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## Formulation of Tigecycline Injection by Lyophilization Technique

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### ABSTRACT

The objective of this experiment is to formulate the tigecycline injection by lyophilization technique for better stability and for long term storage. Lactose monohydrate and lactose spray dried were used with water for injection and hydrochloric acid into 5 ml tubular vials with pre and post purging nitrogen. The filled vials were loaded into lyophilizer and lyophilized them as per cycle. Different composition of additives was used and the different pH concentrations of 4.2, 6.0, 7.0, 7.2 and 8.0 were adjusted with 5, 8.5, 10% hydrochloric acid were tried to formulate the formulation. The formulation F (9) lactose monohydrate which has been used in the ration of 1:2 with drug, the results of the cycle was observed to be optimised. Tigecycline was developed as lyophilised formulation for better stability. The obtained results suggested that a stable formulation for drug tigecycline was developed which was comparable to reference listed product.

**Keywords:** Parenterals, Lyophilization, Tigecycline

### INTRODUCTION

Tigecycline, a novel first in class glycylglycyl [1-3] has shown *in vitro* activity against gram positive, gram negative, aerobic, anaerobic and atypical bacterial species including antibiotic resistant strains. Tigecycline exhibits activity against tetracycline, methicillin resistant *S.aureus* and glycopeptides intermediate *S.aureus*. Penicillin susceptible and resistant *S. pneumonia* [4] and vancomycin resistant enterococci are also susceptible to tigecycline. It is active against more gram negative pathogens, including Enterobacteriaceae, *Acitobacter spp.*, *S.maltophilia* [5-7], *H.influenza* and *N.gonorrhoeae* [8]. Tigecycline's expanded broad spectrum activity is evidenced by its activity against *L.pneumophilla* [9], *Chlamydia* [10], rapidly growing nontuberculosis mycobacteria [11] and anaerobics [12]. After administration of the <sup>14</sup>C-labelled tigecycline to rats its tissue levels with the highest concentration in bone, liver, spleen and kidney exceeded those in plasma and persisted longer [13], tigecycline have a serum half life in a rabbit model of enterococcal endocarditis ranged from 3.3 to 3.6 hr. [14].

Tigecycline is (4*S*, 4*aS*, 5*aR*, 12*aS*)-9-[2-(*tert*-butylamino)acetamido]-4,7-bis(dimethylamino)1,4,4*a*,5,5*a*,6,11,12*a*-octahydro 3,10,12,12-tetrahydroxy-1,11-dioxo-2-naphthacene-carboxamide. The chemical structure of tigecycline has presented in fig.1, and the molecular formula of tigecycline is C<sub>29</sub>H<sub>39</sub>N<sub>5</sub>O<sub>8</sub>, molecular mass 585.65 daltons. This is an orange powder. The pH of a 1% aqueous solution of tigecycline is 7.7 – 8.2. Tigecycline melts at 170°C - 172°C to form a yellow liquid, which decomposes upon further heating to 185°C. The reconstituted solution in water is yellow to orange, essentially free of particulate matter.

Injections are sterile solutions, emulsions or suspensions. These are prepared by dissolving, emulsifying or suspending active substances and excipients in water, in non-aqueous vehicle or mixture of both. Injections are clear, free from particles and emulsions do not show any phase separation.

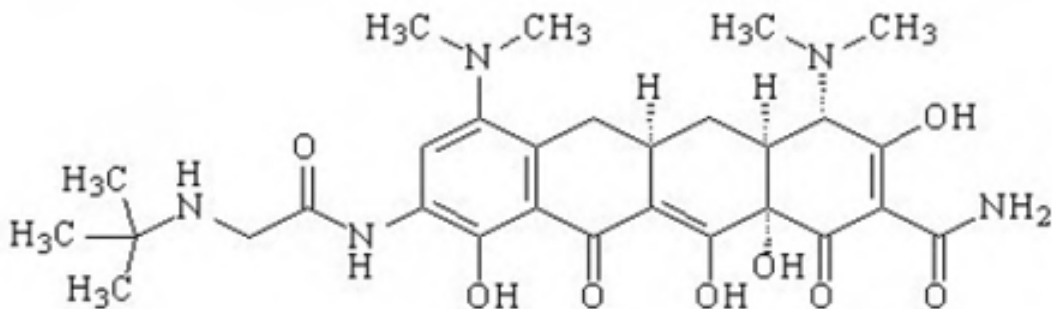


Fig.1: Structure of Tigecycline

Freeze-drying, or lyophilization, is in simple terms a dehydration technique. The aspect of the freeze-drying process that makes it different from other dehydration techniques is that dehydration takes place while the product is in a frozen state under a vacuum. These conditions stabilize the product, minimizing the effects of oxidation and other degradation processes. Freeze-drying has become an accepted method of processing heat sensitive products that require long term storage at temperatures above freezing. Freeze-drying works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to gas. Lyophilization or freeze-drying is often used to stabilize various pharmaceutical products, including virus vaccines, protein and peptide formulations, and liposome and small chemical drugs susceptible to physical and chemical degradation when stored as a ready-to-use solution.

The lyophilization process consists of three stages they are Freezing (Solidification), Primary drying (Ice sublimation), Secondary Drying (Desorption of Unfrozen Water). Secondary drying parameters are based on the quantity and nature of the residual water in the product, the absorption, adsorption and desorption processes.

Tigecycline in liquid injection was found to be unstable. The major objective of this experiment is to formulate the tigecycline injection by lyophilization technique for better stability and for long term storage.

## MATERIALS AND METHODS

Tigecycline is an active ingredient, lactose monohydrate and lactose spray dried were used as lyophilization aid, water for injection as a vehicle for solubility and concentrated hydrochloric acid for acidifying agent. Active ingredient was procured from Natco pharma and all other ingredients used were AR grade.

Table 1: Formulation of tigecycline injection by lyophilization of various batches

Batch	Tigecycline (5% overage)				Lactose spray dried (or) Lactose Monohydrate				Water for Injection				Concentrated Hydrochloric acid			
	Mg/ Vial	Qty/ 50 ml	Qty/ 300 ml	Qty/ 2400 ml	Mg/ Vial	Qty/ 50 ml	Qty/ 300 ml	Qty/ 2400 ml	Mg/ Vial	Qty/ 50 ml	Qty/ 300 ml	Qty/ 2400 ml	Mg/ Vial	Qty/ 50 ml	Qty/ 300 ml	Qty/ 2400 ml
Batch - 1	52.50	1.312 g	-	-	-	-	-	-	QS to 2 ml	QS to 300 ml	-	-	-	-	-	-
Batch - 2	52.50	-	7.875 g	-	50.00*	-	7.50g*	-	QS to 2 ml	-	QS to 300 ml	-	-	-	-	-
Batch - 3	52.50	-	7.875g	-	50.00*	-	7.50g*	-	QS to 2 ml	-	QS to 300 ml	-	5 % Qs to pH6	-	5 % Qs to pH6	-
Batch - 4	52.50	-	7.875 g	-	50.00*	-	7.50g*	-	QS to 2 ml	-	QS to 300 ml	-	10 % Qs to pH6	-	10 % Qs to pH6	-
Batch - 5	52.50	-	7.875g	-	50.00*	-	7.50g*	-	QS to 2 ml	-	QS to 300 ml	-	8.5 % Qs to pH6	-	8.5 % Qs to pH6	-
Batch - 6	52.50	-	7.875 g	-	50.00*	-	7.50g*	-	QS to 2 ml	-	QS to 300 ml	-	8.5 % Qs to pH8	-	8.5 % Qs to pH8	-
Batch - 7	52.50	-	7.875g	-	50.00*	-	7.50g*	-	QS to 2 ml	-	QS to 300 ml	-	8.5 % Qs to pH7	-	8.5 % Qs to pH7	-
Batch - 8	52.50	-	7.875 g	-	100*	-	15g*	-	QS to 2 ml	-	QS to 300 ml	-	8.5 % Qs to pH7.2	-	8.5 % Qs to pH7.2	-
Batch - 9	52.50	-	7.875 g	-	100**	-	15g**	-	QS to 2 ml	-	QS to 300 ml	-	8.5 % Qs to pH4.2	-	8.5 % Qs to pH4.2	-
Batch - 10	52.50	-	7.875 g	-	50**	-	7.5g**	-	QS to 2 ml	-	QS to 300 ml	-	8.5 % Qs to pH4.2	-	8.5 % Qs to pH4.2	-
Batch - 11	52.50	-	-	63.00g	100.00**	-	-	120.00g**	QS to 2 ml	-	-	QS to 2400 ml	8.5 % Qs to pH4.2	-	-	8.5 % Qs to pH4.2

\*Batches used lactose spray dried, \*\*Batches used lactose monohydrate.

### 2.1. Manufacturing Procedure:

Different compositions of lactose monohydrate or lactose spray dried and concentrated hydrochloric acid has been used for the experiment which was placed in table.1. Water for injection (WFI) was collected in a beaker and purged

with nitrogen continuously to reduce the dissolved oxygen until the oxygen level less than 2ppm under 2-8°C condition (except batch F1). 75ml WFI of was collected in a beaker, weighed quantity of lactose monohydrate or lactose spray dried was added and dissolved by stirring until a clear solution was formed (except batch-F1). Tigecycline was weighed and transferred to the above solution and dissolved by stirring for minimum duration of 15 min under 2-8°C condition. The pH of the solution was checked and adjusted the pH with concentrated hydrochloric acid slowly under 2-8°C condition. The solution was diluted and made upto 300ml, by WFI under 2-8°C pH was checked. The final solution was filtered by using 0.22µm membrane filter. The solution was filled into 5ml tubular vials (13mm neck) with pre and post purging nitrogen, and half stopped the vials with 13mm, with lyophilized stopper. The filled vials were loaded into lyophilizer and lyophilized them as per cycle. The samples were collected at the end of the cycle. The samples were withdrawn under the nitrogen atmosphere conditions after the atmospheric pressure was reached, stopped and sealed using flip off aluminium seals and stored the vials at room temperature.

### 2.2. Assay for tigecycline:

HPLC analysis was carried out with a column INTERSIL ODS-3, 5 µm (150mm×4.6mm) with column oven temperature maintained at 40°C, flow rate was 1.5ml/min, detector was UV detector at 247nm and injection volume was 10µl, with the runtime of 8min. 50mg of sample was weighed and transferred into a 50ml volumetric flask and dissolved in about 30ml diluent, sonicated and made up the volume with diluent. 5ml of the above solution was transferred to a 50ml volumetric flask and made up the volume with diluent. The above solution was filtered using 0.22 µ membrane filter, and the sample solution (10µl) was injected with HPLC column by using hypodermic syringe for further analysis. The assay was performed by injecting the sample solution in duplicate into the chromatograph and the peak area was measured for the peaks. The system suitability studies were carried by injecting the standard solution into the chromatograph and chromatogram was recorded. The column efficiency, tailing factor and the relative standard deviation for five replicate injections were determined with respect to standard tigecycline.

### 2.3. Stability studies:

Accelerated stability study was conducted for the optimised batch under various temperature and humidity conditions. The water content, assay and pH were determined and compared with standard conditions.

## RESULTS AND DISCUSSION

Initial trial was done only with drug, Tigecycline and water as the drug is easily soluble in water. The moisture content of the lyophilized vials (Batch F1) was more and the formation of cake was observed in the lyophilized vials which was tight, hence the next cycle was changed in which, the formula included with lactose spray dried, but the drying time had been increased since, the moisture content of the previous batch was more, the results of the batch F2 was observed to be less with drug content in assay. Hence next batch was performed with adjusting the pH of the solution with 5% concentrated hydrochloride solution. The pH of the solution was adjusted to 6.0 before loading into the vials. This cycle was performed with more primary drying time at 5°C. But this batch (F3) was observed to have more pH after reconstitution. Hence next cycle (F4) pH was performed with 10% concentrated hydrochloric acid. The observed results were not fair. The further batches (F5-F8) were planned to observe the effect of pH by adjusting with 8.5% concentrated hydrochloric acid and the results were observed to be out of limits. Hence in batch F9, lactose monohydrate was used in 1:2 ratio with drug, the results of the cycle was observed to be optimised and the next cycle (F10) was planned with 1:1 ratio of lactose monohydrate and drug. But the result of 1:1 ratio of lactose monohydrate with drug was found to be out of limits.

The mean assay value was found to be 105.52%. The retention time of tigecycline is about 5.8 min, the column efficiency is less than 1500 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for five replicate injections is not more than 1.0%. The accelerated stability study was conducted for the optimized batch for 3 months. At 25°C/60% RH the moisture content, pH, and assay was found to be 1.01%, 4.804, and 106.62% respectively. At 40°C/75% RH the moisture content, pH, and assay was found to be 2.05%, 4.83 and 101.90% respectively. At 55°C/90% RH the moisture content, pH, and assay was found to be 3.03%, 4.56 and 98% respectively. The stability study chart of batch F9 which, was optimised has been depicted in fig.2.

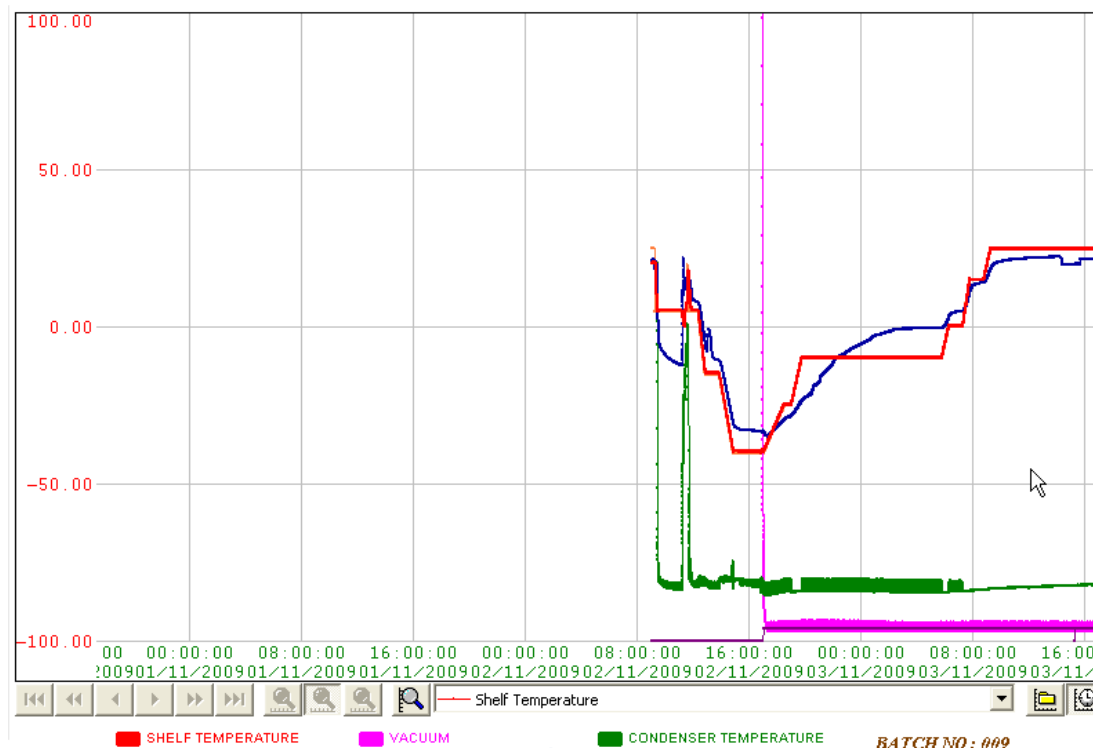


Fig.2: Graphical representation of the optimized condition.

Table2: Evaluation of tigecycline injection by lyophilization technique of optimised batch (Batch-F9).

Parameter	Limit	Tygacil	B.NO: 09	09-3M	09-1M	09-2M	09-3M	09-1M
			INITIAL	25°C/60%RH	40°C/75%RH			55°C/90%RH
Water content	NMT 5.0 %		0.11	1.01%	NA	4.70%	2.05%	3.03%
pH		4.00	4.75	4.804	4.58	4.85	4.83	4.56
Assay (%)	90-120 %		107.9	106.62%	109.8	110.5	101.90%	98.96%

The assay, moisture content and accelerated studies were within the limits.

Table 3: Substances present in the formulation.

Compounds	Standard limit	Observed values						
9-amino minocycline	NMT 0.15%	0.071%	0.070%	0.112%	0.170%	0.140%	0.141%	0.044%
C4 Epimer	NMT 2.0 %	1.012%	1.190%	2.078%	1.961%	1.75%	1.79%	1.570%
Minocycline	NMT 0.15%	0.010%	0.140%	0.490%	0.053%	0.017%	0.001%	0.042%
Un Known	NMT 0.50%	0.046%	0.160%	0.064%	0.065%	0.098%	0.128%	0.101%
Total	NMT 3.0 %	1.214%	1.810%	2.563%	2.55%	2.005%	2.06%	1.75%

In the stability study, it was found to be that, 9-amino minocycline, C4 epimer, minocycline and other unknown compound were found in the optimised batch F9. The 9-amino minocycline was found to be 0.044% and its limit is NMT 0.15%, C4 epimer was found to be 1.57% and its limit is NMT 2.0%, minocycline was found to be 0.042% and its limit is NMT 0.15%, other compound was found to be 0.101% and its limit is NMT 0.50% and the total of these substances was found to be at highest out of seven samples 2.56%, the limit is NMT 3.0%. The observation was found to be present in the optimised batch F9.

## CONCLUSION

Tigecycline in liquid Injection was found to be unstable. Therefore, it was developed as lyophilized formulation for better stability. The lyophilized cycle was optimized with direct four step freezing at -30°C and changing vacuum with post heat up to 40°C. Tigecycline for Injection 50mg was compatible with 5mL clear glass USP Type I vial, bromobutyl rubber closure, nitrogen sparging. The developed formulation was able to withstand three freeze thaw cycles without getting affected product quality. The formulation was stable for 3 months on accelerated stability

studies. In conclusion a stable formulation for drug tigecycline was developed which was comparable to Reference Listed Drug Product (RLD).

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