RESEARCH ARTICLE

Chemometric Assisted UV-Spectrophotometric Quantification of Tigecycline in Parenteral Dosage Form

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ABSTRACT

Relating to the current study, the quality by design (QbD) concept is used for creating and validating an unique, resilient, accurate, and reliable spectrophotometric approach to quantify Tigecycline (TIG) in injections. Fractional factorial design (FFD) was a design implemented to screen the initial parameters. Moreover, the variables went through the central composite design (CCD) to assess the dependency and optimize the design. Several measures were analyzed statistically to determine the appropriateness of the data obtained from the experiments. At 250 nm, by the use of ethanol, TIG displays an absorption maximum. Variables like screening, slit-width, and sampling interval were recognized as critical method and again, evaluation was done by a CCD. A good linearity was produced for TIG within a range of 2 to 12 µg/mL, with R2 less than 0.999. The process was determined to be perfect, having a good average percent recovery (greater than 100%). According to ICH guidelines, validation of the developed method was performed. By the implementation of QbD principles, spectrophotometric techniques were created and planned to, integrate the qualities into the methods. These processes were manifested for being flexible and pertinent for identifying TIG in pharmaceutical dose regimens.

Keywords: Tigecycline, Spectrophotometric, Quality by Design, Validation, Central Composite Design, Fractional Factorial Design.

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INTRODUCTION

The IUPAC name of TIG, (4S,4aS,5aR,12aR)-9-[[2-(tert-butylamino)acetyl]amino]-4,7- bis(dimethylamino)-1,10,11,12a-tetrahydroxy-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide (Figure 1) is a polyketide antibiotic with a broad-spectrum produced by the actinobacteria species *Streptomyces*, similar to tetracycline.[1-3] The mechanism of action involves the reversible attachment with the bacterial ribosome containing 50-S ribosomal subunit, which inhibits the linking with incoming Aminoacyl t-RNA with the ribosomal acceptor site, resulting in a bacteriostatic effect. Moreover, it partially attaches with the bacterial ribosome containing 50S ribosomal subunit and can alter the cytoplasmic membrane, releasing intracellular components from bacterial cells.[4] It is employed for the management of intense infections affecting the body, such as skin infections, infections within the abdomen, and pneumonia caused by bacteria acquired in the community. Its mechanism of action involves eradicating the bacteria responsible for causing these ailments.[5-7]

TIG obtained as parenteral dosage forms along with original samples is evaluated by implementing LC-MS, UV-visible spectroscopy, HPLC methods.[8-11] Although, the recorded UV spectrophotometric method has some limitations such as absence of Sandell's sensitivity, narrow linearity range, and inability to satisfy molar extinction coefficient (ε) etc. Hence, many efforts were performed to develop an advanced and unique method of UV spectroscopy to quantify TIG in parenteral dosage form through QbD approaches.

QbD is combined access ensuring standard integration throughout the procedure to get the planned report. As per ICH-Q8-(R2), QbD is a methodical access to the final product advancement which starts along with a predetermined purpose

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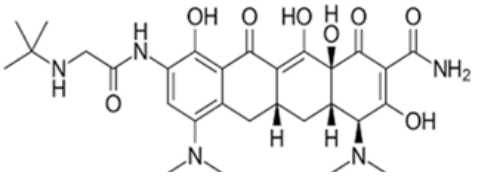

Figure 1: Chemical structure of TIG

and prioritizes understanding the elements and procedure as well as process control, built on trustworthy scientific principles in addition to control of risks.[12] The USFDA established Pharmaceutical Current Good Manufacturing Practices (cGMPs) over 21st century in 2002, which led to the discovery of QbD.[13] Analytical QbD (AQbD) contains six stages of comprehensive development of an analytical method with improved performance and strong resilience.[14]

Utilizing the QbD methodology reduces the duration necessary for creating an effective analytical technique and is also deemed an economical manner of guaranteeing quality at the outset, belonging to the method development perspective. Design of experiment (DoE) is the essential component of QbD that supplies a stable model area of the best performance of the method. This current investigation focuses on utilizing rational experimental designs to decrease variability in the spectrophotometric measurement of TIG. The goal is the identification of optimal solutions. In the beginning, factor screening research utilizing FFD was done to identify crucial technique parameters that affect performance. Method optimization, utilizing the CCD, was done afterward to ensure robustness and accomplish predetermined goals. The goal of the research is to build a novel, appropriate, and exact UV spectrophotometry method for the quantification of TIG available injectables while validating existing procedures related to ICH requirements.[15]

MATERIAL AND METHOD

Standard and Reagent

A pure standard TIG drug (purity < 99.5%) has been received as a gift sample from Ultrakind Biotech Pvt. Ltd., India. Ethanol was procured from Merck Ltd., Jamshedpur, India and employed in purpose of preparation of drug solution and reagent solution. As the retailed parenteral dosage form of TIG (50 mg) was available in the local market it was procured and analyzed by the currently developed method.

Optical Characteristics and Instrumentation

Single beam microprocessor UV-visible spectrophotometer LI-285 (Lasany, India) with ten millimeter matched quartz cuvettes were employed to measure the spectrums. The reagents were weighed using a chemical balance with high precision. The parenteral formulation's ability to dissolve was influenced by ultrasonication (Enertech, India).

Setup of an Analytical Target Profile

Properly reviewing the current literature surveys and medication profiles (physicochemical properties) were conducted to develop a targeted configuration for assessment, providing an advanced overview of classification attributes a procedure analytically. These were essentially entailed the creation of a quick, dependable, and profitable analytical procedure to estimate TIG in the parenteral dosage form. A UV-spectrophotometric approach for quick examination of TIG was chosen depending on the primary goal of this novel research. The reason for choosing the UV spectrophotometric methods was because of uncomplicated and speedy drug analysis compared with other complex techniques analytically.

Cause-effect Relationship Establishment as well as Risk Management

The diagram of the Ishikawa fishbone, is the simplest tool for understanding the cause-effect relationship between probable method factors, which might influence the presentation of the procedure. Regarding the above-mentioned matter, Ishikawa diagram was depicted (undepicted of the illustration) by accentuating various procedural factors that could potentially impact the method characteristics of TIG UV spectrophotometry. In the ongoing investigations, the needy factors influencing analytical qualities were found using a cause-effective relationship, risk assessment matrix, and CNX (Control-Noise-Experimentation) technique. Variations in the solvent utilized, scan speed, detection wavelength, sampling interval, slit width, and sample integrity were identified, being critical method variables (CMVs), linked to high final scores and high-risk variables. The most required method parameters for the CMVs were also assessed with the assistance of a screening model before being submitted utilising an appropriate experiment method to have response surface optimization.

Analysis of Essential Method Variables through FFD

This model (Design Expert 11, Version-11.0.4.0, USA) was used for analyzing the essential parameters to find the high risk variables. A few factors were chosen as essential method variables by analyzing spectrum structure, accuracy, and absorbance. Furthermore, the CMVs were estimated by the assistance of a design screened for building up the critical method parameters (CMPs), and the response surface was optimized, implementing an appropriate experimental methodology. Speed of scanning (X1), width of the slit (X2), and sample interval (X3), otherwise, were analyzed using Design expert software through FFD consisting of no fewer than five trials (One being a centre point). The parameters were examined at both their maximum and minimum values, and the programme was implemented to discover the crucial parameter values that influence the absorbance response variable (Y). Examining the actual against expected values plot, fitting summary plot, Pareto chart, and prediction equation yielded significant parameters.

Method Optimization and Robustness Study by using CCD

The usage of CCD ensured the potency of the procedure for determining optimal method conditions.[16] Thirteen trial runs were acquired with at least five centre points depending on CCD for optimization of CMVs, such as slit width (A)

and sample interval (B), as determined by investigations screened. Observations of the experimentation were analyzed in assistance of absorbance at 250 nm as the response variable. A standard TIG of 10 µg/mL was employed for all of the experiments.

The reported results of the experiment were fixed with a preferable model mathematically using multiple linear regression analysis (MLRA) via design expert software. Advanced models were permitted to research every important impacts and impact of interactions. Single coefficients of design terminologies reported being remarkable $p < 0.05$, according to ANOVA analysis, were determined in formatting the polynomial equation, as well as analyzing the modeling factors, such as comparison between the actual and predicted plot, fit summary, ANOVA following estimation of factors such as coefficient of correlation (R^2), predicted and adjusted R^2, predicted residual sum of squares (PRESS), in sequence manner. In addition, the remaining critical factors such as interaction profiler, prediction profiler, and 3D response surface profiler, were utilized for the determination of the suitability of the design. The most effective resolution was examined by utilizing a numerical desirability function which involved balancing the analyzed variables to achieve the desired outcomes. This was then marked within the designated space of the design region.[17]

A Strategic Planning for the Method Control

Method control, planned strategically, was established depending on the space produced by DoE assessment, where little changes during performing the method were permitted to maintain the method's robustness.

Standard Stock Solution Preparation

The TIG (1000 µg/mL) standard stock solution was formulated by dissolution of exactly weighed 10 mg of TIG with ethanol up to 10 mL. From the above prepared stock solution, 5 mL of the stock solution was incorporated in a 50 mL volumetric flask and diluted up to 50 mL to create a standard solution with a concentration of 100 µg/mL.[18]

Analysing Parenteral Dosage Formulation

The labeled claim for TIG injection is 50 mg (Tigebax 50 mg/ Glenmark Pharmaceutical Ltd.). As per the instruction on the label, 1-mg TIG should be mixed with 0.2 mL of water for injection. The solution was prepared and from this 2 mL (weight equivalent to 10 mg) was taken in a 10 ml volumetric flask and made up to 10 mL with ethanol to produce 10 mg/10 mL solution. The content was ultrasonicated for 30 minutes. This prepared solution was again strained by the use of Whattmann filter paper for the removal of particulate matter, if any. The stained mixture solution was again diluted with ethanol for analysis purposes. The use of a calibration curve of standard TIG obtained the presence of active pharmaceutical ingredients within the sample solution.

Specificity

The perticularity of the procedure of UV spectrophotometry had estimated depending on the entity's assessment and its formulation excipients. Spectrum was estimated for possible interference as a reason of additives.

Linearity

Different tubes were considered using the TIG working standard solution in different 10 mL volumetric flasks and diluted using ethanol to produce a group of concentrated limiting from 2–12 µg/mL. At 250 nm, UV absorbance was determined. The calibration curve was scattered to evaluate the linearity by interpreting the absorbance on the y-axis and concentrated (µg/mL) on the x-axis.

Precision and Accuracy

Searching the clarity of the procedure, recovery studies were performed with 80,100 and 120% of the test concentrated (10 µg/mL) of TIG, by standard addition method. The recovery studies were established triplication occurs at each individual step. Standard drug (TIG), mixed to the recovery solution, intended by implementing calibration curves. A total of 6 replicants of a particular concentrated TIG (10 g/mL) were scanned on the same day to estimate intraday precision, and percent RSD values were determined.[19]

RESULTS AND DISCUSSION

In the above research, a method of UV spectrophotometry has been established to assess the quantity of TIG available within the parenteral dosage form. QbD approaches were utilized for reporting variable factors in the advancement of ultimate spectrophotometric conditions. A standard Ishikawa fishbone diagram was established to identify the variables in the method. Physical evaluation of the design variables were performed. Medication was being estimated to be insoluble in acetone or ether. However, TIG was dissolved in ethanol. Therefore, ethanol was chosen as an appropriate solvent system for future research. Standard TIG solution interpreted absorption maxima (λ_{max}) at 250 nm through ethanol (Figure 2) and was chosen such as detection wavelength.

The sample characteristics were satisfied according to the tested melting point. Although, the method variables such as sampling interval (SV), scanning speed (SS) and slit width (SW) required an investigation systematically to establish the impacts on the robustness of the method. Applying FFD approach assisted in CMVs scanning out of scanning speed,

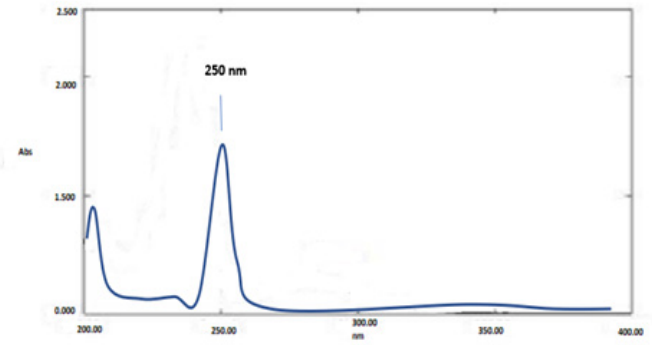

Figure 2: TIG - Standard UV absorption spectrum

slit width, and sampling interval. The evaluation of design via predicted vs actual plots displayed the apt fitness of the preferred method. Model *p*-value (0.0010), R^2 (0.9198) and RMSE (0.0001) also preferred model aptness. Estimating the fit summary displayed predicted R^2 (0.4297) and adjusted R^2 (0.8625) values.

The design CCD was implemented for estimating the CMVs impression on response absorbance. A Total of ten experiments were randomly carried out using a UV-vis spectrophotometer rand The response acquired regarding every experiment and range studied spectrophotometrically are listed below. (Table 1)

Proper evaluation of CCD model implementing varieties of analytical tools statistically were performed and observations were considered by ANOVA, a factor estimating the prediction profiler.

In Figure 3A, perturbation plots for projected models are shown to get the influence of distinct components on a provided response while maintaining every factor fixed at an initial point of reference. The steepest inclination or curve shows the affectability of the feedback to a particular factor, in Figure 3A it was being obtained, the sampling interval (factor B) had the most required effect on absorbance, followed by slit width. Figure 3B accompanies baseline design (blue Points) among the actual vs predicted plot, where the line for the data attained from the experiment was recorded as being good within the range or confines the assurance interims. It refuses the H_0, as the variation in data described by the model effectively, at which the assumed and attained report were reported to be quite equivalent.

Response surfaces plots for slit width and sampling interval are interpreted in Figure 4 (slit width is plotted against the sampling interval). Analyzing optimized models' response

Table 1: Experimental design matrix showing spectrophotometric range studied for robustness study and obtained responses

Run No	*Slit Width (A)*	*Sampling Interval (B)*	*Absorbance (Y)*
1	2	0.5	0.27
2	1.25	1.25	0.25
3	0.18934	1.25	0.22
4	2	2	0.3
5	1.25	2.31066	0.212
6	1.25	1.25	0.25
7	1.25	1.25	0.25
8	1.25	1.25	0.25
9	2.31066	1.25	0.29
10	1.25	1.25	0.25
11	0.5	0.5	0.201
12	1.25	0.18934	0.21
13	0.5	2	0.213
Range	Low	High	
	0.5	2	
	0.5	2	

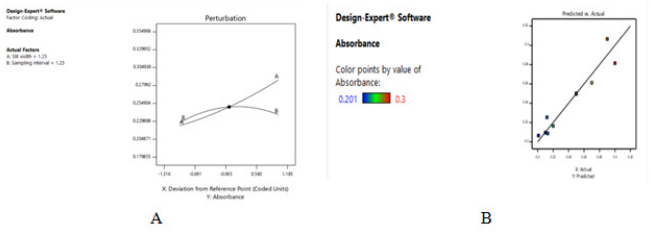

Figure 3: A) Perturbation plot, B) Predicted vs. Actual plot

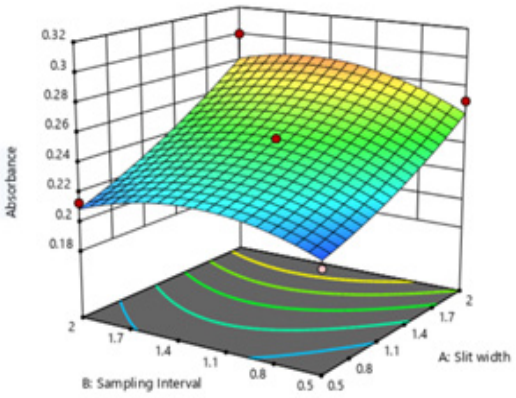

Figure 4: 3-D response surface plot for absorbance against slit width vs. sampling interval

plots and perturbation plots uncovered that factor had a huge response on the analyte absorbance. Further, ANOVA recommended probability value is smaller than 0.0010, indicating the perfectness of dummy addressing the variability and suggesting rejection of the H_0. Aside from, the smaller values for PRESS also ratified the perfectability of the dummy. Factors evaluation assessment is critical for estimating the variable risk among various variables. An obtained probability value smaller than 0.05, prefer a non-zero value obtained by the slope.

Sampling interval × sampling interval (B2) and slit width(A) were found to be the most influencing method variables.

$$Absorbance\,(Y) = 0.2500 + 0.0319A + 0.0056B + 0.0045AB + 0.0058A^2 - 0.0162B^2$$

where, A = Slit Width, B = Sampling Interval.

The characteristics of the optical spectrophotometric methods has tabulated in Table 2. The methods established were reported particularly as selective as the generally applied dosage form. Additives available within the parenteral formulation were observed non-interfere the estimation procedure. Pharmaceutical entities were linear, directing a concentrated limit within 2–12 µg per mL. Regression analysis of linearity results displayed perfect fit overall. The results acquired for factors statistically like R^2, adjusted R^2 and predicted R^2 were observed to be 0.9198, 0.8625 and 0.4297, sequentially. ANOVA preferred the perfectness of the

Table 2: Optical characteristics as well as summary of validation parameters

Parameters	*Obtained values*
Wavelength (nm)	250
Linearity Range (µg/ml)	2–12
Sandell's sensitivity (µg/cm2 /0.001AU)	0.047
Molar extinction coefficient (ltr/ mol.cm)	$0.1129 * 10^{-2}$
Regression equation (Y = ax + b)*	0.0209x + 0.0007
Correlation coefficient (R^2)	0.999
Precision (%RSD., n = 6)	0.3269711
Accuracy (%Recovery ± S.D.)	
80%	1.618378 ± 0.006128
100%	0.387884 ± 0.001633
120%	0.88943 ± 0.004082
% Range of error	
95% confidence limits	± 0.032
99% confidence limits	± 0.042

RSD – Relative standard deviation; S.D. – Standard deviation; AU - Absorbance units,* is Y= ax + b, where Y = absorbance, a = slope, b = intercept and x is the concentration, † is average of three determinations at each level

procedure ($p < 0.05$), regarding linearity data. The percent recovery or improvement of the parenteral dosage formulation were reported and observed to be 100.1% (S.D = ± 0.085, n = 6). In case of accuracy study, mean recovery is limited from 99.6–100.5%. The %RSD was achieved below 2%, in case of intra-day determination, displaying a great extent of exactness of the predicted procedure. Report of the procedure occurred between the prescribed limit, displaying that the process has no interference of additives.

CONCLUSION

A QbD methodology was used to establish a reliable method of UV spectrophotometry method for TIG quantification. Using the QbD procedure the analytical quality of procedure is being confirmed. There were two influential CMVs, slitwidth and sampling interval, those require specific consideration by the analyst while performing the method controls strategically and further experiments regarding continuous development in performance of the method. The reports prefer the research is unique, specific, exact and to-the-point. Statistically investigations of validating the procedure reports prefer the perfectness of the advanced methods in implementing in the quality control laboratory. These methods are appropriate for determination of TIG in parenteral formulation without any obstructions from generally known additives. Henceforth, the design must be employed in regular evaluation purposes.

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