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Original Article

Method Development, Validation and Forced Degradation Studies for Determination of Tigecycline in Bulk and Pharmaceutical Dosage Form using UV Spectroscopy

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ABSTRACT

Objective: The present research work aimed to develop and validate a simple, rapid, accurate and reproducible UV Spectroscopic method for the quantitative estimation of Tigecycline in bulk and pharmaceutical dosage form. **Method:** The method was developed using Acetonitrile as diluent. The wavelength of maximum absorbance (λ_{max}) of Tigecycline was found to be 250nm. Validation of the developed method was performed according to International Conference on Harmonisation (ICH) guidelines on validation of analytical procedures: text and methodology Q2(R1). Assay of Tigecycline marketed formulation was performed and the amount of drug was determined. Forced degradation studies were performed by subjecting Tigecycline to stress conditions such as acid & base hydrolysis, oxidation, thermal degradation and photolysis. The degraded samples were further analyzed by using the developed method to determine the degradation behavior and the amount of Tigecycline degraded. **Results:** The method was found to be linear over the concentration range of 2-30µg/ml with correlation coefficient (r2) 0.999. The analytical method showed good precision with % Relative Standard Deviation (%RSD) below 2. All the other validation parameters such as accuracy, Limit of Detection (LOD) & Limit of Quantification (LOQ), robustness and ruggedness were found to be within the limits. The drug degraded more under thermal stress condition. **Conclusion:** The developed method is simple, rapid with accuracy and reproducibility therefore it can be applied for the routine analysis of Tigecycline in bulk and pharmaceutical dosage form.

Key words: Forced degradation, Tigecycline, UV Spectroscopy, Method development, Validation.

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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] Chemical structure of Tigecycline is shown in Figure 1.

Tigecycline inhibits protein translation in bacteria by binding to the 30S ribosomal sub-unit 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 tetracycline's. 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]

Literature survey reveals that there are only limited reported methods for analysis of Tigecycline using UV-Visible Spectrophotometry[3,4] and RP-HPLC.[5-8] Also there are few RP-HPLC[9,10] and LC-MS/MS[11-14] methods for analysis of Tigecycline in biological samples. The present research work describes the development and validation of a simple, rapid, accurate and precise UV Spectroscopic method for estimation of Tigecycline in bulk and pharmaceutical formulation. This method can also be extended to stress studies to determine the amount of drug degraded under different chemical and environmental conditions.

MATERIALS AND METHODS

Instruments

Elico SL 210 Double Beam UV- Visible Spectrophotometer with 1 cm matched quartz cells are used for performing spectrophotometric measurements and Labman sonicator is used for sonication of the sample solution.

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 Methanol and Acetonitrile were purchased form SD Fine Chemicals, Mumbai, India. Hydrogen Peroxide (H_2O_2), Hydrochloric acid (HCl) and Sodium Hydroxide (NaOH) used were of analytical grade.

Preparation of Standard Stock Solution

Accurately 10 mg of Tigecycline pure drug was weighed and transferred into a 10 ml volumetric flask. The volume was made up to the mark using Acetonitrile (ACN) resulting in 1000 µg/ml concentration primary stock solution. From this 1 ml aliquot was transferred into a 10 ml volumetric

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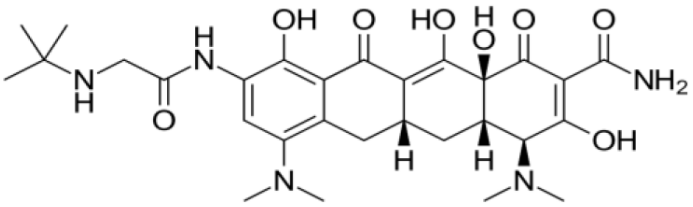

Figure 1: Chemical Structure of Tigecycline.

Table 1: Linearity.

Concentration	Absorbance
2	0.1445
4	0.2619
6	0.3986
8	0.5311
10	0.6749
12	0.8243
14	0.9632
16	1.1136
18	1.2251
20	1.3395
22	1.4786
24	1.5934
26	1.7042
28	1.8253
30	1.9786

flask and the volume was made up to the mark using ACN to obtain 100 µg/ml secondary stock solutions.

Preparation of Working Standard Solutions

From the secondary stock solution different aliquots in the range of 0.2 to 3.0 ml were transferred into series of 10 ml volumetric flasks and further diluted with ACN to obtain working standard solutions ranging from 2-30 µg/ml.

Determination of λ_{max}

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

RESULTS

Method Validation: The developed method was validated according to ICH Guideline Q2 (R1) Validation of analytical procedures: text and methodology. Parameters evaluated were linearity, accuracy, precision, Limit of Detection (LOD) and Limit of Quantification (LOQ), robustness and ruggedness.[15]

Linearity: Linearity was performed by measuring the absorbance of Tigecycline standard solutions in the range of 2 to 30µg/ml at 250nm. Calibration curve was obtained by plotting concentration against respective absorbance values. The observations and calibration curve are shown in Table 1 and Figure 3 respectively.

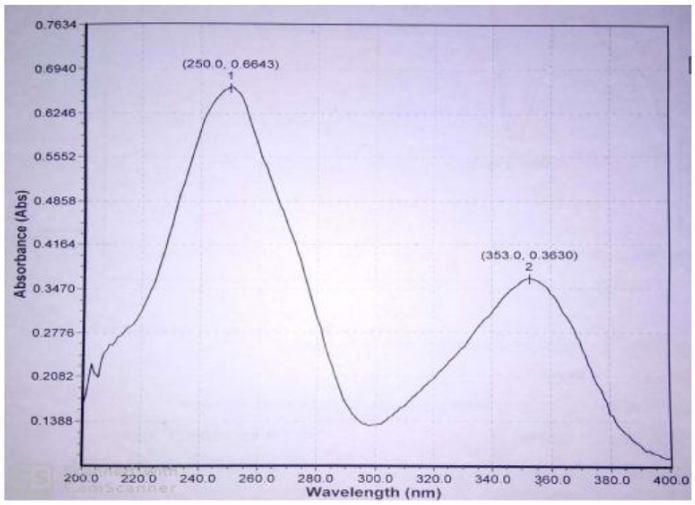

Figure 2: UV Spectrum for determination of Tigecycline $\lambda_{max.}$

Table 2: Accuracy.

% Level	Concentration (µg/ml)		Absorbance	% Recovery	Mean % Recovery
	Test	Standard			
50	5	2.5	0.5389	98.08	98.32
			0.5396	98.21	
			0.5421	98.67	
100	5	5	0.7422	99.19	99.40
			0.7455	99.63	
			0.7437	99.39	
150	5	7.5	0.9381	98.90	99.17
			0.9415	99.26	
			0.9424	99.35	

Table 3: Precision.

S.No	Absorbance
1	0.6778
2	0.6813
3	0.6785
4	0.6742
5	0.6726
6	0.6739
Mean	0.67638
S.D	0.00305
% RSD	0.00451

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 % and 150 %). Three replicates of each concentration level were prepared and absorbance was measured at 250nm. % Recovery was determined. Table 2 shows accuracy data of Tigecycline.

Precision: Precision was examined by measuring the absorbance of six replicates of the same concentration Tigecycline standard solution, on the same day and under the same experimental conditions. Absorbance

of 6 replicates of standard solution was measured at 250 nm and % RSD was calculated. Precision data of Tigecycline is shown in Table 3.

$$\% \text{ RSD} = \text{Standard Deviation/Mean} \times 100$$

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) and 10 (for LOQ) were multiplied by the ratio of standard deviation (σ) and the slope. LOD and LOQ values are given in Table 4.

LOD (Detection Limit) = 3.3 σ/ Slope

LOQ (Quantitation Limit) = 10 σ/Slope

Robustness: It was determined by measuring the absorbance of three replicates of the same concentration Tigecycline standard solution by varying the wavelength i.e. at 250 nm ± 1 nm. % RSD was calculated and reported in Table 5.

Ruggedness: Ruggedness of the method was determined by measuring the absorbance of six replicates of the same concentration Tigecycline standard solution at 250 nm by two different analysts and % RSD was calculated and presented in Table 6.

Assay

Tigecycline lyophilized powder formulation was weighed and powder weight equivalent to 10 mg was transferred into a 10 ml volumetric flask. After dissolving in sufficient amount of ACN the solution was subjected to sonication. The volume was made up to the mark using ACN. From this 0.1 ml was pipetted out into a 10 ml volumetric flask and diluted using ACN. Absorbance of this solution was measured at 250 nm. % Assay was calculated and was found to be 99.6 %.

Table 4: LOD and LOQ.

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

Table 5: Robustness.

λ_{max}	Absorbance	Mean	S.D	% RSD
	0.6427			
249 nm	0.6382	0.6423	0.00092	0.00134
	0.6459			
	0.6683			
250nm	0.6724	0.6702	0.00246	0.00313
	0.668			
	0.6573			
251 nm	0.6508	0.6524	0.00038	0.00055
	0.6492			

Table 6: Ruggedness.

S. No	Analyst I	Analyst II
1	0.6713	0.6864
2	0.6893	0.6726
3	0.6892	0.6822
4	0.6876	0.6836
5	0.6824	0.6869
6	0.6785	0.6756
Mean	0.6797	0.6804
S.D	0.0091	0.0071
% RSD	0.0134	0.0104

Forced Degradation Studies

Forced degradation is the process of subjecting drug to extreme chemical and environmental conditions. Forced degradation studies show the chemical behavior of the molecule and provide an approach to analyze the stability of drug. It provides an insight into degradation pathways and degradation products of the drug substance and helps in elucidation of the structure of the degradation products. Forced degradation studies are done as per the ICH guidelines: ICH Q1A: Stability Testing of New Drug Substances and Products and ICH Q1B: Photo Stability Testing of New Drug Substances and Products. Degradation studies are performed under acidic, basic hydrolysis, oxidation, thermal and photolytic conditions.[16-20]

Acid Degradation

From 100µg/ml Tigecycline standard solution 1 ml was pipetted out in to a 10 ml volumetric flask. To this 1 ml of 0.1 N HCl was added and allowed to stand for 24 hr. Then the solution was neutralized by adding 1 ml of 0.1 N NaOH and the volume was made up to the mark using ACN. Absorbance of the solution was measured at 250 nm and amount of drug degraded was calculated.

Alkali Degradation

From 100µg/ml Tigecycline standard solution 1 ml was pipetted out in to a 10 ml V.F. To this 1 ml of 0.1 N NaOH was added and allowed to stand for 24 hr. Then the solution was neutralized by adding 1 ml of 0.1 N HCl and the volume was made up to the mark using ACN. Absorbance of the solution was measured at 250 nm and amount of drug degraded was calculated.

Oxidative Degradation

From 100µg/ml Tigecycline standard solution 1 ml was pipetted out in to a 10 ml V.F. To this 1 ml of 3 % H_2O_2 solution was added and allowed to stand for 24 hr. Then the volume was made up to the mark using ACN. Absorbance of the solution was measured at 250 nm and amount of drug degraded was calculated.

Thermal Degradation

Tigecycline drug was exposed to 40°C temperature in a hot air oven. After 4 hr required amount of drug was taken and 10µg/ml solution was prepared using ACN as diluent. Absorbance of the solution was measured at 250 nm and amount of drug degraded was calculated.

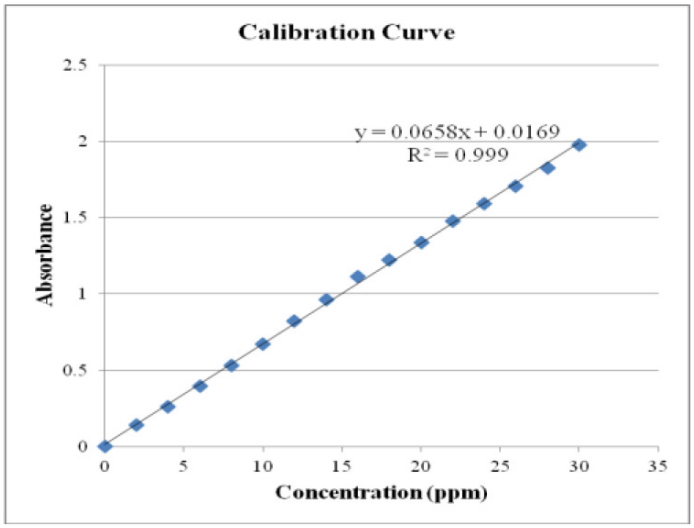

Figure 3: Calibration Curve for Tigecycline.

Table 7: Forced Degradation Data of Tigecycline.

Degradation Condition	% of Drug Degraded
Acidic hydrolysis (0.1 N HCl)	5.87
Basic hydrolysis (0.1 N NaOH)	3.39
Oxidation	7.17
Thermal	12.71
Light	10.27

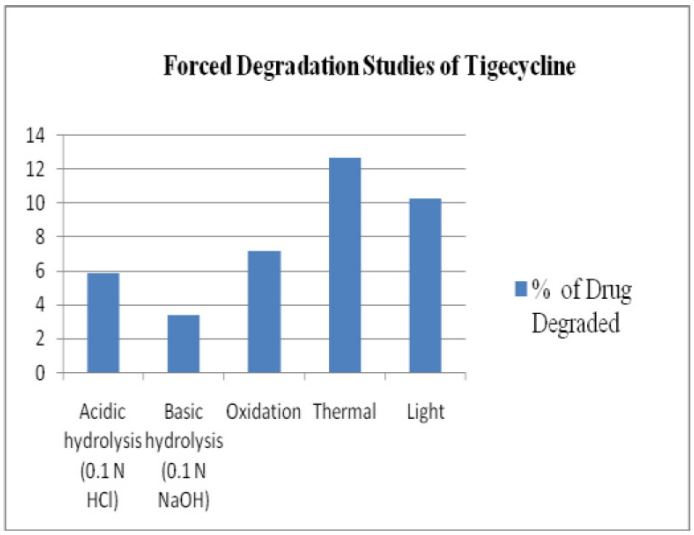

Figure 4: Forced Degradation Studies.

Photolytic Degradation

Tigecycline pure drug was exposed to UV light for 4 hours then required amount of drug was taken and 10µg/ml solution was prepared using ACN as diluent. Absorbance of the solution was measured at 250 nm and amount of drug degraded was calculated.

Graphical representation of the Forced degradation studies data and the observations are shown in Figure 4 and Table 7 respectively.

CONCLUSION

A simple, rapid, accurate and reproducible UV Spectroscopic method was developed and validated as per the ICH guidelines. The method was used for assessing the degradation behavior of the Tigecycline under different stress conditions as per ICH guidelines. Results show that the drug undergoes most degradation under thermal conditions. 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.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest.

ABBREVIATIONS

ACN: Acetonitrile; **ICH:** International Conference on Harmonisation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **%RSD:** % Relative Standard Deviation; **H_2O_2:** Hydrogen Peroxide; **HCl:** Hydrochloric acid; **NaOH:** Sodium Hydroxide.

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