ISSN–2230-7346
Journal of Global Trends in Pharmaceutical Sciences

PHARMACEUTICAL DEVELOPMENT AND CHARACTERIZATION OF NOVEL STERILE INJECTABLE FORMULATIONS FOR CARFILZOMIB INJECTION

Raghuveera Hathibelagal Goruva*, Anjaneyulu Vinukonda[2], Neelesh Chaubey[3], Harish Pandey[4]

[1,3, 4] Department of Pharmacy; Sri Satya Sai University of Technology and Medical Sciences, Sehore, India

[2] Alembic Pharmaceuticals Ltd, Vadodara, India

***Corresponding Author E-mail:** raghuveerahg@gmail.com

ARTICLE INFO

Key Words
Carfilzomib, Injectables Liquid injection, Non aqueous Injection, Headspace Oxygen, Dissolved Oxygen, Ready-to-use parenteral dosage form, Photo stability, In use study, co-solvents.

Access this article online
Website: https://www.jgtps.com
Quick Response Code:

ABSTRACT

Carfilzomib is a peptide epoxy ketone derivative, chemically; it is a tetra peptide epoxy ketone and an analog of epoxomicin. Carfilzomib is commercially available Carfilzomib for Injection, which is a lyophilized formulation available as 30 mg / vial and 60 mg / vial a sterile, white to off - white lyophilized powder and is available as a single - use vial. The marketed KYPROLIS ® (Carfilzomib for Injection) 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.

The present invention relates to a stable non-aqueous, ready-to-use parenteral composition comprising Carfilzomib Injection comprising Carfilzomib or pharmaceutically acceptable salts thereof with pharmaceutically acceptable excipients that substantially increase the solubility, wherein the said injection is free from cyclodextrin derivatives. Further the present invention also relates to a process for the preparation of the stable Carfilzomib injection and methods of treatment thereof.

INTRODUCTION

Carfilzomib is a peptide epoxy ketone derivative, chemically; it is a tetra peptide epoxy ketone and an analog of epoxomicin. Carfilzomib is commercially available as Carfilzomib for Injection, which is a lyophilized formulation available as 30 mg / vial and 60 mg / vial a sterile, white to off - white lyophilized powder and is available as a single - use vial. Each 30 mg vial of Carfilzomib for Injection contains 30 mg of Carfilzomib, 1500 mg sulfobutylether beta - cyclodextrin, and 28.9 mg anhydrous citric acid, and each 60 mg vial contains 60 mg of Carfilzomib, 3000 mg sulfobutylether beta - cyclodextrin, 57.7 mg citric acid , and sodium hydroxide for pH adjustment (target pH 3.5) . Due to stability issues, carfilzomib 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 Carfilzomib for Injection must first be reconstituted with 29 mL and 15

mL of sterile water for injection , which dilutes the amount of carfilzomib to 2 mg / mL , and then further with draw the calculated dose from the vial and diluted into 50 mL using 5 % dextrose Injection , USP intravenous bag . The difficulties with the commercially available Carfilzomib 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 Carfilzomib 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 Carfilzomib product is that the time duration from reconstitution to administration must be completed in 24 hours. Further all the above mentioned reconstitution steps need to be carried out in aseptic conditions, making the process still difficult to follow U.S. Patent. No. 7,737,112 discloses pharmaceutical compositions of practically insoluble proteasome inhibitors. The compositions disclosed in this patent utilize cyclodextrin, water and organic solvents in order to increase the solubility and stability of the practically insoluble proteasome inhibitors. US patent application no. 20140073583 A1 dis closes pharmaceutical compositions comprising peptide epoxy ketone, solvent suitable for injection selected from group consisting ethanol, propylene glycol, polyethylene glycol and mixtures thereof, optionally water and a non-volatile sugar acid as a lyophilized composition. Also dis closed is a liquid composition of carfilzomib. WO patent publication 2015198257 A1 discloses a stable carfilzomib injection comprising carfilzomib or pharmaceutically acceptable salts thereof with citric acid , tertiary butyl alcohol and water for injection to obtain lyophilized product , wherein the said injection is free from cyclodextrin derivatives further the invention also discloses a ready - to use injection comprising carfilzomib , citric acid , dimethylacetamide and Polysorbate 80 , and the injection is free from cyclodextrin derivatives. WO patent publication 2016170489 Al discloses pharmaceutical composition comprising carfilzomib or a pharmaceutically acceptable salt thereof , at least one organic solvent such as dimethylacetamide or propylene glycol or ethanol , and a solubilizer such as hydroxy propyl beta cyclodextrin (HPBCD) , the composition further comprises citric acid , wherein said composition has a water content of less than 2.5 percent w / w . The marketed Carfilzomib 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. Carfilzomib 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 Carfilzomib Injection using minimum solvent(s) and/or alternate excipients. Additionally, further it does not require such cumbersome and expensive procedures of lyophilization, multiple dilutions.

FORMULATION SCREENING STUDIES

The following development data summarizes the development of a new Carfilzomib 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 Kyprolis®

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, and 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 Carfilzomib 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. Proposed formulation of Carfilzomib is stabilized by using 0.2 mg/mL (0.02%) of antioxidant DL Alpha Tocopherol to maintain stability of the formulation over the end of shelf life. The utilized DL Alpha Tocopherol concentration 0.2 mg/mL (0.02%) in the proposed formulation doesn't pose any safety problems for patients. Optimum quantity of Acetic acid as pH modifier and Polysorbate 80 as co-solvent/surfactant with combination of PEG 300 as co-solvent is utilized in the proposed formulation to maintain the physical and chemical stability of the non-aqueous compositions over the end of shelf life. The important property of Surfactants is the formation of micelle when diluted with water for Injection as a result increases the physical stability of formulation and minimizes or inhibits drug crystallization/precipitation over the time. Numerous trials have been performed to determine the effect of Acetic acid quantity on formulation stability with combination of surfactants. From the available physical stability data, it can be concluded that equal to or more than 40 mg Acetic acid is required to keep the diluted solution stable for at least 24 hours or more with the help of surfactants. The observed formulation degradation rate is directly proportional to the quantity of Acetic acid in the formulation. However, surfactants did not show any significant impact on chemical stability, but played critical role on physical stability, the minimum quantity of surfactants (Polysorbate 80 – 400 mg, PEG 300 – 100 mg) is required to keep the diluted solution clear for at least 24 hours and above, with the help of Acetic acid thereby producing stable, ready-to-use, non-aqueous composition.

Conclusion: 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 Carfilzomib Injection, 10 mg/mL differs from innovator (KYPROLIS®) 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 requirements of the KYPROLIS®. The required amount of volume is withdrawn aseptically from the proposed Carfilzomib 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 (0.24 mg/mL to 1.1 mg/mL).

PRIMARY PACKAGING MATERIAL SELECTION

Developmental studies performed did not show any significant interactions with primary Packaging components.

Manufacturing Procedure:

1. Ethanol collected in suitable glass bottle, purged nitrogen to get desired DO level (NMT: 2.0 ppm) parallel bring down the temperature to 2-8°C by using ice bath.
2. 95% of Ethanol Pre-Nitrogen Purged (Dissloved Oxygen- NMT 2.0 PPM) was collected in a cleaned glass Bottle. Maintain the temperature 2°-8°C throughout the process.
3. Added weighed quantity of Alpha tocopherol under stirring, stirred for 20 min under nitrogen purging. Clear solution was observed.

4. Added weighed quantity of Carfilzomib, stirred for 60 min under nitrogen purging. Clear solution was observed.
5. Added weighed quantity of Polysorbate 80 under continuous nitrogen purging, stirred for 15 min. Clear solution was observed.
6. Added weighed quantity of PEG 300 under continuous nitrogen purging, stirred for 15 min. Clear solution was observed.
7. Added weighed quantity of Glacial Acetic acid, under continuous nitrogen purging stirred for 15 min. Clear solution was observed.
8. Made up the volume to 100 % with Ethanol and maintain the temperature 2°-8°C under nitrogen blanketing.
9. Above bulk solution was filtered through 0.22µm filter and was filled in 13 mL/20 mm neck USP Type I clear molded glass vial and stopper under nitrogen and sealed the filled vials.

Observation: Clear colourless solution was observed.

Conclusion: 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 Carfilzomib Injection 10 mg/mL.

DISSOLUTION RATE STUDY OR API SOLUBILITY STUDY

Dissolution rate study was carried out to evaluate the solubility of Carfilzomib in Ethanol. Dissolution rate study was executed to predict the maximum solubility of Carfilzomib in ethanol and placebo solution or vehicle of proposed RTU Carfilzomib injection. As per literature Carfilzomib is practically insoluble in water but soluble in Ethanol.

Objective: To check the maximum solubility of the active ingredient (Carfilzomib) by using ethanol as a solvent.

Procedure: 45 mL of ethanol 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 Carfilzomib was added under stirring and continued stirring for 20 min and found clear solution was observed. Then added another 400 mg of Carfilzomib 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 ethanol 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.

Conclusion: From the above analytical data, it was concluded that a maximum of 38 mg of Carfilzomib API was Soluble in 1 mL of Ethanol. Accordingly, 18.50 mg of Carfilzomib soluble in 0.384 mg of ethanol.

ORDER OF ADDITION STUDY

Objective: To evaluate the process feasibility, order of addition study was carried out. Carfilzomib injection 10 mg/mL contains Carfilzomib as API, DL-A-Tocopherol, Polysorbate 80, PEG 300, Glacial Acetic acid and Ethanol as Excipients. As the Carfilzomib Is Soluble in Ethanol Initially there is a need of 95% Batch Quantity of Ethanol for Solubilization of API.As Carfilzomib API is very much Prone to Oxidation an Antioxidant DL-A-Tocopherol was added to inhibit Oxidation of Carfilzomib prior to addition of API to Ethanol. Polysorbate 80 acts as Surfactant and PEG 300 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 300, glacial acetic acid and Ethanol.

Observation: From the above analytical data, no significant changes were observed in all the trials.

Conclusion: The vehicle in Carfilzomib 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 batch no Batch No: 12020-002 was selected and same shall be recommended for further batches.

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.

Conclusion: 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.

Justification for Manufacturing Procedure at 2°-8°C: Carfilzomib injection 10 mg/mL contains Carfilzomib as API.The excipients present in the formulation are DL-A-Tocopherol, Polysorbate 80, PEG 300, Glacial Acetic acid and Ethanol.As the API is very much sensitive to Thermal degradation and the given Storage temperature of API was at 2°-8°C.

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

COMPONENT COMPATIBILITY STUDY

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

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

Conclusion: 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 Carfilzomib Injection 10 mg/mL.

THERMAL CYCLING STUDY

Objective: 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 Carfilzomib 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.

Conclusion: From the above data it was concluded that only n oxide impurity was increasing during thermal excursion it is purely oxidation related not the thermal, but thermal may act as catalyze to the oxidation.

Recommendations: Based on the data it was concluded that Carfilzomib 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).

PHOTO STABILITY STUDY

Objective: Carfilzomib 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.

Conclusion: 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 Carfilzomib Injection 10 mg/mL was Photo sensitive. Protect from Light is recommended during manufacturing process and storage.

LIGHT SENSITIVITY STUDY

Objective: 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 1: Materials

S. No	Ingredients	Functional Category
1	Carfilzomib	Active Ingredient
2	DL – A- Tocopherol	Antioxidant
3	Super refined Polysorbate 80-LQ-(MH)	Co solvent /Surfactant
4	Super refined Poly Ethylene Glycol 300-LQ- (MH)	Co solvent
5	Acetic acid (Glacial) 100%	pH Modifier
6	Ethanol (Dehydrated Alcohol)	Solvent

Table 2: Rationale for 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**	
DL – A- Tocopherol	0.02%	0.2 mg	0.0048	0.0004	0.024	0.0024	0.075%
Super refined Polysorbate 80-LQ-(MH)	40%	400 mg	9.6	0.96	48	4.8	54%
Super refined Poly Ethylene Glycol 300-LQ- (MH)	10%	100 mg	2.4	0.24	12	1.2	65%
Acetic acid (Glacial) 100%	4%	40 mg	0.72	0.072	3.6	0.36	1%
Ethanol (Dehydrated Alcohol)	38.4%	384 mg	9.21	0.921	46.08	4.608	49.7%

Table 3: Proposed Container Closure System

Particulars	**Specification**	**Manufacturer/Vendor**
Glass vials	13 mL clear vial Fiolax with 20 mm Neck	Schott kaisha Pvt ltd
Rubber Closure	20mm Chlorobutyl Flurotec Laminated Serum Grey Stopper RTU	West pharma
Seal	20 mm flip off seals matte finish	West pharma

DESIGN OF EXPERIMENT

Table 4: Design of experiments are planned as mentioned below to finalize the formulation & process

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.
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

Table 5 : Manufacturing formula and process of Carfilzomib Injection 10 mg/mL was mentioned below

S. No.	Raw materials	Specification	mg/ mL
1.	Carfilzomib	IH	10
2.	DL – A- Tocopherol	USP/ NF	0.2
3.	Super refined Polysorbate 80-LQ-(MH)	USP/ NF	400
4.	Super refined Poly Ethylene Glycol 300-LQ- (MH)	USP/ NF	100

5.	Acetic acid (Glacial) 100%	USP	40
6.	Ethanol (Dehydrated Alcohol)	USP	384
7.	Nitrogen	NF	Q. s

Table 6: Analytical data of above process

Tests	Specification	Results
Description	Clear colorless Solution	Clear colorless Solution
pH	Between 3.0 – 4.0	3.45
Absorbance	NMT 0.05	0.012
Transmittance	NLT 95%	100.633
Assay of Carfilzomib	90%-110%	100.6
Assay of DL-A-Tocopherol	90%-110%	97.9
Assay of Ethanol	90% - 110%	105.83
Related substances		
Acid Impurity	NMT 0.2%	ND
Chloro Impurity	NMT 0.8%	<0.01
N – Oxide Impurity	NMT 1.0%	ND
Diastereomer Impurity	NMT 0.2%	<0.01
Specified unidentified at RRT 0.85	NMT 0.2%	ND
Specified unidentified at RRT 0.97	NMT 0.2%	ND
Any unspecified impurity	NMT 0.2%	ND
Total impurities	NMT 3.0%	0.0194

Table 7: API solubility study

Test parameter↓	Trial -1	Trial -II
Description	Clear colorless Solution	Clear colorless Solution
Assay of Carfilzomib (38 mg/mL)	100.7 %	99.7 %

PROCESS SELECTION

Table 8: Three batches were prepared with change in order of addition. The schematic manufacturing process for three batches is given below.

Batch No: 12020-002	Batch No: 12020-003	Batch No: 12020-004
95% of Ethanol	95% of Ethanol	95% of Ethanol
↓	↓	↓
Alpha Tocopherol	Alpha Tocopherol	Alpha Tocopherol
↓	↓	↓
Carfilzomib	Carfilzomib	Carfilzomib
↓	↓	↓
Polysorbate 80	PEG-300	Glacial acetic acid
↓	↓	↓
PEG-300	Polysorbate 80	Polysorbate 80
↓	↓	↓
Glacial acetic acid	Glacial acetic acid	PEG-300
↓	↓	↓
100 % volume make up with Ethanol	100 % volume make up with Ethanol	100 % volume make up with Ethanol

Table 9: Analytical results are tabulated below.

Test parameter↓	Specifications↓	Batch No: 12020-002	Batch No: 12020-003	Batch No: 12020-004
Description	Clear colorless Solution	Complies	Complies	Complies
pH	Between - 3.0-4.0	3.11	3.15	3.12
Osmolality	200 to 350	212	209	202
Absorbance	NMT 0.05	0.013	0.018	0.018
Transmittance	NLT 95%	99.107	99.422	98.268
Assay of Carfilzomib	90%-110%	101.5	103.0	103.2
Assay of DL-A-Tocopherol	90%-110%	103.9	99.0	99.3
Assay of Ethanol	90% - 110%	97.9	97.4	98.2
Related Substances by HPLC				
Acid Impurity	NMT 0.2%	0.00	0.00	0.00
Chloro Impurity	NMT 0.8%	0.01	0.01	0.02
N – Oxide Impurity	NMT 1.0%	0.23	0.43	0.44
Diastereomer Impurity	NMT 0.2%	0.08	0.09	0.09
Specified unidentified at RRT 0.85	NMT 0.2%	0.00	0.00	0.00
Specified unidentified at RRT 0.97	NMT 0.2%	0.02	0.02	0.02
Any unspecified impurity	NMT 0.2%	0.13	0.06	0.09
Total impurities	NMT 3.0%	0.57	0.68	0.71

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 colourless solution	Complies	Complies	Complies	Complies	Complies	Complies
Assay of Carfilzomib	90%-110%	102.48	102.42	102.29	100.8	100.4	99.3
Assay of Antioxidant	90%-110%.	99.7	99.9	98.0	97.7	96.9	97.0
Ethanol content	90%-110%	101.67	100.56	100.25	99.6	98.50	99.20
Related Substance (By HPLC)							
Acid Impurity	NMT 0.2%	ND	ND	ND	ND	ND	ND
Chloro impurity	NMT 0.2%	BQL	BDL	BDL	BQL	BDL	BDL
N-oxide impurity	NMT 0.5%	0.05	BQL	BQL	0.05	BQL	BQL
Diastereomer impurity	NMT 0.2%	BDL	ND	ND	BDL	ND	ND
Specified impurity	NMT 0.2%	ND	ND	ND	ND	ND	ND
Highest Individual 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

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

Name of Impurity	~RRT	As Such	Acid	Base	Peroxide	Thermal	Photolytic
Acid impurity	0.33	0.0028	0.1168	0.0395	0.0098	0.0590	0.0135
Diol impurity	0.53	0.0087	0.3018	0.0560	0.0274	0.0217	0.0412
Chloro impurity	0.90	0.0056	0.0766	0.0086	0.0083	0.1069	0.0093
N-Oxide impurity	1.12	0.0296	0.0417	0.0612	2.0597	0.0995	0.1312
Max Unknown Imp	--	0.0445	0.8907	0.2176	0.0451	0.0901	0.1898
Total Unknown Imp	--	0.1246	1.8552	0.5055	0.2930	0.4662	0.7439
Total Imp (Known Unknown)	--	0.1713	2.3921	0.6708	2.3982	0.7533	0.9121

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 colourless solution	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
Assay of Carfilzomib	90%-110%.	99.0	99.4	100.0	99.3	99.4	99.3	100.8	99.5
Assay of Antioxidant	90%-110%.	99.3	98.6	98.7	97.9	97.4	97.2	97.5	97.9
Ethanol content	90%-110%.	100.67	99.25	99.8	99.90	98.6	98.20	97.6	98.20
Related Substance (By HPLC)									
Acid Impurity	NMT 0.2%	ND	ND	ND	ND	ND	ND	ND	ND
Chloro impurity	NMT 0.2%	BQL	BDL	BQL	BDL	BQL	BDL	BQL	BDL
N-oxide impurity	NMT 0.5%	0.04	BQL	0.03	BQL	0.05	BQL	0.06	BQL
Diastereomer impurity	NMT 0.2%	BDL	ND	BDL	ND	BDL	ND	BDL	ND
Specified impurity at RRT 0.66	NMT 0.2%	ND	ND	ND	ND	ND	ND	ND	ND
Highest individual 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

Table 13: Results of thermal cycling study:

Test parameters / Time points	Specification limits	Initial	At the end of 3rd Cycle
Description	Clear colourless solution	Complies	Complies
Assay of Carfilzomib	90%-110%.	101.48	100.40
Assay of Antioxidant	90%-110%.	99.7	97.9
Ethanol content	90%-110%.	101.67	99.56
Related Substance (By HPLC)			
Acid Impurity	NMT 0.2%	ND	ND
Chloro impurity	NMT 0.2%	BQL	BDL
N-oxide impurity	NMT 0.5%	0.05	BQL
Diastereomer impurity	NMT 0.2%	BDL	ND
Specified impurity	NMT 0.2%	ND	ND
Highest unspecified impurity	NMT 0.2%	0.05	0.06
Total impurities	NMT 2.0%	0.10	0.12

Table 14: Results of Photo stability study:

Test parameters / Time points	Specification limits	Initial	Direct Exposure	Secondary Packing	Dark Control
Description	Clear colourless solution	Complies	Complies	Complies	Complies
Assay of Carfilzomib	90%-110%.	101.48	90.48	100.00	100.40
Assay of Antioxidant	90%-110%.	99.8	93.7	98.9	99.9
Ethanol content	90%-110%.	101.67	98.5	98.56	99.6
Related Substance (By HPLC)					
Acid Impurity	NMT 0.2%	ND	ND	ND	ND
Chloro impurity	NMT 0.2%	BQL	BQL	BDL	BDL
N-oxide impurity	NMT 0.5%	0.05	0.18	BQL	BQL
Diastereomer impurity	NMT 0.2%	BDL	BDL	ND	ND
Specified impurity	NMT 0.2%	ND	ND	ND	ND
Highest unspecified impurity	NMT 0.2%	0.05	0.10	0.06	0.08
Total impurities	NMT 2.0%	0.10	1.40	0.12	0.10

Table 15: Results of Photo stability 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 colourless solution	Complies	Complies	Complies	Complies
Assay of Carfilzomib	90%-110%.	100.48	99.48	100.00	100.40
Assay of Antioxidant	90%-110%.	99.6	93.7	98.9	99.9
Ethanol content	90%-110%.	99.67	98.5	98.56	99.6
Related Substance (By HPLC)					
Acid Impurity	NMT 0.2%	ND	ND	ND	ND
Chloro impurity	NMT 0.2%	BQL	BQL	BDL	BDL
N-oxide impurity	NMT 0.5%	0.08	0.18	BQL	BQL
Diastereomer impurity	NMT 0.2%	BDL	BDL	ND	ND
Specified impurity	NMT 0.2%	ND	ND	ND	ND
Highest unspecified impurity	NMT 0.2%	0.08	0.10	0.06	0.08
Total impurities	NMT 2.0%	0.10	1.40	0.19	0.10

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

S. No	**DO Level** **(**Dissolved Oxygen**)**	**HSO level** (Headspace Oxygen)
1	1.82 ppm	3% - 4%
2	5 ppm	6%-8%
3	8 ppm	18.0%

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 colourless solution	Complies	Complies	Complies	Complies
Assay of Carfilzomib	90%-110%.	100.48	99.48	100.00	100.40
Assay of Antioxidant	90%-110%.	99.6	93.7	78.2	60.0
Ethanol content	90%-110%.	99.67	98.5	98.56	99.6
Related Substance (By HPLC)					
Acid Impurity	NMT 0.2%	ND	ND	ND	ND
Chloro impurity	NMT 0.2%	BQL	BQL	BDL	BDL
N-oxide impurity	NMT 0.5%	0.08	0.18	0.90	1.0
Diastereomer impurity	NMT 0.2%	BDL	BDL	0.14	ND
Specified impurity at RRT 0.66	NMT 0.2%	ND	ND	0.08	0.09
Highest individual unspecified impurity	NMT 0.2%	0.08	0.10	1.06	10.08
Total impurities	NMT 2.0%	0.10	1.40	2.19	2.26

ND- Not detected; NMT- Not more than

Table 18: Summary of water content results:

Trial -1	
Condition	Water Content
Initial	0.37%
25℃/60%RH 6M	0.39%
2°-8℃ 6M	0.40%
Trial -2	
Condition	**Water Content**
Initial	0.30
25℃/60%RH 1M	0.32
25℃/60%RH 2M	0.31
25℃/60%RH 3M	0.33
2°-8℃ 3 M	0.33

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 – 4.0	3.24	3.37	3.32
3	Assay of Carfilzomib	90%-110%.	99.9%	99.4%	100.4%
4	Assay of Alpha tocopherol	90%-110%.	96.2%	95.8%	97.5%
5	Assay of Ethanol	90%-110%.	105.4%	107.6%	107.7%
6	Related Substances				
6.a	Acid Impurity	NMT 0.2 %	≤ 0.01	≤ 0.01	≤ 0.01
6.b	Chloro Impurity	NMT 0.8 %	0.01	0.01	0.01
6.c	N-Oxide impurity	NMT 1.0 %	0.07	0.17	0.15
6.d	Diastereomer impurity	NMT 0.2 %	ND	ND	ND
6.e	Specified impurity @ RRT 0.85	NMT 0.8 %	ND	0.01	0.01
6.f	Specified impurity @ RRT 0.97	NMT 0.2 %	0.03	0.03	0.02
6.g	Any unspecified impurity	NMT 0.2 %	0.02	0.02	0.01
6.h	Total Impurities	NMT 3.0%	0.17	0.25	0.22

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.

Table 23: Analytical Data in use study:

S. No	Parameters	Specification	Initial	Autoclaved at 121°C for 15mins
1	Description	Clear colorless Solution	Complies	Complies
2	pH	3.0 – 4.0	3.24	4.0
3	Assay of Carfilzomib	90%-110%.	99.9%	83.4%
4	Assay of Alpha tocopherol	90%-110%.	96.2%	87.8%
5	Assay of Ethanol	90%-110%.	105.4%	90.6%
6	Related Substances			
6.a	Acid Impurity	NMT 0.2 %	0.01	0.01
6.b	Chloro Impurity	NMT 0.8 %	0.01	1.01
6.c	N-Oxide impurity	NMT 1.0 %	0.07	0.19
6.d	Diastereomer impurity	NMT 0.2 %	ND	0.01
6.e	Specified impurity @ RRT 0.85	NMT 0.8 %	ND	0.01
6.f	Specified impurity @ RRT 0.97	NMT 0.2 %	0.03	1.03
6.g	Any unspecified impurity	NMT 0.2 %	0.02	0.02
6.h	Total Impurities	NMT 3.0%	0.17	3.25

Table 23: Analytical Data Extractable volume study:

Carfilzomib 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					0.197448 (0.211 mL)

Consider density value: 0.934 g/mL

Table 24: Stability data:

S. No	Parameters	Specification	Initial	5±3°C - 6M	25°C /60RH-6M
1	Description	Clear colorless Solution	Complies	Complies	Complies
2	pH	3.0 – 4.0	3.24	3.37	3.32
3	Assay of Carfilzomib	90%-110%.	99.9%	99.4%	100.4%
4	Assay of Alpha tocopherol	90%-110%.	96.2%	95.8%	97.5%
5	Assay of Ethanol	90%-110%.	105.4%	107.6%	107.7%
6	Related Substances				
6.a	Acid Impurity	NMT 0.2 %	≤ 0.01	≤ 0.01	≤ 0.01
6.b	Chloro Impurity	NMT 0.8 %	0.01	0.01	0.01
6.c	N-Oxide impurity	NMT 1.0 %	0.07	0.17	0.15
6.d	Diastereomer impurity	NMT 0.2 %	ND	ND	ND
6.e	Specified impurity @ RRT 0.85	NMT 0.8 %	ND	0.01	0.01
6.f	Specified impurity @ RRT 0.97	NMT 0.2 %	0.03	0.03	0.02
6.g	Any unspecified impurity	NMT 0.2 %	0.02	0.02	0.01
6.h	Total Impurities	NMT 3.0%	0.17	0.25	0.22

Table 25: Comparative Physicochemical testing of formulation Carfilzomib Injection 10 mg/mL (RTU) to the Reference Product Kyprolis (Carfilzomib for Injection 60 mg/Vial) Results

S. No	Parameters	Specification	Carfilzomib Injection 10 mg/mL (RTU)	RLD KyprolisT(Carfilzomib for Injection 60 mg/Vial))
1	Description	Clear colorless Solution	Complies	Complies
2	pH	3.0 – 4.0	3.24	3.37
3	Assay of Carfilzomib	90%-110%.	99.9%	99.4%
4	Assay of Alpha tocopherol	90%-110%.	96.2%	95.8%
5	Assay of Ethanol	90%-110%.	105.4%	107.6%
6	Related Substances			
6.a	Acid Impurity	NMT 0.2 %	≤ 0.01	≤ 0.01
6.b	Chloro Impurity	NMT 0.8 %	0.01	0.01
6.c	N-Oxide impurity	NMT 1.0 %	0.07	0.17
6.d	Diastereomer impurity	NMT 0.2 %	ND	ND
6.e	Specified impurity @ RRT 0.85	NMT 0.8 %	ND	0.01
6.f	Specified impurity @ RRT 0.97	NMT 0.2 %	0.03	0.03
6.g	Any unspecified impurity	NMT 0.2 %	0.02	0.02
6.h	Total Impurities	NMT 3.0%	0.17	0.16

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

Objective: To know the effect of oxygen different studies were performed and Stability data was generated. 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.

Observation: There were Noteworthy changes observed in the Chemical properties of drug product i.e. Assay of alpha tocopherol was decreasing 43.6%, Assay of Carfilzomib was decreasing by 4.5% from initial to 3 months. Related Substances was Increasing by 2.26% from Initial to 3Months.

Conclusion: 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.

Conclusion: As per forced degradation data of API there is only increase in Diol Impurity due to water Hydrolysis. Whereas we have not observed any hydrolytic impurities in both the batches. So the Water content limit was freeze to 1.5% and based on Scale up and placebo trials can be relooked the proposed limit.

IN USE STUDY

Objective: The Objective of 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 Carfilzomib 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 Carfilzomib Injection 10 mg/ mL with fill volume of 12.5 mL.

Study Procedure:

a. Take a vial of Carfilzomib 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.

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 according to pack Insert of KYPROLIS (Carfilzomib for Injection 60 mg/vial) and vial 2 (6 Punctures) as a worst-case Scenario.

Conclusion and Recommendations: Carfilzomib 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 Carfilzomib 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.

Conclusions: Based on the results and summary, In the Carfilzomib 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 carfilzomib) 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

As per decision trees for sterilization methods (CPMP/QWP/054/98), Those products intended to sterile, should be terminally

sterilized in their final container as clearly stated in the European Pharmacopeia and in the CPMP notes for guidance. Carfilzomib is a tetra peptide epoxy ketone. 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. Carfilzomib 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.

Conclusion: Upon autoclaving of Carfilzomib 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 chloro impurity and Specified Impurity are crossed the proposed specification limit. Hence it was concluded that terminal sterilization is not feasible for Carfilzomib Injection 10 mg/ mL. Sterilization by aseptic filtration will be evaluated by performing filter validation study

EXTRACTABLE VOLUME STUDY

Objective: To perform the residual volume study of Carfilzomib injection 10 mg/mL.

Observation: 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 Carfilzomib 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 justifiable.

STABILITY DATA

Carfilzomib 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 Carfilzomib Injection 10 mg/mL was given below

Conclusion: 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 PHYSICO CHEMICAL TESTING BETWEEN FORMULATION CARFILZOMIB INJECTION 10 MG/ML (RTU) AND US REFERENCE PRODUCT

To demonstrate the equivalence of formulation Carfilzomib Injection 10 mg/mL (RTU) to the Reference Standard Product Kyprolis™ (Carfilzomib for Injection 60 mg/Vial,) pharmaceutical equivalence testing was conducted. Testing included the drug product key parameters Results are summarized in Table 25.

Observation and Conclusion: Results of formulation Carfilzomib Injection 10 mg/mL (RTU) is comparable to that of respective Reference Product (Kyprolis) Carfilzomib for Injection 60 mg/Vial. Hence Carfilzomib Injection 10 mg/mL (RTU) is pharma equivalent to that of Reference Product

REFERENCES:

1. Excipients and Their Use in Injectable Products, PDA Journal of Pharmaceutical Science and Technology July 1997, 51 (4) 166-171.
2. Solubilizing Excipients in Oral and Injectable Formulations March 2004, Pharmaceutical Research 21(2):201-30.
3. In Vitro Hemolysis: Guidance for the Pharmaceutical Scientist Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20627
4. Coleman, M. P., Et. Al. (2008). Cancer survival in five continents: a worldwide population-based study (CONCORD). The Lancet Oncology, 9(8), 730–756. doi:10.1016/s1470-2045(08)70179-7
5. Ataollahi M. R. Et. Al. (2015). Breast cancer and associated factors: a review. Journal of Medicine and Life, 8(4), 6 – 11. PMCID: PMC5319297, PMID: 28316699
6. Malvia, S., Bagadi, S. A., Dubey, U. S., &Saxena, S. (2017). Epidemiology of breast cancer in Indian women. Asia-Pacific Journal of Clinical Oncology,

13(4), 289–295. doi:10.1111/ajco.12661
7. http://www.breastcancerindia.net/statistics/stat_global.html
8. Chhabra, S &Tembhare, Amardeep. (2009). Current Status of Endometrial Carcinoma (Risk To Recurrence). Journal of MGIMS. 14. 18-23.
9. Pack insert of Reference Listed Drug Kyprolis™ (Carfilzomib) for Injection 60 mg/Vial(Applicant: Onyx)
10. ICH Q8 (Pharmaceutical Development Guideline)
11. ICH Q9 (Quality Risk Management)
12. ICH Q10 (Pharmaceutical Quality System)
13. CFR - Code of Federal Regulations Title 21
14. D. O. Edem, "Palm oil: biochemical, physiological, nutritional, hematological, and toxicological aspects: a review," Plant Foods for Human Nutrition, vol. 57, no. 3-4, pp. 319–341, 2002.
15. C. E. Elson and A. A. Qureshi, "Coupling the cholesterol- and tumor-suppressive actions of palm oil to the impact of its minor constituents on 3-hydroxy-3-methylglutaryl coenzyme a reductase activity," Prostaglandins Leukotrienes and Essential Fatty Acids, vol. 52, no. 2-3, pp. 205–208, 1995.