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Stabilisation Attempts of Carfilzomib Formulations and Evaluation

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ABSTRACT

Carfilzomib is an anti-cancer drug acting as a selective proteasome inhibitor. Chemically, it is a tetrapeptide epoxyketone and an analog of epoxomicin. Commercially, Carfilzomib is available as a lyophilized drug product in the global markets. Carfilzomib is a modified tetrapeptidyl epoxide, isolated as the crystalline free base. Carfilzomib is practically insoluble in water, and very slightly soluble in acidic conditions. Based on the drug substance nature, it is found very unstable in the liquid dosage form. The drug substance is very sensitive to hydrolytic degradation in the presence of water. Therefore, an attempt for stabilizing the Carfilzomib formulations via simple formulations were attempted. Based on the available data, it is concluded that nonaqueous formulations evaluation needs to be worked out for the better control of impurities.

INTRODUCTION:

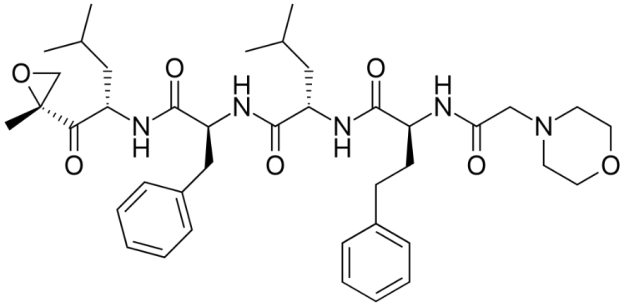
Cancer is a disease of uncontrolled cell division, invasion, and metastasis[1]. It is generally considered to be due to the clonal expansion of a single neoplastic cell. Cancers are classified in two ways[2]: Carfilzomib (marketed under the trade name Kyprolis in the US, developed by Onyx Pharmaceuticals) is an anti-cancer drug acting as a selective proteasome inhibitor. Chemically, it is a tetrapeptide epoxy ketone and an analog of epoxomicin. Bortezomib (Velcade) was approved in 2003. This was the first proteasome inhibitor approved for use in the U.S. Its boron atom binds the catalytic site of the 26S proteasome[3]. Carfilzomib (Kyprolis) was approved by the FDA for relapsed and refractory multiple myeloma in 2012[4]. It irreversibly binds to and inhibits the chymotrypsin-like activity of the 20S proteasome. Ixazomib (Ninlaro) was approved by the FDA in 2015 for use in combination with lenalidomide and dexamethasone for the treatment of multiple myeloma after at least one prior therapy. It is the first orally-available proteasome inhibitor[5].

Carfilzomib is derived from epoxomicin, a natural product that was shown by the laboratory of Craig Crews at Yale University to inhibit the proteasome[6]. The Crews laboratory subsequently invented a more specific derivative of epoxomicin named YU101[7], which was licensed to Proteolix, Inc. Scientists at Proteolix invented a new, distinct compound that had potential use as a drug in humans, known as carfilzomib. Proteolix advanced carfilzomib to multiple Phase 1 and 2 clinical trials, including a pivotal phase 2 clinical trial designed to seek accelerated approval[8]. Clinical trials for carfilzomib continue under Onyx Pharmaceuticals, which acquired Proteolix in 2009[8].

Carfilzomib is an irreversible proteasome inhibitor. It selectively blocks the chymotrypsin-like activity of the 20S proteasome. With this, the degradation of unwanted proteins in the cell is blocked, leading to the buildup of polyubiquitinated proteins. This causes cell cycle arrest, apoptosis, and cell death, which is more in myeloma cells, as the protein production is enhanced in this cancer[9].

It differs from bortezomib in the following aspects: (1) its binding is more selective than bortezomib; (2) it is irreversible; and (3) because of 1 and 2, it is less prone to the development of resistance[9].

The chemical name for carfilzomib is (2S)-N-((S)-1-((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-ylcarbamoyl)-2-phenylethyl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-4-methylpentanamide. Carfilzomib has the following structure:



Carfilzomib is a crystalline substance with a molecular weight of 719.9. The molecular formula is $C_{40}H_{57}N_5O_7$. Carfilzomib is practically insoluble in water and very slightly soluble in acidic conditions.

Carfilzomib is approved by the DCGI for used in relapsed myeloma. DCGI has mandated that a Phase IV trial with this agent be conducted by the parent company. In India, the drug product is approved as the Lyophilized powder Injection 60 mg/vial. Lyophilization is a time consuming, tedious, and involves cumbersome procedures. Further, it involves expensive technology to develop a lyophilized product. One of the main disadvantages of lyophilization is the expensive and the lyophilization cycle development and criticality of the freeze drying process, which requires very low temperatures, can be quite costly. Further, the product needs to be handled with the precautions while dispensing, manufacturing, and lyophilization. The lyophilization cycle recipe needs to be set carefully based on the load of the vials that goes into the lyophilizer. Hence, an attempt to develop a non-lyophilized drug product such as liquid formulation which would offer convenience for practitioners by avoiding the reconstitution step when preparing the drug for administration.

As per the literature available, Carfilzomib's active substance is a non-polar ionizable weak base, is practically insoluble in water. The epoxide group of carfilzomib degrades over time in the aqueous solution therefore, dosage form development focused on the identification of compositions that could provide adequate stability of carfilzomib while enhancing the solubility necessary for the required therapeutic dose. Hence, an attempt is made to evaluate the simple aqueous based formulations of Carfilzomib.

MATERIALS AND METHODS:

Carfilzomib was procured from Laurus Pharma Labs, Ahmedabad. DMSO was sourced from Sigma-Aldrich, L-Arginine, and Citric Acid was received as a gift sample from Merck. Polysorbate 80 and Sorbitol 70% Solution were purchased from the commercial sources. All required chemicals used were of standard grade.

Preparation of Carfilzomib Formulations

A total of 3 formulations were prepared. The concentration was chosen of carfilzomib is 5 mg/mL based on the solubility. Initially, the drug substance was dissolved in the solvent. One by one excipient was added in the required amount of purified water and finally, the volume is made to 100% using water. Citric acid is used as an acidifier in the formulation.

Table No. 1: Formulation of Carfilzomib Injection.

Sr. No.	Ingredients	ACF1	ACF2	ACF3
1	Carfilzomib	5 mg/mL	5 mg/mL	5 mg/mL
2	Dimethyl Sulfoxide	200 mg/mL	200 mg/mL	200 mg/mL
3	Polysorbate 80	80 mg/mL	80 mg/mL	80 mg/mL
4	L-Arginine	--	400 mg/mL	--
5	Sorbitol 70% Solution	--	--	400 mg/mL
6	Citric Acid	0.25 mg/mL	0.25 mg/mL	0.25 mg/mL
7	Purified Water	q.s. to 1 mL	q.s. to 1 mL	q.s. to 1 mL

Evaluation of Carfilzomib Formulations

Physical evaluation

***Description*:** This is a physical observation made by an individual.

pH: pH was measured using a pH meter at about 25°Ctemperature.

Light Transmission: All the formulations were tested for light transmission at650 nm using a UV spectrophotometer.

Chemical Evaluation:

Assay: HPLC method was adopted to measure the active drug content from the 3 formulations. The active obtained is expressed as a percent of the labelled amount of Carfilzomib content. The obtained value of drug content is expected to be within limits of 90.0% to 110.0% (General compendia like USP & BP requirement).

Related Substances: % content of known and unknown impurities were determined by the HPLC method.

RESULTS AND DISCUSSION:

The results are compiled in Table No.2. A clear colourless solution was observed from all the three formulations. The pH of all 3 formulations was observed in the range of 3.2 to 3.6 due to the presence of citric acid in the formulation. It is also noted from the pH trend that all the three formulations indicated that formulation stability is towards the acidic nature. Light transmission measured for the three formulations found close to 100 % indicating the clear transmission of the liquid formulation when each of the formulations was transmitted through a UV spectrophotometer at 650 nm. With respect to the chemical analysis of all the three formulations, it was observed that all the three formulations have shown satisfactory assay levels indicating the correct input of % content of the carfilzomib vs label claim. It also indicates that the analytical method employed for estimating the % content of carfilzomib is correct. From the related substances analysis, it was observed that 3 known impurities were observed in all the three formulations in a significant amount, and the Chloro impurity level was found satisfactory. Also, the content of the single highest unknown impurity is found high in all three formulations.

Table No.2: Physical and Chemical Evaluation of Aqueous Carfilzomib Formulations.

Sr. No.	Formulation Codes	Description	pH	LT (in%)	Assay (in %)	Related Substances
1	ACF1	@	3.47	98.5	97.9%	Acid Impurity: 0.32% Chloro Impurity: 0.18% Diol Impurity: 0.62% N-Oxide Impurity: 0.21% Single Highest UNK Imp: 0.24% Total Imp: 1.82%
2	ACF2	@	3.58	99.1	98.8%	Acid Impurity: 0.31% Chloro Impurity: 0.12% Diol Impurity: 0.42% N-Oxide Impurity: 0.18% Single Highest UNK Imp: 0.28% Total Imp: 1.41%
3	ACF3	@	3.38	98.4	98.2%	Acid Impurity: 0.37% Chloro Impurity: 0.16% Diol Impurity: 0.46% N-Oxide Impurity: 0.24% Single Highest UNK Imp: 0.22% Total Imp: 1.64%

ACF stands for Aqueous Carfilzomib Formulations.

@: Description: A clear colorless solution. LT is Light Transmission.

CONCLUSION:

All the studied three formulations found that there was no physical description complications were observed during the evaluation. Tested parameters of pH and light transmission were found satisfactory with respect to the results. It is concluded that the presence of citric acid has helped to maintain acidic pH helping the drug substance for the better stability. However, Chemical evaluation such as Assay test parameter result was found satisfactory. But with respect to impurities formation such as acid impurity, diol & N-Oxide impurity levels were found on the higher side. It is also observed that the % content of unknown impurities is on the higher side in all the three formulations. However, overall control of Chloro impurity is observed in all the three formulations. From the above experiment, it can be concluded that carfilzomib stability needs fine tuning with respect to a lesser quantity of water to arrest the degradation impurities in the formulation. Compared to the three formulations, ACF2 has lesser impurity levels when compared to the other two formulations. This indicates that the level of water in the formulation is playing an important role with respect to the stability. As an alternate, the scope of developing nonaqueous carfilzomib shall be attempted.

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