Acta Scientific Pharmaceutical Sciences

Volume 2 Issue 6 June 2018

Research Article

Quantitative Estimation of Carfilzomib by UV-AUC Method

Pritam S Jain*, Swati D Yeole, Najeef A Ansari and Sanjay J Surana

R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, India

***Corresponding Author:** Pritam S Jain, R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, India.

Received: April 06, 2018; **Published**: May 29, 2018

Abstract

Aim: The aim of this work is to establish a novel, simple, precise and sensitive UV-AUC spectrophotometric method has been developed for estimation of Carfilzomib from bulk and in-house formulation. Moreover, this work is performed to validate as per ICH guidelines.

Materials and Methods: Carfilzomib working standard was obtained as gift sample from Cipla Pharmaceuticals, Mumbai. In Methods, a stock standard solution was prepared by dissolving 100 mg of Carfilzomib in 100 mL of ethanol to obtain a concentration of 1000 µg/mL. After suitable dilution, 100 µg/mL of Carfilzomib prepared and scanned in the UV-visible range 400 - 200 nm; Carfilzomib showed a maximum wavelength at 258 nm. The Method applied was area under curve (AUC in which area under curve was integrated in the wavelength range of 256.80 - 261.20.

Results: The drug follows linearity in the concentration range 100 - 600 µg/mL with correlation coefficient value 0.999. The developed method was implemented to the marketed formulation and the evaluated % amount of drug was found in good consistency with the label claim.

Conclusion: The proposed method was a rapid, economical and commercial quality-control tool for routine analysis of Carfilzomib in bulk and in pharmaceutical formulations.

Keywords: Carfilzomib; UV Method; Validation; Quantitative Determination

Introduction

Carfilzomib is an epoxomicin derivate having potential antineoplastic activity. The chemical name for carfilzomib is(2S)-N-((S)-1-((S)-4-methyl-1-((R)-2-methyloxiran2-yl)-1-oxopentan-2-ylcarbamoyl)-phenylethyl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-4-methylpentanamide.

Carfilzomib is a tetrapeptide epoxyketone proteasome inhibitor that irreversibly binds to and inhibits the chymotrypsin-like activity of the 20S catalytic core subunit of the proteasome. Carfilzomib is a supplementary with a low rate of serum enzyme rise during treatment and has been implicated to rare situations of clinically apparent, acute liver injury some of which have been fatal. The proposed method was validated as per ICH guidelines According to International Conference on Harmonization (ICH).

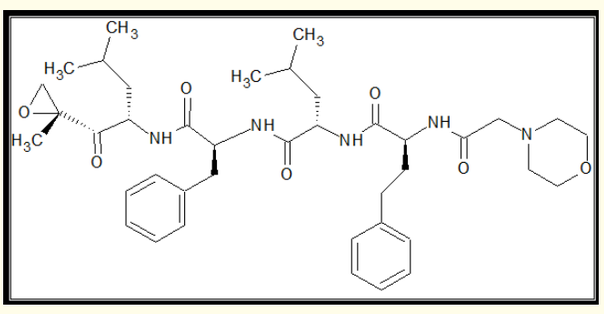

Figure 1: Chemical structure of Carfilzomib.

Materials and Methods

Materials

Carfilzomib working standard was obtained as gift sample from Cipla Pharmaceuticals, Mumbai. As the tablet formulation was not available in Indian market; tablet containing 60 mg Carfilzomib were prepared in-house using direct compression technique. Prepared tablets were used as pharmaceutical formulation for further analysis.

Instrument

A UV-VIS double beam spectrophotometer (UV-2450, Shimadzu, Japan) connected to a computer having spectra manager software UV Probe version 2.21 with 1 cm quartz cells was used. An electronic balance (Model Shimadzu AUX 120) was used for weighing purpose.

Preparation of stock standard solution and determination of λ_{max}

The stock standard solution of Carfilzomib was prepared by weighing, 100 mg of Carfilzomib transferred into a 100 mL of volumetric flask and volume made up to the mark with the ethanol, achieving concentration of 1000 µg/mL.

From the standard stock solution 1 mL of solution was transferred into 10 mL volumetric flask and volume was make up with the same solvent to get the concentration of 100 µg/mL. The final solution was scanned in UV range (200 nm - 400 nm). The spectrum showed maximum absorbance at 258 nm (λ_{max}).

Validation of the method

The method was validated with respect to various parameters such as linearity, accuracy, Limit of detection and quantification, precision and ruggedness.

Linearity

Aliquots 1, 2, 3, 4, 5 and 6 mL of standard stock solution of carfilzomib was transferred into series of 10 mL volumetric flasks and the volume was made up to the mark with ethanol. The calibration curve was obeyed in the concentrations range of 100 - 600 µg/mL and the graph was plotted between concentration versus AUC.

Accuracy

To study the accuracy of the expected method and to confirm the interference from the excipients used in the dosage form, recovery analysis was performed by the standard addition method. It was carried out by adding noted amount of standard drug to the manufactured in-house tablet formulation at 80, 100 and 120% level. It was reanalyzed by the proposed method. The % recovery of noted.

Precision

The precision of the proposed method was determined in terms of intra-day and inter-day precision. Intra-day precision was resolute by examine the 200, 300 and 400 µg/mL of Carfilzomib solution for three times in the similar day. Inter-day precision was estimated by 200, 300 and 400 µg/mL of Carfilzomib for three days.

Sensitivity

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the drug were calculated by using the equation designated by International Conference on Harmonization (ICH) guidelines.

LOD = 3.3 x N/B

LOQ = 10 x N/B

Where 'N' is standard deviation of intercept and 'B' is the slope.

Repeatability

Repeatability was determined by analyzing 300 µg/mL concentration of Carfilzomib solution for six times.

Ruggedness

Ruggedness of the introduced process is determined for 300 µg/mL concentration of Carfilzomib by examining of aliquots from uniform slot by two analysts using same operational and environmental conditions.

Determination of Carfilzomib in bulk

From the stock standard solution of 1000 µg/mL withdrawing an appropriate volume of 3 mL this solution was transferred to 10 mL volumetric flask and volume was adjusted to mark using same solvent. The solution was analyzed on spectrophotometer in the UV range 200 - 400 nm. The concentrations of the drug were evaluated with the help of linear regression equations.

Application of proposed method for pharmaceutical formulation

For analysis of tablet formulation, weighed 20 tablets of Carfilzomib and average weight calculated equivalent to 100 mg taken in 100 mL volumetric flask and the volume was made up to the mark with ethanol to obtained 1000 µg/mL concentration. From the above solution 1 mL was withdrawn and transferred into 10 mL volumetric flask and volume was made up to the mark with same solvent to get 100 µg/mL concentration. The spectrum was reported at 258 nm. The concentrations of the drug were evaluated by linear regression equation.

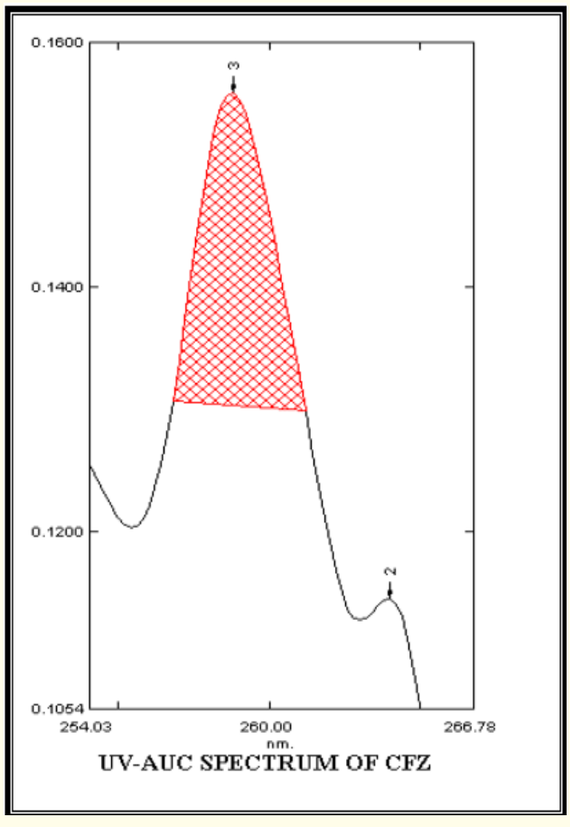

Figure 2: UV-AUC Spectrum of CFZ.

Results and Discussion

Method Validation

The validated method was validated as per ICH guidelines. Preparation of analyzed solution has already discussed in the earlier section.

Linearity studies

Carfilzomib showed a good correlation coefficient and linear regression data for the calibration curves (Figure 3) over the concentration range 100 - 600 µg/ml for Carfilzomib. Linear regression equation was found to be

Y =0.0008 X+0.0666 (r2=0.999). The result is summarized in table 1.

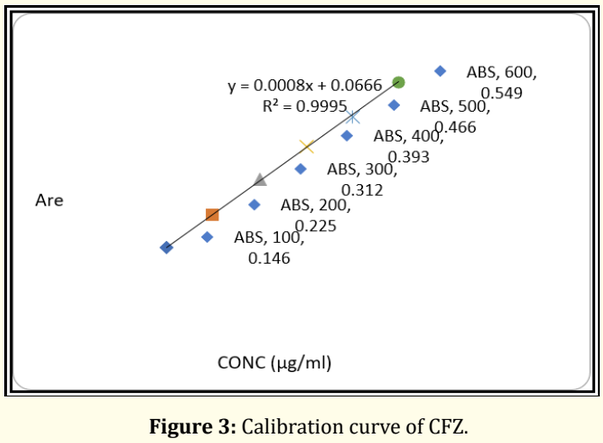

Figure 3: Calibration curve of CFZ.

y=0.0008x+0.0666

r^2 = 0.9995

Sr. no.	Concentration (µg/ml)	Mean ± S.D. (n = 6)	%R.S.D.
1	100	0.146 ± 0.0021	1.36
2	200	0.225 ± 0.0032	1.42
3	300	0.312 ± 0.0047	1.52
4	400	0.393 ± 0.0029	0.74
5	500	0.466 ± 0.0038	0.82
6	600	0.549 ± 0.0080	1.45

Table 1: Linearity study of Carfilzomib.

* Average of six estimations.

Accuracy

The % recovery of Carfilzomib at three concentrations level 80, 100 and 120% were calculated and the results discussed in table 2.

Pre-analyzed sample solution (µg/ml)	Amount of drug added (µg/ml) (n = 3)	%Recovery	%R.S.D.
300	240	99.86	0.79
	300	99.33	1.25
	360	99.09	1.26

Table 2: Recovery studies.

*Average of three estimations.

Precision

Intra-day and Inter-day precision was observed by analyzing three replicates of three different concentration 200, 300 and 400 µg/mL of carfilzomib showed the % RSD less than 2. Results given in table 3.

Component	Concentration (µg/ml)	Intra-day precision* (n = 3)		Inter-day Precision* (n = 3)	
		Conc. found	%R.S.D.	Conc. Found	%R.S.D.
Carfilzomib	200	199.66	0.95	199.25	0.62
	300	299.25	1.25	299.66	0.63
	400	398.83	0.47	399.25	0.31

Table 3: Precision studies.

*Average of three estimations.

Sensitivity

The sensitivity of the proposed method in terms of LOQ and LOD for were found to be 40.06 µg and 119.23 µg, respectively

Repeatability

The % amount found of Carfilzomb was 99.66 % with % R.S.D. less than 2 by performing 300 µg/mL of six replicates. Results expressed in table 4.

Component	Amount taken (µg/ml) (n = 6)	Amount found* (%)	%R.S.D.
Carfilzomib	300	99.66±0.99	0.99

Table 4: Repeatability studies.

Ruggedness

Ruggedness of the method was determined by performing six times for the same concentration solution. The % RSD was found to be less than 2. The result putted in table 5.

Component	Amount taken (µg/ml) (n = 3)	Amount Found (%) *	
		Analyst I ± S.D.	Analyst II ± S.D.
Carfilzomib	300	99.91 ± 0.86	98.41 ± 0.80

Table 5: Ruggedness studies.

*Average of Three estimations

Determination of Carfilzomib in bulk

Determined the Carfilzomib from bulk by analyzing the 300 µg/mL solution. The concentrations of the drug were calculated from linear regression equations. The % amount found was between 98.17 % to 100.01%. The result denoted in table 6.

Concentration (µg/ml)	Amount found (µg)	Amount found (%)
300	299.25	99.75
	298	99.33
	300.5	100.16
	299.25	99.75
	294.25	98.08
	299.25	99.75
Mean± S.D.	298.4 ± 2.18	99.47 ± 0.72
%R.S.D.	0.73	0.73

Table 6: Analysis of Carfilzomib in bulk.

Application of proposed method for pharmaceutical formulation

The % amount found anticipated from in-house formulation show that there is no interference from excipient present in the formulation. The % amount was found 99.75.

Component	Amount Taken (µg/mL)	Amount found (µg/mL) ± SD	% Amount found	%RSD (n = 6)
Carfilzomib	300	299.25 ± 1.76	99.75	0.59

Table 7: Analysis of formulation.

Conclusion

UV-Spectrophotometric is simple, accurate, and economical and least calculations are involved for estimation of concentrations of Carfilzomib in bulk and formulation. All these methods can routinely be used for determination of the drugs in their respective pharmaceutical matrix.

The validation procedure approve that this is a pertinent method for their quantification in the plant material formulation. It is also used in routine quality control of the raw materials as well as formulations containing this entire compound.

Acknowledgement

The authors are thankful to the Principal and management, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur (M.S.), India for providing the required facilities to carry out this research work.

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