wjpmr, 2022,8(11), 141-153

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

PHARMACEUTICAL DEVELOPMENT AND CHARACTERIZATION OF NOVEL STERILE INJECTABLE FORMULATIONS FOR ANTINEOPLASTIC AGENT

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Article Received on 01/09/2022 **Article Revised on 21/09/2022** **Article Accepted on 11/10/2022**

ABSTRACT

Trilaciclib dihydrochloride, is a kinase inhibitor, chemically; he chemical name for trilaciclib is 2'-{[5-(4-methylpiperazin-1-yl)pyridin-2-yl]amino}-7',8'dihydro-6'H-spiro[cyclohexane-1,9'-pyrazino[1',2':1,5]pyrrolo[2,3-d]pyrimidin]-6'-one. Trilaciclib dihydrochloride is commercially available COSELA (trilaciclib) for injection, which is a lyophilized formulation available as 300 mg / vial and (trilaciclib) for injection is a yellow lyophilized cake supplied in a single-dose vial. Each vial contains one 300 mg strength single-dose vial. The current investigation was designed to alternative stable liquid composition of Trilaciclib using minimum solvent(s) or low or no use of excipients. The present invention provides a stable, non-aqueous, ready-to-use parenteral composition comprising: Trilaciclib or pharmaceutically acceptable salt thereof, acidifying agent, optionally a surfactant, one or more solvents or co-solvents.

KEYWORDS -: Trilaciclib, Liquid injection, Non aqueous Injection, Headspace Oxygen, Dissolved Oxygen, Ready-to-use parenteral dosage form, Photo stability, In use study. Thermal cycle study, co-solvents.

INTRODUCTION

Each single-dose vial contains the equivalent of 300 mg of trilaciclib (provided as 349 mg of trilaciclib dihydrochloride) and the following inactive ingredients: citric acid monohydrate (75.6 mg) and mannitol (300 mg); hydrochloric acid and sodium hydroxide to adjust pH. Trilaciclib is commercially available as Trilaciclib for Injection, which is a lyophilized formulation available as 300 mg / vial and is a sterile, preservative-free, yellow lyophilized cake in a single-dose vial for intravenous infusion after reconstitution and dilution.

Due to stability issues, Trilaciclib containing com positions must be lyophilized before storage and reconstituted before use. The reconstituted solution should be diluted further. The reconstituted or diluted compositions are not stable and must be used within 24 hours after reconstitution. It requires initial reconstitution, two dilutions prior to intravenous infusion and the same needs to be carried out under aseptic conditions.

Prior to administration, the Trilaciclib for Injection must first be reconstituted, reconstitute each 300 mg vial with 19.5 mL of 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP using a sterile syringe to obtain a concentration of 15 mg/mL of trilaciclib. Gently swirl the vial for up to 3 minutes until the sterile lyophilized cake is completely dissolved. Do not shake. Inspect the reconstituted solution for discoloration and particulate matter. Reconstituted COSELA solution should be a clear, yellow solution. Do not use if the reconstituted solution is discolored, cloudy, or contains visible particulates. If needed, the unused reconstituted solution in the vial can be stored at 20°C to 25°C (68°F to 77°F) for up to 4 hours prior to transfer to the infusion bag. Do not refrigerate or freeze. Discard any unused portion after use.

The difficulties with the commercially available Trilaciclib formulation are complex administration process involving multiple steps. As described above, the person administering the drug must first reconstitute the vial with sterile water for injection and then subsequently transfer the reconstituted solution into an intra venous bag. While reconstituting, the medical practitioner must gently swirl and / or invert the vial slowly for about one minute, or until complete dissolution of any cake or powder occurs. The prescribing information for Trilaciclib for Injection gives clear instructions not to shake the vial to avoid foaming. Possibility of foaming during reconstitution may pose risk of dosing error. A further difficulty of the Trilaciclib product is that the time duration from reconstitution to administration must be completed in 12 hours.

The marketed Trilaciclib product has many limitations, such as long manufacturing procedure including drug

dissolution and long lyophilization cycle to obtain the lyophilized product. Further the lyophilized product requires multiple dilutions and the reconstituted or diluted composition develops frothing or foam formation, if proper care is not taken during reconstitution. If foam is formed, then the health professional needs to wait 5 minutes until the foam subsides from reconstituted solution. This is a cumbersome procedure and complication to health care professionals. Trilaciclib has low aqueous solubility and hence considering the above drawbacks,

Therefore, still there is a need to develop an alternative stable ready - to - use, liquid composition of Trilaciclib Injection using minimum solvent(s) and/or alternate excipients. Additionally, further it does not require such cumbersome and expensive procedures of lyophilization, multiple dilutions.

MATERIALS AND METHODS

Table 1: Materials.

S. No	Ingredients	Functional category
1	Trilaciclib	Active Ingredient
2	Tocopherol	Antioxidant
3	Polysorbate	Co solvent /Surfactant
4	Polyethylene glycol	Co solvent
5	Acetic acid	pH Modifier
6	N,N dimethylacetamide	Solvent

Formulation screening studies

The following development data summarizes the development of a new Trilaciclib formulation i.e. new strength (10 mg/mL) and new dosage form (Ready to Dilute Liquid Injection) intended for same route of administration, same indication and prescribed for Patients in-line with reference drug

Formulation rationale

The development studies were aimed at developing a drug product formulation matching the chemical characteristics of RLD product. The qualitative and quantitative composition of the proposed drug product is not same as that of RLD, the proposed product pharmaceutically and therapeutically equivalent when compare to the RLD product. The product would be developed to comply with general requirements for injectable drug products and products containing most commonly used non –aqueous solvents (for IV administration) were selected based on literature

Rationale for selecting excipients

The excipients utilized in the proposed formulation are selected based on the physio-chemical properties, functionality, historic experience in manufacturing of such dosage forms and the compatibility of excipients with active ingredient in the formulation over the time at recommended storage condition.

Active ingredient Trilaciclib in its pure form is very hydrophobic in nature and does not retain its stability over the time and results in denaturation. Hence, to solubilize and stabilize the API, necessary excipients are selected and optimized in the formulation, which will aid in maintaining the stability of the product over the end of shelf life.

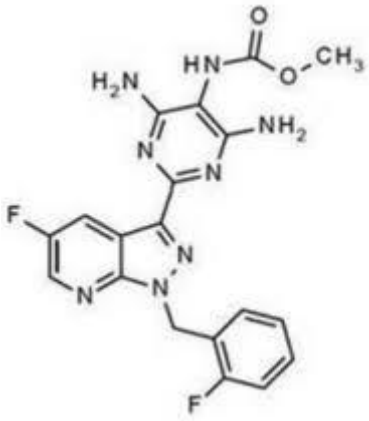
Table 2: Selecting Excipients with IIG limits.

Ingredients	% w/v	Qty /mL	Concentration of inactive ingredients post dilution				Maximum potency in IIG for intra-venous infusion Route Qty /mL (%)
			Low concertation		High concentration		
			0.24 mg/mL	% w/v	1.2 mg/mL	% w/v	
Tocopherol	0.02%	0.2 mg	0.0048	0.0004	0.024	0.0024	0.075%
Polysorbate 80	30%	300 mg	9.6	0.96	48	4.8	54%
Polyethylene glycol 400,	8%	80 mg	2.4	0.24	12	1.2	65%
Acetic acid	4.5%	45 mg	0.72	0.072	3.6	0.36	1%
N,N dimethylacetamide	25.4%	254 mg	9.21	0.921	46.08	4.608	49.7%

Inference: As the proposed formulation is ready to dilute IIG limits evaluated against to the post dilution concentration and found all the excipient levels are well below the IIG limits proposed under intravenous infusion route.

Rationale for strength: Proposed test product Trilaciclib Injection, 10 mg/mL differs from innovator in the dosage form and the strength (quantitative change to the active substance). The selected 10 mg/mL strength falls within the approved dosage and administration.

The required amount of volume is withdrawn aseptically from the proposed Trilaciclib Injection, 125 mg/12.5 mL and diluted into an infusion bag of 50 mL or 100 mL sterile water for injection before use to administer the pre-defined concentration (0.20 mg/mL to 1.1 mg/mL).



Trilaciclib dihydrochloride is a water-soluble yellow solid, with molecular formula of $C_{24}H_{30}N_8O$•2HCl, a molecular weight of 519.48 g/mol (Free base: 446.56 g/mol),

Primary Packaging material selection: Packaging components.

Table 3: Proposed container closure system.

Particulars	Specification	Manufacturer/Vendor
Glass vials	20 mL vial	Piramal
Rubber Closure	20mm Rubber stopper	West
Seal	20 mm flip off seals	West

Design of experiment

Table 4: Experiments are planned as mentioned below.

Experiment	Description of study	Objective
Process selection	To study the feasibility of process	To define a process of manufacturing
Order of Addition	To Study the order of addition	To Define the Order of addition of Excipients
SS vessel compatibility / hold time stability study	Compatibility of the product with the SS 316L vessel/parts	To find out the effect of SS 316L parts/vessel on product during manufacturing / Hold process.
Tubing compatibility study	Compatibility of the product with the tubing	To find out compatibility of the bulk solution of the product with different Pharma tubing's.
Filter compatibility study	Compatibility of the product with the sterilizable grade filter	To find out compatibility of the bulk solution of the product with sterilizable grade filter for physical and chemical properties.
Gasket compatibility study	Compatibility of the product with the silicone gasket	To find out compatibility of the bulk solution of the product with silicone gasket (used as a process aid during filtration & filling) for physical and chemical properties.
Thermal cycling study	Thermal Cycling study	To study the effect of temporary excursion of temperature on the formulation
Photostability study	Impact of light on the formulation	To know the effect of light on drug product & also to finalize the pack configuration
Oxygen sensitivity study	To study the effect of oxidation on the product	To find out the effect oxidation on the product.
In-use stability study	Stability of product during patient usage.	To study the product characteristics during actual usage (During the course of treatment for which it intended for) as per RLD pack insert recommendation.
Lab Scale Stability Study	To establish the shelf life the product	To estimate the stability of the product as per ICH guidelines in real time & accelerated conditions

Manufacturing process selection

Table 5: Manufacturing Formula and Process of Trilaciclib Injection 10 mg/mL.

S. No.	Description	Specification	mg/ mL
1.	Trilaciclib	IH	10
2.	Tocopherol	USP/ NF	0.2 mg
3.	Polysorbate 80	USP/ NF	300 mg
4.	Polyethylene glycol 400,	USP/ NF	80 mg
5.	Acetic acid	USP	45 mg
6.	N,N dimethylacetamide	USP	254 mg
7.	Nitrogen	NF	Q. s

Manufacturing procedure

- N, N dimethylacetamide collected in suitable glass bottle, purged nitrogen to get desired DO level parallel bring down the temperature to 2-8°C by using ice bath.
- 95% of Polyethylene glycol 400, Pre-Nitrogen Purged was collected in a cleaned glass Bottle. Maintain the temperature 2°-8°C throughout the process.
- Added weighed quantity of tocopherol under stirring, stirred for 30 min under nitrogen purging. Clear solution was observed.
- Added weighed quantity of Trilaciclib, stirred for 30 min under nitrogen purging. Clear solution was observed.
- Added weighed quantity of Polysorbate 80 under continuous nitrogen purging, stirred for 30 min. Clear solution was observed.
- Added weighed quantity of PEG 400 under continuous nitrogen purging, stirred for 30 min. Clear solution was observed.
- Added weighed quantity of Acetic acid, under continuous nitrogen purging stirred for 45 min. Clear solution was observed.
- Made up the volume to 100 % with N,N dimethylacetamide and maintain the temperature 2°-8°C under nitrogen blanketing.
- Above bulk solution was filtered through 0.2µm filter and was filled in 20 mL/20 mm neck USP Type I clear glass vial and stoppered under nitrogen and sealed the filled vials.

Table 6: Analytical data of above process.

Tests	Specification	Results
Description	Clear yellow Solution	Clear colorless Solution
pH	Between 3.0 – 6.0	4.45
Absorbance	NMT 0.05	0.0122
Transmittance	NLT 95%	99.63
Assay of Trilaciclib	90%-110%	98.6
Assay of Tocopherol	90%-110%	99.9
Related substances		
Impurity -I	NMT 0.2%	ND
Impurity -II	NMT 1.0%	<0.01
Impurity -III	NMT 1.0%	ND
Diastereomer Impurity	NMT 0.2%	<0.01
Any unspecified impurity	NMT 0.2%	ND
Total impurities	NMT 3.0%	0.014

Inference: - From the above process, all the excipients and Drug substances were easily solubilized and were found feasible with respect to analytical data. However, considering the analytical results like assay, impurity profile, etc. were also found satisfactory and within the specification limit, hence the manufacturing procedure was proposed for scale up and Submission batches of Trilaciclib Injection 10 mg/mL.

Dissolution rate study: Dissolution rate study was carried out to evaluate the solubility of Trilaciclib in N,N dimethylacetamide. Dissolution rate study was executed to predict the maximum solubility of Trilaciclib in N, N dimethylacetamide and placebo solution or vehicle of proposed ready to use Trilaciclib Injection.

Procedure: 45 mL of N,N dimethylacetamide was taken in 100 mL glass beaker and brought down to the temperature of 5°C ± 3°C by using ice bath under nitrogen purging. 1.5 g of Trilaciclib was added under stirring and continued stirring for 20 min and found clear solution was observed. Then added another 400 mg of Trilaciclib under stirring and stir for another 30 minutes and found almost clear solution with two to three undissolved particles observed at the bottom of the glass beaker and volume made up to 50 mL with N,N dimethylacetamide and stirred for 10 minutes found clear solution. Then in-process samples were submitted for analysis sample was filtered by using 0.2-micron syringe filter. Continue stirring was continued for another 30 minutes and clear solution was observed and in-process

samples were submitted for analysis. Sample was filtered by using 0.2-micron syringe filter.

Table 7: API Solubility study.

Test parameter↓	Trial -1	Trial -II
Description	Clear yellow Solution	Clear yellow Solution
Assay of Trilaciclib	99.74 %	96.7 %

Inference: From the above analytical data, it was concluded that a maximum of 38 mg of Trilaciclib API was Soluble in 1 mL of N, N dimethylacetamide. Accordingly, 19.50 mg of Trilaciclib soluble in 0.384 mg of Polyethylene glycol 400.

Order of addition study: Trilaciclib injection 10 mg/mL contains Trilaciclib as API, Tocopherol, Polysorbate 80, PEG 400, Acetic acid and N, N dimethylacetamide as Excipients.

As the Trilaciclib is Soluble in N, N dimethylacetamide Initially there is a need of 95% Batch Quantity of N, N dimethylacetamide for Solubilization of API.As Trilaciclib API is very much Prone to Oxidation an Antioxidant DL-A-Tocopherol was added to inhibit Oxidation of Trilaciclib prior to addition of API to Polyethylene glycol 400.

Polysorbate 80 acts as Surfactant and PEG 400 acts as a Co-solvent in Formulation. Polysorbate 80 are likely to form the Micelles post to dilution of Formulation. However, there won't be Significant Difference in changing the order of addition of these Co-solvents'.

Glacial Acetic acid was used as pH Modifier in the formulation to maintain acidic environment in the formulation to prevent Degradation of formulation.

However, process feasibility was performed by modifying the order of addition Polysorbate 80, Polyethylene glycol 400, glacial acetic acid and N,N dimethylacetamide.

Table 8: The schematic manufacturing process for three batches is given below.

Trial -1	Trial -2	Trial -3
95% of N,N dimethylacetamide	95% of N,N dimethylacetamide	95% of N,N dimethylacetamide
↓	↓	↓
Tocopherol	Tocopherol	Tocopherol
↓	↓	↓
Trilaciclib	Trilaciclib	Trilaciclib
↓	↓	↓
Polysorbate 80	PEG-400	Acetic acid
↓	↓	↓
PEG-400	Polysorbate 80	Polysorbate 80
↓	↓	↓
Acetic acid	Acetic acid	PEG-400
↓	↓	↓
100 % volume make up with N,N dimethylacetamide	100 % volume make up with N,N dimethylacetamide	100 % volume make up with N,N dimethylacetamide

Table 9: Results are tabulated below.

Test parameter↓	Specifications↓	Trial -1	Trial -2	Trial -3
Description	Clear yellow Solution	Complies	Complies	Complies
pH	Between 3.0-6.0	4.11	4.15	412
Osmolality	200 to 350	209	205	208
Absorbance	NMT 0.05	0.015	0.012	0.019
Transmittance	NLT 95%	98.10	98.42	99.76
Assay of Trilaciclib	90%-110%	101.5	103.0	103.2
Related Substances by HPLC				
Impurity -I	NMT 0.5%	0.03	0.04	0.06
Impurity -II	NMT 1.0 %	0.01	0.01	0.02

Test parameter↓	Specifications↓	Trial -1	Trial -2	Trial -3
Impurity -III	NMT 1.0%	0.13	0.63	0.54
Any unspecified impurity	NMT 0.2%	0.14	0.02	0.08
Total impurities	NMT 3.0%	0.86	0.91	0.67

Inference: From the above analytical data, no significant changes were observed in all the trials. The vehicle in Trilaciclib Injection 10 mg/ mL is Ethanol. Hence 95% of Ethanol was taken initially and then added remaining components. For process feasibility purpose, manufacturing process of trial II was selected and same shall be recommended for further batches.

Solution bulk hold time studies: In a Pharmaceutical manufacturing process, SS 316 vessel is the widely used component for Compounding, filtration and to hold the solution certain period of time. In order to establish the compatibility of drug solution with the SS vessel, compatibility study was carried out and the solution was held for 24 hours at 2-8°C and 24 hours at 20-25°C.

Table 10: Results of Hold time solution at 2-8°C and 24 hours at 20-25°C.

Test parameters	Specification limits	Hold time at 2-8°C			Hold time at 20-25°C		
Time points		0 hrs.	12hrs.	24 hrs	0 hrs.	12hrs.	24 hrs
Description	Clear yellow solution	Complies	Complies	Complies	Complies	Complies	Complies
Assay of Trilaciclib	Between 90 to 100	99.48	98.42	97.29	98.8	98.4	98.3
Related Substance							
Impurity -I	NMT 0.2%	ND	ND	ND	ND	ND	ND
Impurity -II	NMT 0.2%	BQL	BDL	BDL	BQL	BDL	BDL
Impurity -III	NMT 0.5%	0.05	BQL	BQL	0.05	BQL	BQL
Any individual unspecified impurity	NMT 0.2%	0.05	0.05	0.05	0.05	0.05	0.05
Total impurities	NMT 2.0%	0.10	0.10	0.05	0.10	0.10	0.05

Inference: Based on the above results of hold time of the bulk solution at 20-25°C and 2-8°C conditions is found to be stable temperature for manufacturing of bulk solution.

Table 11: Rationale for Manufacturing Procedure at 2°-8°C.

Name of Impurity	~RRT	As Such	Acid	Base	Peroxide	Thermal	Photolytic
Impurity -I	0.13	0.0018	0.1568	0.0295	0.0048	0.0790	0.0155
Impurity -II	0.23	0.0087	0.6018	0.0460	0.0574	0.0617	0.0512
Impurity -III	0.30	0.0046	0.0606	0.0066	0.0073	0.0069	0.0083
Impurity -IV	1.32	0.0296	0.0417	0.0612	2.0597	0.0995	0.1312
Max Unknown Imp	--	0.0245	0.5907	0.6176	0.0451	0.0301	0.5898
Total Unknown Imp	--	0.1246	1.8552	0.5055	0.2930	0.4662	0.2439
Total Imp (Known Unknown)	--	0.1413	1.3921	0.3758	2.3982	0.633	0.6121

Inference: There is raise of thermal Degradant in the Forced Degradation Studies which will affect the Impurity Profile. Since the Manufacturing Process of Trilaciclib Injection 10 mg/mL was proposed at 2°-8°C,

Material of construction compatibility study

Below are the DOE's designed to establish the compatibility of process components with the drug product Trilaciclib Injection 10 mg/mL, 12.5 mL

- Tubing compatibility study
- SS vessel compatibility study
- Filter compatibility study
- Gasket compatibility study

Table 12: Results of component compatibility study.

Test parameters	Specification limits	Silicon Tubing	SS 316 Vessel	PVDF filter	PTFE Gasket

Time points		0 hrs.	24 hrs	0 hrs.	24 hrs	0 hrs.	24 hrs	0 hrs.	24 hrs
Description	Clear yellow solution	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
Assay of Trilaciclib	Between 90 to 110 %	98.0	97.4	98.0	98.3	98.4	98.3	98.8	97.5
Related Substance									
Impurity -I	NMT 0.2%	0.07	0.06	0.05	0.03	0.06	0.05	0.08	0.03
Impurity -II	NMT 0.2%	BQL	BDL	BQL	BDL	BQL	BDL	BQL	BDL
Impurity -III	NMT 0.5%	0.04	BQL	0.03	BQL	0.05	BQL	0.06	BQL
Any unspecified impurity	NMT 0.2%	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total impurities	NMT 2.0%	0.14	0.18	0.11	0.15	0.10	0.18	0.18	0.19

Inference: Based on the above data it was concluded that Silicon Tubing, SS 316 Vessel, PVDF filter and PTFE Gasket was compatible up to 24 hours for Trilaciclib Injection 10 mg/mL.

Thermal cycling study: This study evaluated the effects of temperature variation on the product when cycled through temperature conditions that simulate the short-term excursions outside the proposed label storage conditions likely to be encountered during drug product distribution.

This study was performed by subjecting the Trilaciclib Injection 10 mg/mL samples to temperature cycling. The study was performed by storing samples at –20°C ± 5°C for 48 hours followed by 25°C ± 2°C/60% RH ± 5% RH for 48 hours for a total of three cycles.

Table 13: Results of thermal cycling study.

Test parameters / Time points	Specification limits	Initial	At the end of 3rd Cycle
Description	Clear yellow solution	Complies	Complies
Assay of Trilaciclib	NLT 90.0% and NMT 110.0%	99.45	98.60
Related Substance			
Impurity -I	NMT 0.2%	0.03	0.05
Impurity -II	NMT 0.2%	0.04	0.08
Impurity -III	NMT 0.5%	0.05	0.05
Any unspecified impurity	NMT 0.2%	BDL	ND
Total impurities	NMT 2.0%	0.13	0.14

Inference: Based on the data it was concluded that Trilaciclib Injection can withstand the "Temporary Excursion of Temperatures" during shipping. Based on the data, the product storage condition is 2°-8°C (36°-46°F). Avoid freezing is recommended during shipping or transportation of the product.

Photo stability study

Objective: Trilaciclib Injection 10 mg/mL was evaluated for its photo stability in the primary and simulated secondary pack profile as per ICH guidelines. The drug product exposed to light providing an overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200watt hours/square meter. Aluminum foil wrapped samples as dark control and the samples protected by secondary pack (card board box) exposed to similar light conditions also included as part of study.

Table 14: Results of photo stability study.

Test parameters / Time points	Specification limits	Initial	Direct Exposure	Secondary Packing	Dark Control

Test parameters / Time points	Specification limits	Initial	Direct Exposure	Secondary Packing	Dark Control
Description	Clear yellow solution	Complies	Complies	Complies	Complies
Assay of Trilaciclib	NLT 90.0% and NMT 110.0%	99.0	91.8	99.10	98.40
Related Substance					
Impurity -I	NMT 0.2%	0.04	0.11	ND	ND
Impurity -II	NMT 0.2%	BQL	0.12	BDL	BDL
Impurity -III	NMT 0.5%	0.05	0.15	BQL	BQL
Any unspecified impurity	NMT 0.2%	0.05	0.11	0.04	0.08
Total impurities	NMT 2.0%	0.12	1.30	0.11	0.15

Inference: There were noteworthy changes observed in the Chemical properties of drug product in direct exposure sample in Photostability study. From the above analytical data, dark Control and Secondary pack sample were found in compliance with the specification. It was inferred that Trilaciclib Injection 10 mg/mL was Photo sensitive. Protect from Light is recommended during manufacturing process and storage.

Light sensitivity study: Light sensitive study or Photosensitivity study was carried out to evaluate the product stability at room light for particular period of time. Data was generated for drug product exposed to regular light in its primary container and also a dark control and sample was stored at 25ºC.

Table 15: Results of Light sensitivity study.

Test parameters / Time points	Specification limits	Initial	48 hrs light at exposure 2-8°C	48 hrs light exposure at 25ºC to 30ºC	Dark Control
Description	Clear yellow solution	Complies	Complies	Complies	Complies
Assay of Trilaciclib	Between 90 to 110 %	98.8	99.78	97.60	98.40
Related Substance					
Impurity -I	NMT 0.2%	0.04	0.06	0.09	0.03
Impurity -II	NMT 0.2%	BQL	BQL	BQL	BQL
Impurity -III	NMT 0.5%	0.05	0.06	0.09	0.04
Any unspecified impurity	NMT 0.2%	0.02	0.04	0.05	0.03
Total impurities	NMT 2.0%	0.10	0.11	0.18	0.12

Inference: There were no noteworthy changes observed in the physical and chemical properties of drug product in light exposure sample in light exposure study. Not found any significant changes with the sample which was loaded at 2-8°C for 48 hours in presence of light when compare to the initial results and also sample which was loaded at room temperature for 48 hours in presence of light and dark control samples found comparable and compliance with the specification.

Oxygen sensitivity study: to know the effect of oxygen different studies were performed and Stability data was generated.

Table 16: Below trails were executed with the different DO and HSO.

S. No	Do Level (Dissolved Oxygen)	HSO level (Headspace Oxygen)
1	1.72 ppm	3% - 5%
2	5 ppm	5%-8%
3	6 ppm	15.0%

Some of the chemical molecules are prone to oxidation and there by lose their potency at an early stage. Dissolved Oxygen in the Bulk Solution and Headspace Oxygen in the vials during filling play a key role on product stability.

Table 17: Results of oxygen sensitivity Study:

Test parameters / Time points	Specification limits	Initial	DO:1.82 ppm HSO: 3% - 4%	DO:5 ppm HSO : 6% - 8%	DO: 8 ppm HSO: 18%
Description	Clear yellow solution	Complies	Complies	Complies	Complies
Assay of Trilaciclib	Between 90 to 110 %	98.8	99.8	98.00	98.30

Test parameters / Time points	Specification limits	Initial	DO:1.82 ppm HSO: 3% - 4%	DO:5 ppm HSO : 6% - 8%	DO: 8 ppm HSO: 18%
Related Substance					
Acid Impurity	NMT 0.2%	ND	ND	ND	ND
Impurity -I	NMT 0.2%	BQL	BQL	BDL	BDL
Impurity -II	NMT 0.2%	0.06	0.07	0.10	1.0
Impurity -III	NMT 0.5%	BDL	BDL	0.14	ND
Any unspecified impurity	NMT 0.2%	0.09	0.11	1.16	8.08
Total impurities	NMT 2.0%	0.11	1.30	1.19	1.26

Inference: Based on the above data it was concluded that Dissolved oxygen content and Head space oxygen content plays an important role on stability of the product. It was recommended that Dissolved oxygen content should be less than 2.0 ppm and Head space oxygen should be less than 5.0% during manufacturing.

Effect of water content: For Non aqueous formulations water content is a critical parameter whereas available forced degradation data proposed non-aqueous formulation is sensitive to hydrolysis. So utmost precautions to be taken while executing the batches like all the contact parts coming in contact with formulation should be free from moisture while manufacturing the batches. However enough precautions were taken while manufacturing the development batches and the data as presented below.

Table 18: Summary of water content rresults:

Trial -1	
Condition	**Water Content**
Initial	0.27%
25°C/60%RH 6M	0.31%
2°-8°C 6M	0.39%
Trial -2	
Condition	**Water Content**
Initial	0.20
25°C/60%RH 1M	0.22
25°C/60%RH 2M	0.30
25°C/60%RH 3M	0.31
2°-8°C 3 M	0.32

Inference: As per forced degradation data of API there is only increase in Impurity due to water Hydrolysis.

In use study: The study is to recommend a period of time during which a multi-dose/multi-use product can be used while retaining quality within an accepted specification of partially used vials (Needle Punched Vials) of Trilaciclib Injection 10 mg/ mL supplied as a multi dose vial during usage period.

As per innovator pack insert the minimum dose mentioned as 20 mg x with lower BSA considered as 1.6, accordingly minimum dose to be considered as 32 mg (3.2 mL). So, 4 doses can be withdrawal from the proposed formulation of Trilaciclib Injection 10 mg/ mL with fill volume of 12.5 mL.

Study procedure

a. Take a vial of Trilaciclib Injection 10 mg/mL.
b. Pierce the vial and withdraw 0.2 mL sample from each vial as per sample withdraw schedule in the protocol with a new sterile disposable 21-gauge syringe, each piercing and withdrawal shall be performed with a new sterile disposable 21-gauge syringe.
c. The pierced vials shall be stored at as per the given sample plan. Retain in original carton until time of use to protect from light.

At the end of 28 days submit the sample for analysis for the required test parameters.

Table 19: Sample withdrawal schedule for Vial 1.

Time period	0 days	7 days	14 days	28 days
Product withdraw qty	0.2 mL	0.2 mL	0.2 mL	0.2 mL

Table 20: Sample withdrawal schedule for Vial 2.

Time period	0 days	4 days	9 days	14 days	21 days	28 days
Product withdraw qty	0.2 mL	0.2 mL	0.2 mL	0.2 mL	0.2 mL	0.2 mL

Table 21: Analytical Data in use study.

S. No	Parameters	Specification	Initial	Vial 1	Vial 2
1	Description	Clear colorless Solution	Complies	Complies	Complies
2	pH	3.0 – 6.0	4.20	4.37	4.32
3	Assay of Trilaciclib	Between 90 to 110	98.9%	99.3%	98.2%
4	Related Substances				

4.1	Impurity -I	NMT 0.2%	ND	ND	ND
4.2	Impurity -II	NMT 0.2%	BQL	BQL	BDL
4.3	Impurity -III	NMT 0.5%	0.08	0.09	0.12
4.4	Impurity -I	NMT 0.2%	BDL	BDL	0.14
4.5	Any unspecified impurity	NMT 0.2 %	0.02	0.02	0.01
4.6	Total Impurities	NMT 3.0%	0.17	0.25	0.22

Inference: All the parameters were found to be within the proposed specification limit. There is no significant change was observed in the analytical results at the end of 28th day for vial 1 (4 Punctures) as per the dose requirement for minimum Body Surface Area Trilaciclib for Injection and vial 2 (6 Punctures) as a worst-case Scenario.

Conclusion and Recommendations

Trilaciclib Injection 10 mg/mL 12.5 mL was found to be physically and chemically stable for about 28 days after its first opening at 2°C-8°C in which Four Doses can be withdrawn for minimum body Surface area.

It is recommended that Trilaciclib Injection 10 mg/mL 12.5 mL can be used for about 28 days after its first opening at 2°C-8°C with respect to physical and chemical stability of the product. With respect to Microbiological stability, In-use stability will be performed in submission batches to ensure the in-house product microbial withstand property (Preservative action).

Preservative effectiveness study: Antimicrobial preservatives are substances added to non- sterile dosage forms to protect them from microbiological growth or from microorganisms that are introduced inadvertently during or subsequent to the manufacturing process. In the case of sterile articles packaged in multiple-dose containers, antimicrobial preservatives are added to inhibit growth of microorganisms that may be introduced from repeatedly withdrawing individual doses. All useful antimicrobial agents are toxic substances. For maximum protection of patients, the concentration of the preservative shown to be effective in the final packaged product should be below a level that may be toxic to human beings.

The concentration of an added antimicrobial preservative can be kept at a minimum if the active ingredients of the formulation possess an intrinsic antimicrobial activity. Antimicrobial effectiveness, whether inherent in the product or whether produced because of the addition of an antimicrobial preservative, must be demonstrated for all injections packaged in multiple-dose containers or for other products containing antimicrobial preservatives.

Table 22: Acceptance criteria.

For Category 1 Products	
Bacteria	Not less than 1.0 log reduction from the initial calculated count at 7 days, not less than 3.0 log reduction from the initial calculated count at 14 days, and no increase from the 14 days count at 28 days.
Yeasts and molds	No increase from the initial calculated count at 7 days, 14 days and 28 days.

Inference: Based on the results and summary, In the Trilaciclib Injection 10mg/mL drug itself is cytotoxic which is proteasome inhibitor causing Cell cycle arrest or Apoptosis which results in reduction of Microbial growth proves self-antimicrobial efficacy and meets the acceptance criteria USP <51> "Antimicrobial Effectiveness Testing" for an Injectable product.

Placebo product (without Trilaciclib) also subjected for PET study and results are proving Alcohol which acts Self preservative and meets the acceptance criteria USP <51> "Antimicrobial Effectiveness Testing" for an Injectable product.

Selection of sterilization process

Trilaciclib is a kinase inhibitor. High temperature terminal sterilization is not feasible for peptides, therefore sterile filtration and aseptic processing are applied to achieve product sterility. However, perform the autoclave suitability study to establish the method of sterilization of finished product. Trilaciclib Injection 10 mg/ mL was autoclaved at 121ºC for 15 min to check the physical and chemical parameters. Both microbiological lethality and degradant formation are directly dependent on cumulative thermal exposure, and therefore, sterilization conditions are well suited for the development of a design space.

Table 23: Analytical data.

S. No	Parameters	Specification	Initial	Autoclaved at 121°C for 15 mins
1	Description	Clear yellow Solution	Complies	Complies
2	pH	3.0 – 6.0	4.25	4.0
3	Assay of Trilaciclib	Between 90.0 % to 110.0 %	99.9%	73.4%
4	Assay of Alpha tocopherol	Between 90.0 % to 110.0 %	96.2%	77.8%
5	Related Substances			
	Impurity -I	NMT 0.2%	0.01	1.11
	Impurity -II	NMT 0.2%	0.01	1.21
	Impurity -III	NMT 0.5%	0.07	0.79
	Any unspecified impurity	NMT 0.2 %	0.03	1.03
	Total Impurities	NMT 3.0%	0.17	3.25

Inference: Upon autoclaving of Trilaciclib Injection 10 mg/ mL, there was a significant change observed with relative substances and Assay of Alpha Tocopherol were not met the specification when compare to the control sample results. Assay of Alpha Tocopherol was not detected in autoclaved at 121°C for 15 min and impurity and Specified Impurity are crossed the proposed specification limit. Hence it was concluded that terminal sterilization is not feasible for Trilaciclib Injection 10 mg/ mL. Sterilization by aseptic filtration will be adopted,

Extractable volume study: To perform the residual volume study of Trilaciclib Injection 10 mg/mL.

Table 23: Analytical data extractable volume study.

Trilaciclib Injection Extractable Volume Study					
S. No	Fill volume (gm)	Weight of Total vial (gm)	Empty vial weight [A]	Weight of Empty vial after removing solution (gm) [B]	Residual volume (gm) [B-A]
1.	12.33480	25.69664	13.36184	13.58261	0.22077 (0.236 mL)
2.	12.33031	25.49561	13.1653	13.36572	0.20042 (0.214 mL)
3.	12.32210	25.59238	13.27028	13.49174	0.22146 (0.237 mL)
4.	12.32470	25.57774	13.25304	13.42502	0.17198 (0.184 mL)
5.	12.32826	25.55928	13.23102	13.40363	0.17261 (0.184 mL)
Average					0.1975 (0.211 mL)

Inference: From the data it was observed that still small quantity was left in the vial after removal of drug product. It is evident that slight residual quantity of Trilaciclib Injection 10 mg/mL (12.5 mL) was available in the vial after withdrawal of drug product. However, the 12.5 mL of drug solution can be withdrawn from the target fill volume as 13.2 mL is admissible.

Stability data

Trilaciclib Injection 10 mg/mL of lab scale batch size of fill volume 12.5 mL was prepared and charged into stability in both accelerated and real-time condition as per ICH guideline. Lab scale stability data of Trilaciclib Injection 10 mg/mL was given below

Table 24: Stability data.

S. No	Test parameters	Specification	Initial	5±3°C - 6M	25°C /60%RH-6M
1	Description	Clear yellow Solution	Complies	Complies	Complies
2	pH	3.0 – 6.0	5.14	5.17	5.32
3	Assay of Trilaciclib	Between 90 to 110 %	98.9%	98.7%	99.4%
4	Assay of Alpha tocopherol	Between 90 to 110 %	97.2%	98.8%	98.5%
5	Related Substances				
	Impurity -I	NMT 0.2%	0.02	0.04	0.09
	Impurity -II	NMT 0.2%	0.01	0.03	0.09
	Impurity -III	NMT 0.5%	0.07	0.17	0.15
	Any unspecified impurity	NMT 0.2 %	0.08	0.10	0.15
	Total Impurities	NMT 3.0%	0.89	0.90	1.89

Inference: From the above analytical results of stability study data, it can be concluded that the tested parameters are in compliance with the specification of the drug product till 6M accelerated condition.

Comparative Physicochemical testing between novel formulation Trilaciclib Injection 10 mg/mL (Ready to

use) and Reference listed drug product lyophilized product (Trilaciclib for Injection)

To demonstrate the equivalence of formulation Trilaciclib Injection 10 mg/mL (RTU) Ready to use to the Reference listed drug product lyophilized product (Trilaciclib for Injection), pharmaceutical equivalence testing was conducted. Testing included the drug product key parameters Results are summarized in Table 25.

Table 25: Comparative physicochemical testing of results.

S. No	Parameters	Specification	Trilaciclib Injection 10 mg/mL (Ready to use)	RLD Lyophilized product
1	Description	Clear yellow Solution	Complies	Complies
2	pH	3.0 – 6.0	4.24	4.37
3	Assay of Trilaciclib	Between 90 to 110 %	97.6%	98.4%
4	Assay of Alpha tocopherol	Between 90 to 110 %	98.6%	98.9%
5	Related Substances			
	Impurity -I	NMT 0.2%	0.03	0.04
	Impurity -II	NMT 0.2%	0.01	0.03
	Impurity -III	NMT 0.5%	0.13	0.18
	Any unspecified impurity	NMT 0.2 %	0.04	0.045
	Total Impurities	NMT 3.0%	0.19	0.16

Inference

Results of formulation Trilaciclib Injection 10 mg/mL (RTU)is comparable to that of respective Reference Product Trilaciclib for Injection. Hence Trilaciclib Injection 10 mg/mL (RTU) Ready to use is pharma equivalent to that of Reference Product (Lyophilized product)

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