Research Article

UV Spectrophotometric Method Development and Validation for Estimation of Tigecycline in Spiked Human Plasma

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ABSTRACT

A new, rapid, precise and accurate UV Spectrophotometric method was developed for determination of Tigecycline in spiked human plasma. The analyte was extracted by protein precipitation method using Acetonitrile. Absorbance of the analyte extracted was measured at 250 nm using UV-Visible Spectrophotometer. % Recovery of the method was determined. The developed method was validated for linearity, accuracy, precision, robustness. The method was found to be linear in the range of 2 to 10 □g/ml with correlation coefficient (r2) 0.999. All validation parameters were within the acceptable range. Therefore, the developed method can be used for routine bio analytical estimation of Tigecycline.

Keywords: Acetonitrile, Bio analytical, Human Plasma, Protein Precipitation, Tigecycline, UV Spectrophotometer.

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] Figure 1 shows chemical structure of Tigecycline.

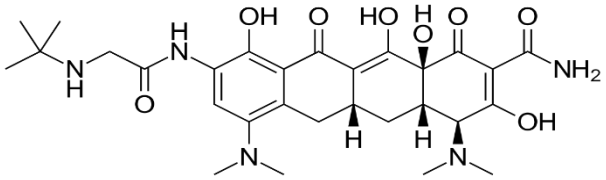

Figure 1: Chemical Structure of Tigecycline

Tigecycline binds to the 30S ribosomal subunit and interferes with the entry of amino-acyl tRNA molecules into the A site of the ribosome, which inhibits protein translation in bacteria. This blocks incorporation of amino acid residues into elongating peptide chains, thereby preventing protein synthesis and eventually bacterial cell growth. Compared to Tetracyclines, Glycylcyclines appear to bind more effectively. Tigecycline carries a glycylamido moiety attached to the 9-position of minocycline. The substitution pattern is not present in any naturally occurring or semi-synthetic tetracycline and imparts certain microbiologic properties to Tigecycline. It shows 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]

From literature survey it is evident that there are several quantitative analytical methods for determination of Tigecycline in various biological samples such as human bone, human skin, human serum, human plasma, cerebrospinal fluid, rabbit plasma, rat brain tissues, turkey plasma and rabbit aqueous humor/vitreous humor/plasma etc[3-9]. These methods include RP- HPLC[3], LC-MS/MS[4-9] which are complex and expensive to use in conventional bio analytical laboratories. Therefore, it was decided to develop and validate an alternate simple, rapid, precise and accurate method using UV Spectroscopy for determination of Tigecycline in spiked human plasma.

MATERIALS AND METHODS

Chemicals and Reagents

Tigecycline pure drug was obtained as a gift sample from Gland Pharma, Hyderabad, India. Blood was collected from individuals not taking drug. Plasma was separated and refrigerated. Acetonitrile used in the study was of HPLC grade purchased from SD Fine Chemicals, Mumbai, India.

Instruments

The instrument used was Elico SL 210 Double Beam UV-Visible Spectrophotometer with silicon photo diode detector. The data acquisition was done on Spectratreats software. Other equipments used in the study was REMI R-8C centrifuge and vortex mixer.

Preparation of Standard Solutions

Accurately 10 mg of Tigecycline was weighed and transferred in to a 10ml volumetric flask and dissolved in Acetonitrile (ACN). The volume was made up to the mark using ACN to produce 1000 µg/ml stock solution. From this stock solution 0.02, 0.04, 0.06, 0.08 & 0.1 ml aliquots were pipetted out and transferred into individual 10 ml volumetric flasks and the volume was made up to the mark with ACN to get working standard solutions with concentrations of 2, 4, 6, 8 & 10 µg/ml respectively.

Determination of Working Wavelength (λmax)

The λmax (wavelength of maximum absorption) of Tigecycline was determined by scanning 10 µg/ml solution using UV Spectrophotometer within the wavelength region of 220 to 400 nm against ACN as blank. Tigecycline showed characteristic absorption maxima at 250 nm. The absorption curve is shown in Figure 2.

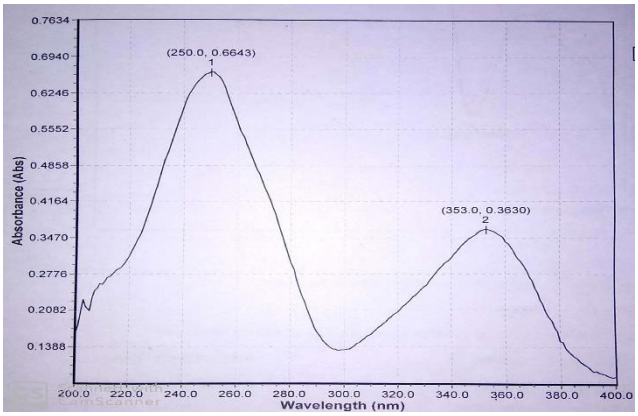

Figure 2: UV Spectrum of Tigecycline

Procedure for Extraction of Tigecycline from Spiked Human Plasma

Protein precipitation method was used for extraction of Tigecycline from spiked human plasma. Plasma sample was thawed at room temperature and from this 1 ml of plasma was placed in polyethylene tubes with conic bottom. Tigecycline standard solution 1 ml was spiked to plasma. To this solution 2 ml of Acetonitrile was added for protein precipitation. Obtained solution was vortex mixed for 30 seconds and subjected to centrifugation at 15000 rpm for 10 minutes at 4°C. The supernatant was collected and evaporated to dryness. The residue obtained was reconstituted in ACN (diluent) and absorbance was measured at 250 nm using UV Spectrophotometer. % Recovery was determined using the absorbance values of Tigecycline extracted from spiked human plasma and Tigecycline standard solution.

RESULTS

Validation

The method validation was done as per the FDA guidelines. The linearity, accuracy, recovery, and robustness parameters were tested.

Linearity

Linearity of the method was determined by analyzing five standard solutions covering the range of 2 to 10µg/ml. Absorbance of the standard solutions was measured at 250 nm using ACN as blank. Calibration curve was plotted by taking concentration against absorbance. The linearity data is presented in Table no1 and calibration curve is shown in Figure no 3.

Table 1: Linearity Data of Tigecycline

Concentration (µg/ml)	Absorbance
0	0.1445
2	0.2619
4	0.3986
6	0.4707
8	0.5311
10	0.6749

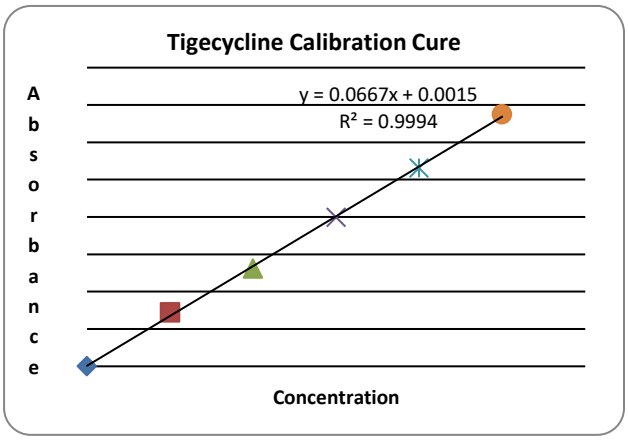

Figure 3: Calibration curve of Tigecycline

Accuracy

The accuracy of the method was determined by calculating the recoveries of Tigecycline by the standard addition method at three levels. Known amounts of standard solutions of Tigecycline were added at 50%, 100% & 150% concentration to pre quantified sample solution of Tigecycline and the amount of drug recovered was estimated. Each level has been analyzed in triplicate. Results are shown in Table 2. The recovery was found to be in between the predefined acceptance criteria of 80.0–120.0%.

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.

% RSD = Standard Deviation/Mean X 100

% Recovery (Extraction Efficiency)

% Recovery should be evaluated for methods that employ sample extraction. Recovery is reported as the % of the known amount of an analyte carried through the sample extraction and the processing steps involved in the method. Recovery is determined by comparing the analyte response in a biological sample that is spiked with analyte and processed, with the response of standard analyte solution. Recovery of analyte does not need to be 100 % but the extent of recovery should be consistent. % Recovery was found to be 89.54%.

Table 2: Accuracy Data of Tigecycline

Concentration added	%Recovery	Mean %Recovery
50%	88.08	88.75
	88.91	
	89.27	
100%	87.19	87.76
	87.63	
	88.46	
150%	89.95	89.33
	88.59	
	89.47	

Table 3: Precision Data of Tigecycline

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

DISCUSSION

Tigecycline was extracted from human plasma by protein precipitation method using Acetonitrile as it has maximum extraction efficiency (% Recovery) compared to other solvents like perchloric acid, formic acid- methanol, trichloroacetic acid etc. Figure 3 shows Tigecycline % Recovery from human plasma in different analytical instruments.

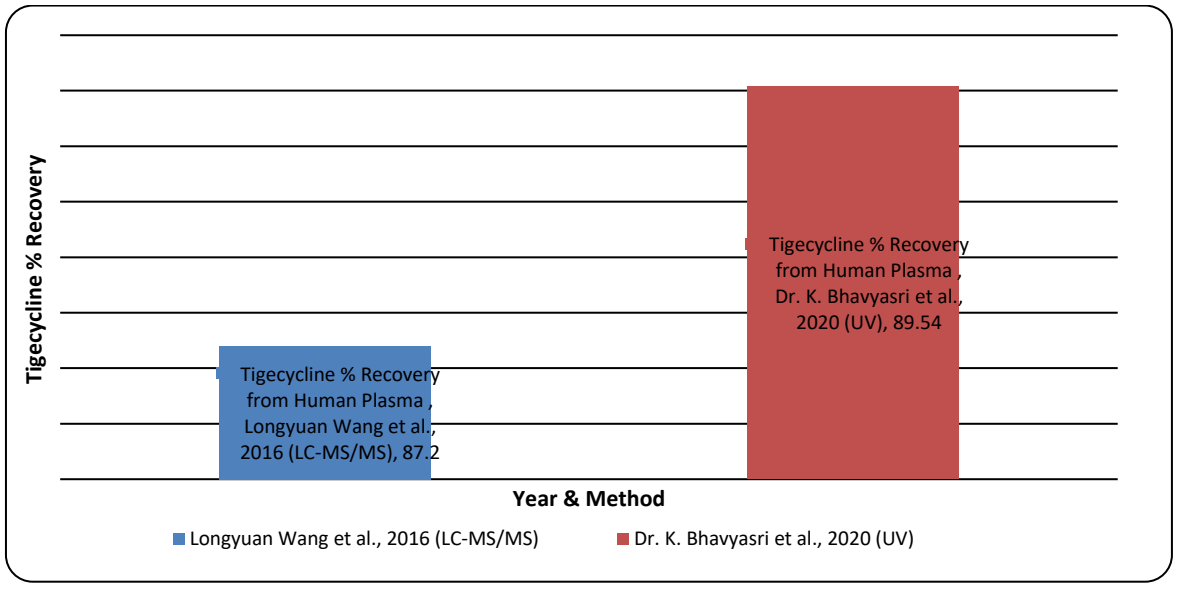

Figure 3: Tigecycline % Recovery from Human Plasma in Analytical Instruments

CONCLUSION

A new, rapid, precise and accurate protein precipitation process with UV Spectrophotometric measurement was developed and validated for analysis of Tigecycline in spiked human plasma. The results demonstrated that the developed method has good recovery. Therefore, this technique is useful for quantitative analysis of Tigecycline in biological samples and could be used in routine analysis for most of bio analytical purposes.

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Conflict of Interest: None declared.