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Development and Validation of RP-HPTLC Method for Determination of Tigecycline hydrochloride in Bulk and its Formulation

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ABSTRACT: Tigecycline is a semisynthetic intravenous antibiotic. In this study a new simple, accurate, precise and selective high performance thin layer chromatographic (HPTLC) method has been developed and validated for the determination of Tigecycline hydrochloride in bulk and its formulation Tigecycline is a semisynthetic intravenous antibiotic. Chromatographic separation was performed on aluminum plate percolated with RP-18 Silica Gel 60 F25S using, Methanol: Acetonitrile: Water (3: 3: 4 v/v/v) as mobile phase & 1ml Triethylamine as a plate modifier. Densitometry scanning done at 245 nm. This system was found to give compact spot for Tigecycline (Rfvalue = 0.7 ± 0.02). The calibration curve was found to be linear between 1000- 6000 ng/band. The limit of detection and quantitation were found to beng/band and 2.3 ng /band respectively. The proposed method can be applicable for theestimation of Tigecycline during stability studies.. **KEYWORDS:** HPTLC, Tigecycline hydrochloride, Method development, Validation

I. INTRODUCTION

Tigecycline, a member of the glycylcycline class of antimicrobial agents, carries a glycylamido moiety attached to the 9-position of minocycline(Grayson et al., 2017; Yaghoubi et al., 2022), which is a semisynthetic intravenous antibiotic (Groman, 2015). Tigecycline binds to bacterial 30S ribosomal subunit to inhibit protein synthesis in bacteria, and has shown a broad-spectrum of antibacterial activity against anaerobic bacteria, Gram-negative, and Gram-positive including methicillin-resistant Staphylococcus aurous (Bradford, 2004). It is indicated for the treatment of complicated skin andskin-structure infections and complicated intra-abdominal infections (Townsend et al., 2007). 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(dimethyl amino)-3,10,12,12a- tetrahydroxy-1,11- dioxo-1,4,4a,5,5a,6,11,12a- octahydrotetracene-2-carboxamide.1 Chemicalstructure of Tigecycline is shown in Figure 1.

A review of the literature reveals the analytical procedures used to determine TGN. Quantification of Tigecycline in Bulk and its Dosage Form Using a Stability Indicating Method, ultrafast LC for estimation of Tigecycline in injection formulation, and UPLC for estimation of Tigecycline in Human plasma were all done using high performance liquid chromatography. Few Analytical studies performed for determination of Tigecycline using UV spectroscopy.

However, no HPTLC method has been produced to date. Therefore, attempts were made to develop and validate a cost effective, economical and environment friendly method for the determination of Tigecycline. In RP-HPTLC technique, the mobile phase was prepared by the simple mixture of water and methanol (green solvent) as compared to normal phase HPTLC technique. The adaptation of reverse phase methodology over normal phase helped in avoiding the non-polar fractions from the sample in the TLC, which gives a very clear elution pattern. The aim of the practical is to develop simple, accurate and precise chromatographic methods for Tigecycline in bulk. These methods are developed and validated as per ICH guidelines.

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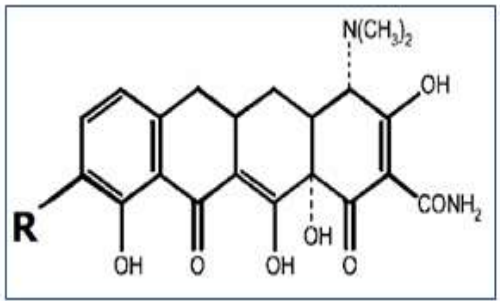

Figure 1: Chemical Structure of Tigecycline

II. MATERIAL AND METHOD

Chemicals

Pure Tigecycline provided by BDR Pharmaceuticals International PVT. LTD, Vadodara, India as a gift sample. Methanol, Acetonitrile, Waterand Triethylamine were used as solvents to prepare the mobile phase. All the chemicals used were of AR grade used without further purification and aluminum backed TLC plates pre-coated with RP- 18 silica gel 60 F254 (0.2mm thick) were purchased from E. Merck Ltd., Mumbai (India).

Equipments

The sample was transferred to the plate using a 100 µl sample syringe (CAMMG Hamilton, Switzerland) by a Linomate V sampler applicator with continuous nitrogen gas flow (CAMMG). The plate used for application was Aluminium plate coated with Silica Gel RP-18 60 F254S plates (10×10 cm, 6mm) (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India) as stationary phase. The plate was prewashed with methanol in twin through chamber and dried at 115◦C for 5 min before chromatography. The applied plate was placed for development in automatic developing chamber (ADC 2 CAMMG) after mobile phase saturation.TLC scanner 3 was used to scan the developed plate, which was connected to the scanning software of the winCAT data processor software (version 1.4.10).

Stock sample preparation

The stock standard solution was daily prepared by accurately weighing 10mg of TGN. Weighed powder was transferred into 10mL volumetric flask, dissolved and diluted up to mark with methanol to obtain concentration 1000µg/ml.

Sample preparation for analysis

To prepare 10 µg/ml solution of sample by withdrawing 0.1 ml from standard stock solution in 10 ml volumetric flask and diluted with methanol.

Formulation preparation for analysis

A Tigecycline powder for injection was taken (lable claim to 50mg/vial) and reconstituted with 5 ml Methanol. Then transferred to a 100mL volumetric flask and 70mL methanol and sonicate for 5 minutes. After ultrasonic vibration, volume was made up to mark with same solvent and filtered through 0.45µm Whatman filter paper. From the filtrate, an appropriate volume was taken and diluted with same solvent to get the final concentration of 2800ng/band.

Validation of method

As per ICH guidelines the developed method was validated. The method validation parameters as per guidelines are linearity, precision, accuracy, ruggedness, detection of limit and quantification.

Linearity

The linearity was evaluated by taking six sets of aliquots ranging 1000 to 6000 ng concentration of Tigecycline hydrochloride from standard stock solution. This concentration applied on plate and tested through plotting peak area versus drug concentration. The linearity of curve was observed.

Precision

The intra-day, inter-day and repeatability parameter studied as in precision. The sample application for calculation in intra-day and inter-day as 1400ng/band, 2100ng/band, 2800ng/band.

Accuracy

The term accuracy refers to the degree to which the measured and true values are similar. The accuracy was measured by spiking of pre-analyzed sample with 80%, 100%, 120% extra Tigecycline standard. To study drug recovery in formulation at different level and re-analyzed the sample.

Robustness

The robustness of the HPTLC method was tested by incorporating small changes in the composition of the mobile phase, chamber saturation time, and slight change in the solvent migration distance, the results were examined.

Limit of detection and limit of quantitation

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In analytical method lowest amount of sample that can be detected known as Limit of Detection (LOD) whereas the lowest amount of sample that can be quantified known as Limit of Quantification (LOQ). The detection was calculated by (3.3 x A.S.D)/b andQuantification limit was calculated by (10 x A.S.D)/b where, b, correspond to graph slope.

III. RESULT AND DISCUSSION

After the development of novel method the different analytical prospects are achieved, as discussedfollows.

Optimization and development of TLC

Initially, Methanol and Acetonitrile solvent were chosen on the basis of drug polarity, then the Water is added ,in varying ratios was tested but tailing was observed, was overcome by Triethylamine used as modifier. In the end, the mobile phase Methanol: Acetonitrile: Water (3: 3: 4 v/v/v) gave good, sharp, well resolved and symmetrical peak with Rf valueof 0.70± 0.02 for Tigecycline (TGN). The mobile phase containing chamber was saturated for 20 min at room temperature and plate activated at 5 min.

Study of calibration curve.

The developed method of linearity was linear in range of 1000 – 6000 ng/band (fig.2). It shows the good linear relationship with applied sample concentration. The correlation coefficient was found to be 0.998 with linear equation of 0.5680x + 1868.6**(table 1).**

Precision

The precision was described by relative standard deviation (RSD %). The intraday and inter-day precision RSD value was found to be 0.59-1.10 and 0.80-1.7 respectively (**table 2**).

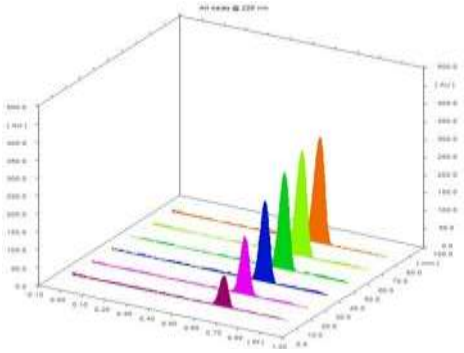

Figure 2: Densitogram of TGN in optimized mobile phase

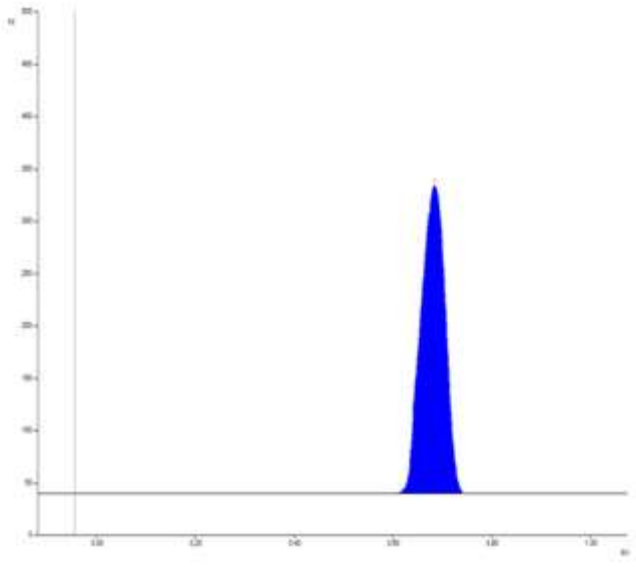

Figure 3: Linearity of TGN

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Accuracy or recovery

The recovery studies by analytical technique of pre- analyzed sample spiked with standard TGN is known as recovery or accuracy. The results of accuracy were shown in table. The % recovery was found to be 99.94 -100.19 % (**Table 3**)

Table 1: Results of validation parameter (as perICH guidelines) for TGN

Parameters	Result
Linearity	0.998
%RSD (precision)	< 2 %
Recovery	100.08
Limit of detection	0.7 ng/band
Limit of quantification	2.328 ng/band
Robustness	Method was robust

Table 2: Precision Studies

Concentration	Intra-day %RSD (should be <2)	Inter-day %RSD (shouldbe <2)
1400	0.96	1.38
2100	1.1	0.80
2800	0.58	1.73

Table 3: Result of accuracy study

Drug	% amount of standard drugadded	%Recovery	%RSD
TGN	80	100.18	0.43
	100	100.11	0.26
	120	99.94	0.10

Robustness

For developed HPTLC method, the standard deviation and percentage of RSD of the robustness parameter was calculated. The results of robustnessgiven in (**table 4**)

Limit of detection and limit of quantitation For the developed HPTLC method, the limit of detection and limit of quantification were found to be 0.76 ng/ml and 2.3 ng/ml, respectively.

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Table 4 .Robustness Studies

Robustness parameter	RF	% RSD
Change in Mobile Phase Composition		
(Methanol: Acetonitrile: Water: trimethylamine)		
4:4:2:0.1 v/v/v	0.73	1.21
5: 1: 4:0.1 v v/v/v	0.69	1.1
3.5: 3.5: 3 :0.1v/v/v/v	0.72	1.3
Mean	0.71	1.20
Mobile Phase Volume		
9 mL	0.72	1.68
10 mL	0.68	1.33
11 mL	0.71	0.94
Mean	0.70	1.31
Duration of saturation		
15 min	0.72	1.56
20 min	0.74	1.14
25 min	0.68	0.68
Mean	0.71	1.12

IV. CONCLUSION

The developed TLC/Densitometry method is a simple, accurate, and repeatable quantitative analysis for estimating Tigecycline hydrochloride in pharmaceutical formulation. The procedure has been validated as per ICH guidelines Q2 (R1). The method is precise and there is no intervention from any of the sample in the study. It can be concluded that the system established offers many advantages such as quick, simple.
Mobile phase and simple preparation measures, more sensitive and comparative short runtime.

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Conflict of Interest
None.

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