JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Area Under the Curve Spectrophotometric Method for Determination of Tigecycline in Pharmaceutical Formulation

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ABSTRACT:

A simple, accurate, and precise Area under curve spectrophotometric method was developed for determination of Tigecycline in pharmaceutical dosage form.. The principle for AUC curve method is "the area under two points on the mixture spectra is directly proportional to the concentration of the component of interest". The area selected between 249 to 256 nm for determination of Tigeccycline. The drug follows Beer-Lambert's law over the concentration range of 4-20 µg/ml for Tigecycline. The % estimation of the drugs was found near to 100 % representing the accuracy of the method. The recovery of the Tigecycline found near to 100 %. Validation of the proposed method was carried out for its accuracy, precision, and specificity according to ICH guidelines. The proposed methods can be successfully applied in routine work for the determination of Tigecycline in its pharmaceutical dosage form.

Key words: Area Under Curve, Spectrophotometric method, Tigecycline, Validation.

Article history:
Received 22 March 2012
Accepted 31 March 2012
Available online 13 April 2012

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INTRODUCTION:

Tigecycline chemically is, (4S,4aS,5aR,12aS)-9-[2-(tert-butylamino)acetamido]-4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12atetrahydroxy-1,11-dioxo-2-naphthacene carboxamide **(Figure.1)**[1-5] **.**It is tetracycline antibiotic having class of glycylcycline derivative. It exhibits its antibacterial action by reversible binding to the 30S subunits of the ribosome, thus preventing the binding of aminoacyl tRNA and the incorporation of amino acids into the elongating peptide chains. Tigecycline is used in Complicated Skin and Skin Structure Infections, Complicated Intra-abdominal Infections, Community Acquired Pneumonia (mild to moderate infections only)[6-10].Literature survey revealed many analytical methods for its estimation. Tigecycline has been quantitatively assayed in biological fluids by HPLC and LC/MS/MS [11-19]. Determination of drug in pharmaceutical dosage form has been reported by the method that includes fluorimetry.

Literature survey revealed no spectroscopic methods reported for determination of the drug. The aim of the present work was to develop a simple, sensitive, accurate, and precise AUC method for routine analysis. The proposed method was validated according to ICH guidelines [20].

MATERIAL AND METHODS:

Chemicals

A standard sample of Tigecycline was obtained as gift from Zydus Pharmaceutical Technology Centre, Cadila Healthcare Ltd., Ahmedabad, Gujarat, India. Lyophilized form of injection of Tigecycline 50 mg/vial was procured from local pharmacy.

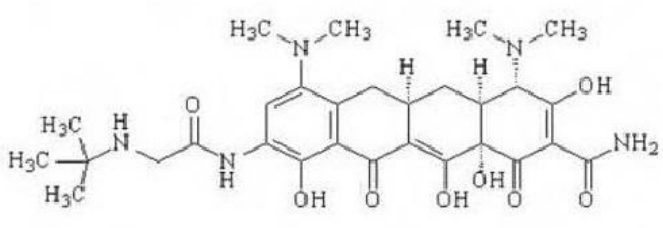

Figure 1: Chemical structure of Tigecycline

Methanol (S.D. Fine Chemicals, Mumbai, India) was used. All chemicals and reagents were of analytical reagent (AR) grade.

Instrumentation

A Shimadzu (Kyoto, Japan) model UV-1700 double beam UV-Visible spectrophotometer attached with computer operated software UV probe 2.0 with spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Sartorius CP224S analytical balance (Gottingen, Germany) and ultra sonic cleaner (Frontline FS 4, Mumbai, India) were used during the study.

Preparation of standard and Sample stock solution:

Accurately weighed portion of Tigecycline (10 mg) was transferred to a separate 100 mL volumetric flask, dissolved, sonicated and diluted to the mark with methanol to obtain standard solution having concentrations of Tigecycline (100 µg/mL).After proper dilution, 10 µg/ml of Tigecycline was scanned in the UV-region i.e. 400 to 200 nm. In UV – Spectrophotometric method two wavelengths 240 nm to 256 nm were selected for determination of Area Under Curve [AUC] of Tigecycline (**Figure 2).** For Sample solution, one vial of lyophilized form of Tigecycline injection was taken, (label claim =50 mg/vial) and reconstituted with 5 ml methanol. Then transferred to 50 ml volumetric flask and made up to mark with methanol. From above solution 10 ml was taken into 100 ml volumetric flask and made up to volume with methanol to get a final concentration 100µg/ml.

Study of linearity curves

Standard solution of Tigecycline (0.4, 0.8, 1.2, 1.6, and 2.0) was pipette out in to a separate series of 10 ml volumetric flask. The volume was adjusted to the mark with methanol and mixed. The area under curve for solutions was measured between 240 to 256 nm against methanol as blank. From using this area the 'X' value of the drug was determined at the selected AUC range.

Assay Procedure:

Pharmaceutical formulation of Tigecycline was purchased from local pharmacy. Sample Stock solution was prepared as described earlier. Appropriate dilutions were made with Methanol from stock solution for area under the curve spectrophotometric method.

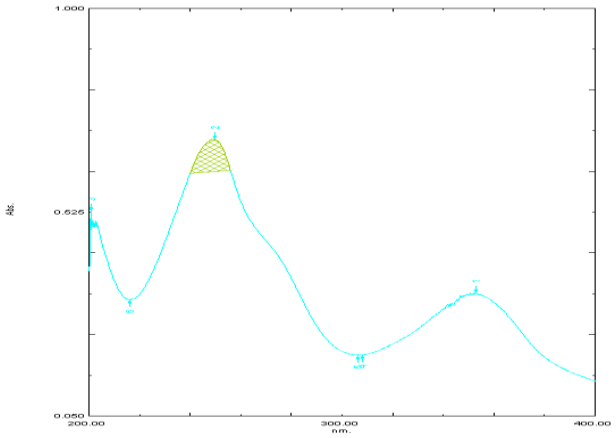

Figure 2: Wavelength range selected for AUC method of Tigecycline

RESULTS AND DISCUSSION

The area under the curve spectra for Tigecycline was recorded at the wavelength of 240-256 nm [Figure. 2].

Linearity and Range:

Under the experimental conditions described, the graph obtained for area under the curve spectra showed linear relationship **(Figure. 3).** Regression analysis was made for the slope, intercept and correlation-coefficient values. The regression equations of calibration curves were y = 0.0697x – 0.0246 (r^2 =0.9991) at 240-256 nm for area under the curve spectrophotometry. The range was found to be 4-20 µg/ml for area under the curve spectrophotometric method. **(Table 1)**

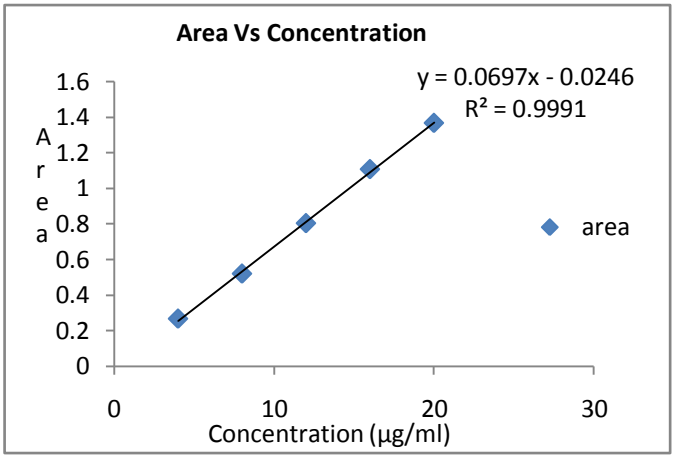

Figure 3: Calibration Curve of Tigecycline at 240 nm – 256 nm for Area Under Curve Method

Table 1: Regression analysis data for Tigecycline by the Area Under Curve method

Parameters	Area Under Curve
wavelength range(nm)	240 -256
Concentration range (µg/ml)	4-20
Slope (m)	0.0697
Intercept (c)	0.0246
Correlation coefficient (r^2)	0.9991

Table 2: Results of Intra and Inter Day Precision

Parameter	Inter- day Precision		Intra-day Precision	
	SD*	% RSD*	SD*	% RSD*
Area Under Curve	0.005	1.33	0.001	0.13

*n=6

Table 3: Data of recovery studies

Level of Mean Recovery (%)	% Mean Recovery	SD*	% RSD
50%	99.32	0.5755	0.5794
100%	99.69	0.5477	0.5494
150%	99.27	0.6818	0.6868

*n=3

Table 4: Assay results for the determination of Tigecycline in pharmaceutical formulation

Parameter	Label Claim (mg/vial)	Amount Found(mg/vial)	% lable claim (mg/vial)
Area Under Curve	50	49.70	99.39

Precision:

To determine the precision of the method, a Tigecycline solution at a concentration 12 µg/ml was analyzed each six times for area under the curve spectrophotometric method. Solutions for the standard curves were prepared fresh everyday **(Table 2).**

Sensitivity:

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3 x σ / S and LOQ = 10 x σ /S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.02 µg/ml and 0.08 µg/ml respectively for area under the curve method.

Recovery:

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of Tigecycline to reanalyzed solutions of commercial injectables **(Table 3.)**

Analysis of the Marketed Formulation:

There was no interference from the excipients commonly present in the injectables. The drug content was found to be 99.39 % for area under the curve spectrophotometric method. It may therefore be inferred that degradation of Tigecycline had not occurred in the marketed formulations that were analyzed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of Tigecycline in pharmaceutical dosage form **(Table 4).** The summary of the validation parameters is depicted in **(Table 5)**

Table 5: Summary of validation parameters

Parameters		**Area Under Curve**
Wavelength		240-256 nm
Concentration range(µg/mL)		4 - 20
Regression equation (*Y)		Y=0.0697X - 0.0246
Slop (m)		0.0697
Intercept (c)		0.0246
Correlation coefficient(r^2)		0.9991
LOD (µg /mL)		0.02
LOQ (µg /mL)		0.08
Accuracy (Recovery=3)%	Level-1	99.32± 0.57
	Level-2	99.69± 0.54
	Level-3	99.27± 0.68
Repeatability (RSD, n = 6)%		0.08
Precision (RSD)%		
Interday (n = 6)		0.18 – 1.33
Intraday (n = 6)		0.11 – 0.13

CONCLUSION

No any spectrophotometric methods have been described for the determination of Tigecycline. Therefore simple, fast and reliable Are Under Curve spectrophotometric method was developed for the routine determination of Tigecycline. The developed method can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

ACKNOWLEDGEMENT

The authors are thankful to managements of Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Mehsana for providing needed facilities for this work.

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