit of quantification (LOQ) were estimated from the signal-to-noise ratio. The LOD and LOQ were found to be 1.8 and 5.42µg/ml respectively.

Table 6: LOD and LOQ results for Tigecycline.

Sample	LOD (µg/ml)	LOQ (µg/ml)
Tigecycline	1.8	5.42

3.1.7 .ROBUSTNESS

Robustness of the developed method was demonstrated by purposely altering the experimental conditions. Robustness of method was carried out with variation of flow rate ±0.1 ml/min (Figure 7&8), mobile phase 2% (Fig.9&10) and detection wavelength ± 2 nm (Fig.11&12). The results were incorporated in Tab.7,8&9. It indicates that there was no effect on the results, hence the developed method is said to be more robust.

Table 7: Effect of flow rate.

Flow rate	Drug name	t_R (min)	Peak Area	Asymmetry	Efficiency
1.1ml/min	Tigecycline	7.666	2540602	0.99	57185
1.3 ml/min		7.639	2492449	0.99	57396

Table 8: Effect of mobile phase

Mobile phase	Drug name	t_R (min)	Peak Area	Asymmetry	Efficiency
Buffer/ACN-1 81/19	Tigecycline	7.020	2340602	0.99	52985
Buffer/ACN-2 85/15		7.689	2592449	0.99	57396

Table 9: Effect of detection wave length.

Detection wave length(nm)	Drug name	t_R (min)	Peak Area	Asymmetry	Efficiency
245 nm 249 nm	Tigecycline	7.638 7.659	2510022 2503304	0.99 0.9	57540 57616

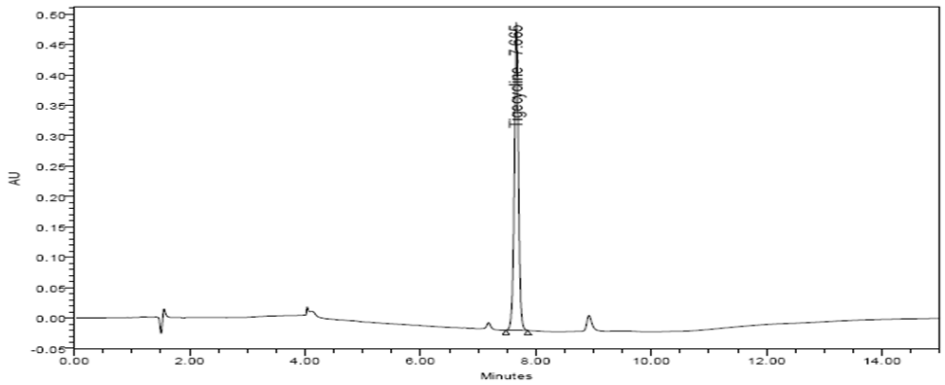

Fig.7: Chromatogram for effect of flow rate- 1.1ml/min.

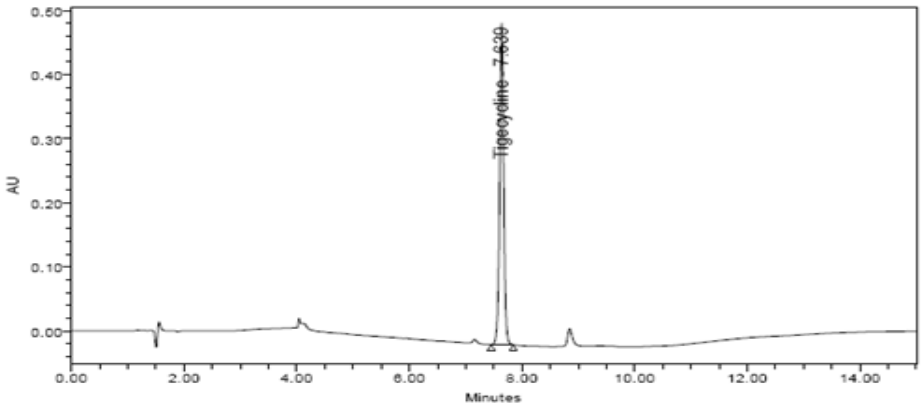

Fig 8: Chromatogram for effect of flow rate- 1.3 ml/min.

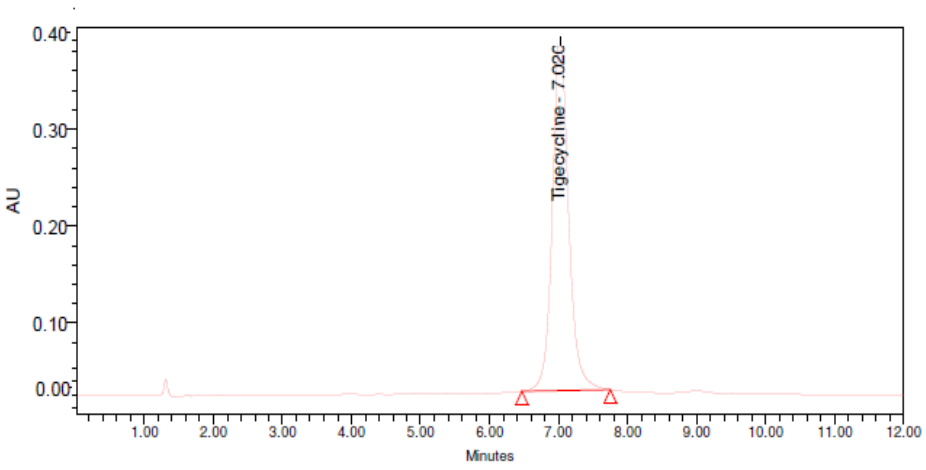

Fig.9: Chromatogram for effect of mobile phase-1.

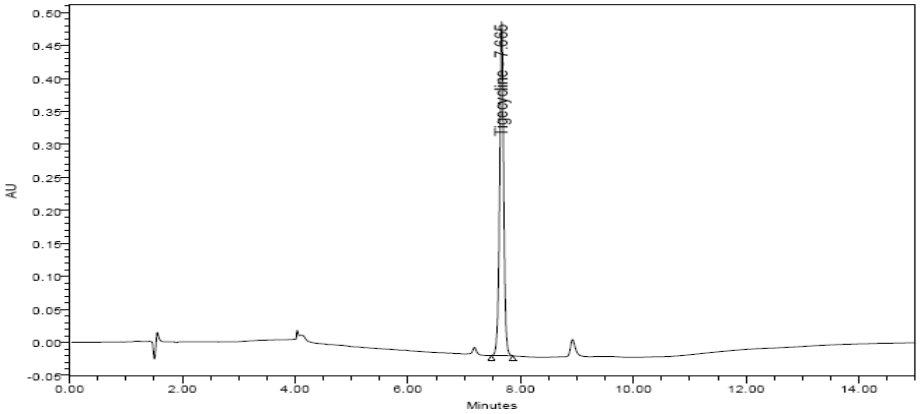

Fig 10: Chromatogram for effect of mobile phase-2.

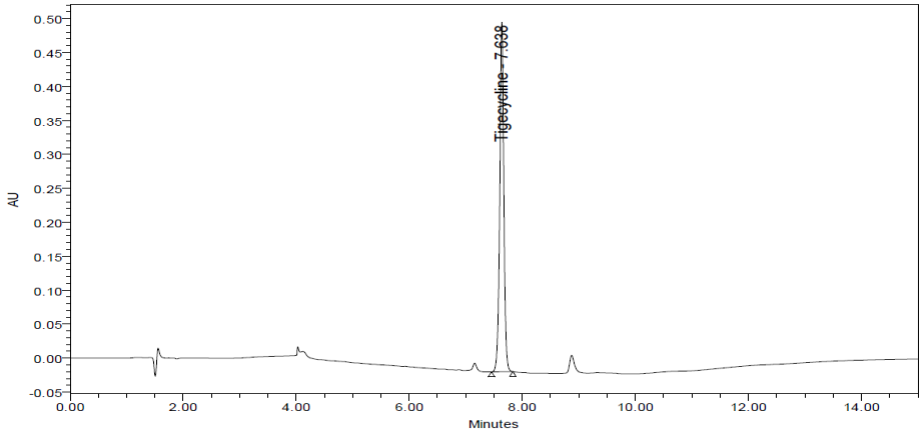

Fig 11: Chromatogram for effect of detection wave length (245nm).

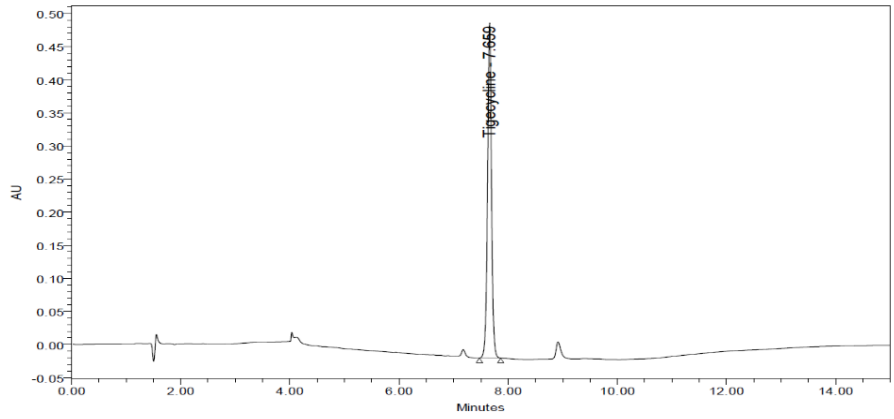

Fig 12: Chromatogram for effect of detection wave length (249nm).

4. CONCLUSION

The RP-LC method developed for the analysis of Tigecycline in their pharmaceutical preparations is simple, precise, and accurate. The method is useful for routine analysis due to short run time.

5. ACKNOWLEDGEMENT

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