RP-HPLC Method Development and Validation for Determination of Tigecycline in Bulk and Pharmaceutical Dosage form

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To develop and validate a new, simple, rapid, precise and accurate Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for the quantitative determination of Tigecycline in bulk and pharmaceutical dosage form.

Study Design:
Place and Duration of the Study: RBVRR women’s college of pharmacy, Barkatpura, Hyderabad, between June 2019 and July 2020.

Methodology: The RP-HPLC method was developed on Sunsil C18 150 mm x 4.6mm x 5µ column using acetonitrile : water (pH maintained at 3.5 with acetic acid) [70:30] as mobile phase at flow rate 0.8 ml/min and UV detection at 250 nm.

Results: Tigecycline exhibited linearity over the concentration range of 5-40 µg/mL (R2 > 0.999). The analytical method showed good precision with % RSD below 2. The method showed suitable accuracy and robustness.

Conclusion: Validation of the developed method was done as per International Conference on Harmonization (ICH) Q2R1 guidelines.

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1. 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,5,6,11,12a-octahydrotetracene-2-carboxamide [1].

Tigecycline inhibits protein translation in bacteria by binding to the 30S ribosomal subunit 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 tetracyclines. 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 [2-3].

The present research work describes the development and validation of a simple, rapid, accurate and precise RP-HPLC [4-13] method for estimation of Tigecycline in bulk and pharmaceutical formulation.

2. MATERIALS AND METHODS

2.1 Instruments

Shimadzu HPLC (LC-20AD Multi-solvent delivery system, SPD-20A UV-Visible detector, LC solution software). Labman sonicator was used for sonication of the sample solution. Thermo scientific pH meter was used to measure pH. Vacuum pump filter was used for filtration of mobile phase solvents and they were provided by RBVRR women's college of pharmacy, barkatpura, Hyderabad, India.

2.2 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 water, methanol, acetonitrile and glacial acetic acid were purchased from SD Fine Chemicals, Mumbai, India.

3. CHROMATOGRAPHIC CONDITIONS

The isocratic mobile phase consisted of Acetonitrile: Water (pH adjusted to 3.5 with Acetic acid) [70:30], flowing through the column at constant flow rate 0.8 ml/min. Sunsil C18 column (150 mm x 4.6mm x 5µm) was used as the stationary phase. 250 nm was selected as the detection wavelength for UV-Visible detector.

4. PREPARATION OF TIGECYCLINE STANDARD SOLUTIONS FOR RP-HPLC METHOD

4.1 Preparation of Standard Stock Solution

Accurately 10 mg of Tigecycline standard drug was weighed and transferred into a 10 mL volumetric flask. The volume was made up to the mark using the mobile phase resulting in 1 mg/mL concentration primary stock solution. From this, 1mL was pipetted out and transferred into a 10 mL volumetric flask and diluted to obtain 100 µg/mL secondary stock solution.

![Fig. 1. Chemical structure of tigecycline](image-url)
4.2 Preparation of Working Standard Solutions

From the secondary stock solution 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 & 4.0 mL aliquots were transferred into series of 10 mL volumetric flasks and further diluted with mobile phase to obtain 5, 10, 15, 20, 25, 30, 35 and 40μg/mL working standard solution.

4.3 Determination of λmax

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

Fig. 2. UV Spectrum for determination of Tigecycline λmax

5. RESULTS AND DISCUSSION

5.1 RP-HPLC Method Development

Based on the drug solubility and pKa value the following chromatographic conditions have been selected to initiate the method development trials for determination of Tigecycline.

Trial 1:

Chromatographic conditions:

| Parameter            | Specification                      |
|----------------------|------------------------------------|
| Column               | Sunsil C18 150 mm x 4.6mm x 5μ     |
| Mobile Phase         | Methanol: Water [70:30]            |
| Flow rate            | 0.8 ml/min                         |
| Detection            | UV-Visible Spectrophotometer at 250 nm |
| Temperature          | 25°C                               |
| Injection Volume     | 10 μL                              |
| Run time             | 20 minutes                         |
| Pump Mode            | Isocratic                          |
Table 1. Trial-1 Chromatogram Data

| Ret. Time | Peak Area | Theoretical Plate count | Tailing Factor |
|-----------|-----------|--------------------------|---------------|
| 13.371 mins | 781779 | 3051.953 | 1.299 |

Inference: Improper baseline and bad peak shape were observed.

Trial 2:
Chromatographic conditions:

Column : Sunsil C18 150 mm x 4.6mm x 5μ
Mobile Phase : Acetonitrile: Water [50:50]
Flow rate : 0.8 ml/min
Detector : UV-Visible Spectrophotometer 250 nm
Temperature : 25°C
Injection Volume : 10 µL
Run time : 20 minutes
Pump Mode : Isocratic
**Table 2. Trial- 2 Chromatogram data**

| Ret. Time | Peak Area | Theoretical Plate count | Tailing Factor |
|-----------|-----------|--------------------------|---------------|
| 4.947 mins | 701269 | 3375.749 | 1.346 |

**Inference:** Better peak shape compared to the initial trial but improper baseline and delayed retention time was observed.

**Trial 3:**

**Chromatographic conditions:**

- **Column:** Sunsil C18 150 mm x 4.6mm x 5µ
- **Mobile Phase:** Acetonitrile: Water [70:30]
- **Flow rate:** 0.8 ml/min
- **Detector:** UV-Visible Spectrophotometer 250 nm
- **Temperature:** 25°C
- **Injection Volume:** 10 µL
- **Run time:** 20 minutes
- **Pump Mode:** Isocratic

![Fig. 5. Chromatogram of Trial – 3](image)

**Table 3. Trial- 3 Chromatogram data**

| Ret. Time | Peak Area | Theoretical Plate count | Tailing Factor |
|-----------|-----------|--------------------------|---------------|
| 3.721 mins | 941127 | 4326.174 | 1.243 |

**Inference:** Baseline was straight and also good peak shape was observed. Considering this, further method optimization was done.

**6. METHOD OPTIMIZATION:**

**Optimized Chromatographic conditions:**

- **Column:** Sunsil C18 150 mm x 4.6mm x 5µ
- **Mobile Phase:** Acetonitrile: Water (pH adjusted to 3.5 with Acetic acid) [70:30]
- **Flow rate:** 0.8 ml/min
- **Detector:** UV-Visible Spectrophotometer 250 nm
- **Temperature:** 25°C
- **Injection Volume:** 10 µL
- **Run time:** 20 minutes
- **Pump Mode:** Isocratic
Inference: Good peak shape and Rt were observed. Also system suitability parameters plate count and tailing factor were within the limits.

7. METHOD VALIDATION:

The developed method was validated according to ICH Guideline Q2 (R1). The following parameters were evaluated:

Specificity: It is the ability to assess the analyte unequivocally in the presence of other components which may be expected to be present. A blank (only diluent without drug) was injected into HPLC. No peaks were observed.

Linearity: Linearity was performed by injecting Tigecycline working standard solutions in the range of 5 to 40μg/ml in HPLC and response was recorded. Calibration curve was obtained by plotting concentration against respective peak area values. R² value was determined which was found to be 0.9995.

Precision: Precision was assessed by injecting six replicates of 10μg/ml Tigecycline standard solution, on the same day, and under the same experimental conditions. Peak area of six replicates of standard solution was obtained from chromatograms. % RSD was determined.

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 % & 150 %). Three replicates of each concentration level were prepared. These solutions were injected in HPLC and response was recorded. % Recovery was determined.

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) & 10 (for LOQ) were multiplied by the ratio of standard deviation (σ) and the slope obtained from calibration curve.

LOD (Detection Limit) = 3.3 σ/ Slope
LOQ (Quantitation Limit) = 10 σ/Slope

Table 4. Optimized trial chromatogram data

| Ret. Time | Peak Area | Theoretical Plate count | Tailing Factor |
|-----------|-----------|--------------------------|---------------|
| 2.749 mins | 1121869 | 4191.541 | 1.351 |

Table 5. Linearity data of Tigecycline RP-HPLC Method

| Concentration | Peak Area |
|---------------|-----------|
| 5 μg/ml       | 538764    |
| 10 μg/ml      | 1125843   |
| 15 μg/ml      | 1634598   |
| 20 μg/ml      | 2191785   |
| 25 μg/ml      | 2723598   |
| 30 μg/ml      | 3320164   |
| 35 μg/ml      | 3938749   |
| 40 μg/ml      | 4461695   |

Robustness: Robustness of the method was determined by injecting three replicates of 10 μg/ml Tigecycline standard solution by varying
the flow rate in chromatographic conditions at 0.8 ± 0.1 ml/min and pH 3.5 ± 0.1. Chromatograms were obtained and % RSD was calculated.

**System Suitability Test:** Tigecycline working standard solution was prepared as per the procedure and five replicates were injected into the HPLC system. The system suitability parameters were evaluated from the obtained chromatograms by calculating the % RSD of Rt, peak areas, tailing factor and theoretical plates, which were found to be within range.

![Blank chromatogram](image)

![Calibration curve](image)

**Fig. 7. Blank chromatogram**

**Fig. 8. Calibration curve of Tigecycline RP-HPLC Method**

**Table 6. Precision data of Tigecycline RP-HPLC Method**

| Injections | Peak Area | Intra Day Precision | Inter Day Precision |
|------------|-----------|---------------------|---------------------|
| 1          | 1125218   |                     | 1082637             |
| 2          | 1094654   |                     | 1134523             |
| 3          | 1121027   |                     | 1112271             |
| 4          | 1098719   |                     | 1086945             |
| 5          | 1089425   |                     | 1079948             |
| 6          | 1109263   |                     | 1094639             |
| Mean       | 1106384.3 |                     | 1098493.8           |
| Standard Deviation | 14569.6 |                     | 19285.2             |
| % RSD      | 1.31%     |                     | 1.75%               |
Table 7. Accuracy Data of Tigecycline RP-HPLC Method

| % Level | Sample | Standard Spiked | % Recovery | Mean % Recovery |
|---------|--------|----------------|------------|----------------|
| 50      | 5 μg/ml| 2.5 μg/ml      | 100.4      | 99.3           |
|         |        |                | 100.2      |                |
|         |        |                | 97.2       |                |
| 100     | 5 μg/ml| 5 μg/ml        | 98.6       | 98.6           |
|         |        |                | 99.2       |                |
|         |        |                | 100.4      |                |
| 150     | 5 μg/ml| 7.5 μg/ml      | 98.6       | 99.4           |

Table 8. LOD & LOQ of Tigecycline RP-HPLC Method

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

Table 9. Robustness Data of Tigecycline RP-HPLC method at 0.8 ± 0.1ml/min flow rate

| Flow rate | Peak Area | Mean   | S.D   | % RSD |
|-----------|-----------|--------|-------|-------|
| 0.8±0.1 ml/min | 998742 | 1013467 | 11514.36 | 1.136 |
| 0.8-0.1 ml/min  | 1026937 | 1013048.66 | 5322.76 | 0.531 |

Fig. 9. Chromatogram of Tigecycline Sample Solution

%Assay = Peak area of sample / Peak area of Standard x Concentration of Standard (µg/ml) / Concentration of sample (µg/ml) x 100. %Assay = 1080610/1086330 x 10/10 x 100. %Assay = 99.47 %
Table 10. Robustness Data of Tigecycline RP-HPLC Method at pH 3.5 ± 0.1

| Flow rate   | Peak Area | Mean       | S.D   | % RSD        |
|-------------|-----------|------------|-------|--------------|
| pH 3.5 +0.1 | 996731    | 1001163.66 | 9422.52 | 0.941        |
|             | 1014263   |            |       |              |
| pH 3.5-0.1  | 995134    | 997241     |       |              |
|             | 1011597   | 1001324    | 7314.85 | 0.730        |

7.1 ASSAY

Preparation of Tigecycline Sample Solution for RP-HPLC Method: Tigecycline lyophilized powder formulation equivalent to 10 mg was transferred into a 10 mL volumetric flask. It was dissolved in sufficient amount of mobile phase and sonicated. Then using mobile phase the volume was made up to the mark. The solution was filtered and from this 0.1 ml was pipetted out into a 10 mL volumetric flask and diluted using mobile phase. This solution was injected into HPLC and chromatogram was obtained. % Assay was calculated using the peak area.

8. CONCLUSION

A new, simple, rapid, precise and accurate RP-HPLC method was developed and validated as per the ICH guidelines. 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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