International Journal of Multidisciplinary Research and Growth Evaluation

International Journal of Multidisciplinary Research and Growth Evaluation
ISSN: 2582-7138
Received: 16-04-2021; Accepted: 19-05-2021
www.allmultidisciplinaryjournal.com
Volume 2; Issue 3; May-June 2021; Page No. 214-217

Identification of deactivating agent concentration and exposure time for Carfilzomib in drug substance and drug product

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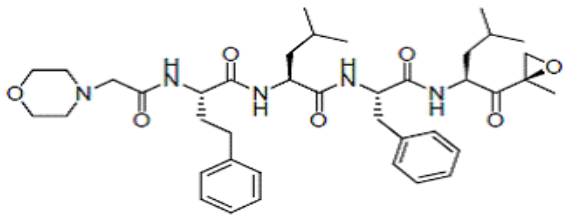
Abstract
The Indian health-care facilities (HCFs) made some guidelines related to Cytotoxic drugs so called Cytotoxic policy for patient safety and health-care worker safety, and environmental monitoring program as per the available international guidelines.Carfilzomib is indicated for the treatment of patients with multiple myeloma and chemically it is a tetra peptide epoxy ketone and an analog of epoxomicin. Analytical method for the detection of Deactivating agent and concentration play an impartment role in the pharmaceuticals especially with cytotoxic molecules after completion of manufacturing and testing is mandatory to follow the safety protocol to dispose those materials. The present invention provides to identify the suitable deactivating agent for the neutralization of Carfilzomib injection 10 mg/mL and Carfilzomib API with respect to concentration and time. This method developed based by RP-HPLC.

Keywords: Carfilzomib, Deactivating Agent, Sodium Hypochlorite Solution, Parenteral dosage form, RP-HPLC. Cytotoxic waste

Introduction
Carfilzomib is indicated for the treatment of patients with multiple myeloma. It is modified tetra peptidyl epoxide, isolated as the crystalline free base. 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 is a crystalline substance with a molecular weight of 719.9. The molecular formula is C40H57N5O7. Carfilzomib is practically insoluble in water and very slightly soluble in acidic conditions.
Carfilzomib has the following structure:



Materials
Carfilzomib API - A gift sample from Hetero Drugs Ltd, Carfilzomib Injection - Purchased from Medlife Ltd, Sodium hypochlorite solution – purchased from Nice chemical Pvt Ltd,
Potassium dihydrogen phosphate, 1-Octane sulphonic acid sodium salt anhydrous, Orthophosphoric acid , Acetonitrile AR grade or equivalent, Water Ultrapure grade water.

Equipment(S)/Instrument(S)
Volumetric flask, Measuring cylinders, Volumetric pipettes Glass, Class-A (both bulb and graduated), Micro pipettes-Ependorff, Balance with sensitivity of 0.01 mg, HPLC system Agilent, pH meter, Thermo scientific Column-Agilent Poroshell 120,EC-C18, (4.6 mm ×150 mm).

Methods

Assay Method [4, 5]

Analytical method for the estimation of Carfilzomib in Drug substance and Drug product were developed by RP-HPLC.

Principle: Reverse phase liquid chromatography with gradient elution and UV detector.

Buffer Preparation:

Weigh accurately about 2.72 g of Potassium dihydrogen phosphate and 6.0 g of 1-Octane Octane sulphonic acid sodium salt anhydrous in 2000mL of water and mix well to dissolve completely and adjust the pH to 3.5 with dilute Orthophosphoric acid solution and filter through 0.22 µm filter.

Mobile phase A

Mix Buffer and Acetonitrile in the ratio of 1400:600(v/v) respectively. Sonicate and degas for 10 minutes.

Mobile phase B

Mix Buffer and Acetonitrile in the ratio of 600:1400(v/v) respectively. Sonicate and degas for 10 minutes.

Table 1: Chromatographic conditions

Column	:	Agilent Poroshell 120, EC-C18 (4.6mm ×150 mm), 4 µm
UV detection	:	210 nm
Flow rate	:	1.0 mL/min
Column temperature	:	35°C
Auto sample temperature	:	10.0°C
Runtime	:	15 minutes
Injection volume	:	10 µL
Elution mode	:	Gradient

Table 2: Gradient Programme

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	30	70
12	40	60
13	30	70
15	30	70

Diluent: Prepare a degassed mixture of water and acetonitrile in the ratio of 30:70(v/v).

Standard preparation: (60 ppm)

Accurately weigh and transfer about10 mg of Carfilzomib WS/RS into a 25 mL volumetric flask and add about 10mL of acetonitrile to dissolve completely and make up to the volume with acetonitrile and mix well. Further transfer 3 mL of above solution to 20 mL volumetric flask dilute and make up to the volume with diluent and mix well.

Sample preparation: (60 ppm)

Accurately weigh and transfer about 2.0 g of Sample into a 50 mL volumetric flask and add about 20 mL of acetonitrile to dissolve completely and make up to the volume with acetonitrile and mix well. Further transfer 3 mL of above solution to 20 mL volumetric flask dilute and make up to the volume with diluent and mix well.

Procedure

1. Equilibrate the column using mobile phase to get a stable baseline.
2. Inject blank (one injection) and standard preparation (five injections)into chromatographic system and check the system suitability parameters.

System Suitability

a. USP Tailing factor/Asymmetry for Carfilzomib peak from first injection of standard solution as recorded by software should be NMT 2.0.
b. USP Plate count /Theoretical plates for Carfilzomib peak from first injection of Standard Solution as recorded by software should be NLT 2000.
c. % RSD of Carfilzomib peak areas from five replicate injections of Standard Solution should be NMT 2.0
d. If system suitability parameter pass, then inject sample solution into the chromatographic system and record the chromatograms.

Table 3: Sequence of Injections

S. No	Description of the solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Sample preparation	2
4	Standard preparation (Bracketing)	1

Calculation for Assay

Calculate the % of labeled amount of Carfilzomib by using the following formula:

$$\% \text{ labelled amount of Carfilzomib} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{100}{LC} \times Wt/mL$$

Where,

AT : Area of peak response of Carfilzomib in the sample Preparation

AS : Average area of peak response of Carfilzomib in Standard Preparation

WS : Weight of Standard taken in mg

DS : Dilution of standard solution

DT : Dilution for sample solution

WT : Weight of sample taken in g

P : % Potency of Carfilzomib Standard in % w/w on as is basis

LC : Label claim of Carfilzomib in Carfilzomib injection (in mg/mL)

Wt/mL : Weight per mL of Sample

Method for deactivating agent[1,3]

The analytical method used for the estimation of Carfilzomib in Drug substance and Drug product will be used to detect the concentration at the end. To determine the same different concentrations of Sodium hypochlorite with different exposure times were studied as per below data. Above samples of said concentration shall be separately filled into flint vials, stoppers and seal. Labelled the vials and performed analysis by HPLC. At which concentration and respective time the API gets neutralized was explained in the results & discussion mentioned below.

Table 4: Selection of concentration of deactivating agent for Carfilzomib Injection 10 mg/mL

Sample description	Deactivating agent (with %)	Amount of deactivating solution added	Amount of drug product to be added	Total volume	Final concentration of deactivating agent
Sample A	5 % Sodium hypochlorite solution	NA	5 mL	5 mL	NA
Sample B		1.67 mL	5 mL	6.67 mL	25.0 %
Sample C		5 mL	5 mL	10 mL	50.0 %
Sample D		15 mL	5 mL	20 mL	75.0 %
Sample E		28.3 mL	5 mL	33.3 mL	85.0 %
Sample I		95 mL	5 mL	100 mL	950 %

Table 5: Duration of exposure of deactivating agent for Carfilzomib Injection 10 mg/mL

Sample description	Amount of deactivating agent	Amount of drug solution	Duration of exposure
Sample F	28.3 mL	5 mL	10 min
Sample G	28.3 mL	5 mL	15 min
Sample H	28.3 mL	5 mL	30 min
Sample K	95 mL	5 mL	60 min
Sample M	95 mL	5 mL	120 min

Table 6: Duration of exposure of deactivating agent for Carfilzomib (API)

Sample description	Amount of deactivating agent	Amount of drug Substance	Duration of exposure
Sample N	30 mL	10 mg	10 min
Sample O	30 mL	10 mg	15 min
Sample P	30 mL	10 mg	30 min
Sample Q	30 mL	10 mg	60 min

Results & discussion

Table 7: Analytical results of selection of concentration of deactivating agent for Carfilzomib Injection 10 mg/mL

Sample description	Deactivating agent (with %)	Amount of deactivating solution added	Amount of drug product to be added	Total volume	Final concentration of deactivating agent	Description	Active/ Principal peak results
Sample A	5 % Sodium hypochlorite solution	NA	5 mL	5 mL	NA	Complies	99.5 %
Sample B		1.67 mL	5 mL	6.67 mL	25.0 %	Light yellow color solution	10.2 %
Sample C		5 mL	5 mL	10 mL	50.0 %		14.3%
Sample D		15 mL	5 mL	20 mL	75.0 %		4.0 %
Sample E		28.3 mL	5 mL	33.3 mL	85.0 %		3.8 %
Sample I		95 mL	5 mL	100 mL	950 %		2.3 %

Table 8: Analytical results of Duration of exposure of deactivating agent for Carfilzomib Injection 10 mg/mL

Sample description	Deactivating agent (with %)	Amount of deactivating agent	Amount of drug solution	Total volume	Duration of exposure	Description	Active/ Principal peak results
Sample F	5 % Sodium hypochlorite solution	28.3 mL	5 mL	33.3 mL	10 min	Light pale yellow color solution	4.1 %
Sample G		28.3 mL	5 mL	33.3 mL	15 min		4.0 %
Sample H		28.3 mL	5 mL	33.3 mL	30 min		4.0 %
Sample K		95 mL	5 mL	100 mL	60 min		2.3 %
Sample M		95 mL	5 mL	100 mL	120 min		Not Detected

Table 9: Analytical results of Duration of exposure of deactivating agent for Carfilzomib (API)

Sample description	Deactivating agent (with %)	Amount of deactivating agent	Amount of drug Substance	Duration of exposure	Description	Active/Principal peak results
Sample N	5 % Sodium hypochlorite solution	30 mL	10 mg	10 min	Light pale yellow color solution	Not Detected
Sample O		30 mL	10 mg	15 min		Not Detected
Sample P		30 mL	10 mg	30 min		Not Detected
Sample Q		30 mL	10 mg	60 min		Not Detected

Conclusion

Carfilzomib Injection 10 mg/mL was tested for deactivating agent with Sodium hypochlorite solution (with 5 % w/v active chlorine).

Based on the analytical results it was concluded that the drug product was completely deactivated with 95% Sodium hypochlorite exposure to the Carfilzomib Injection 10 mg/mL after 120 min

Based on the analytical results it was concluded that the drug substance (Carfilzomib API) was completely deactivated in the ration of 3 ml: 1 mg (Sodium hypochlorite: drug substance) upon exposure of after 15 min.

Recommondations

Carfilzomib Injection 10 mg/mL

From the above conclusion, 5 % Sodium hypochlorite solution with 95% Sodium hypochlorite exposure to the Carfilzomib Injection 10 mg/mL for 120 minutes was recommended for deactivation of carfilzomib for Injection 10 mg/mL.

Carfilzomib (API)

From the above conclusion, 1 mg of Carfilzomib was completely deactivated with 3 ml of 5 % Sodium hypochlorite solution for minimum exposure of 15 min was recommended for Carfilzomib.

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