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DEVELOPMENT AND VALIDATION OF BIOANALYTICAL METHOD FOR ESTIMATION OF CARFILZOMIB IN HUMAN PLASMA USING RP-HPLC

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ABSTRACT

Objective: This study points to build up and validate a simple methodology to quantify the most used drug Carfilzomib for the treatment of cancer, in human plasma for preclinical studies and validate as per ICH guidelines.

Methods: Carfilzomib was isolated from plasma samples by liquid-liquid extraction method. Chromatographic separation was achieved on Agilent Column (100 mm ×4.6 mm, 2.5 µm). The mobile phase consisted of 0.05 % orthophosphoric acid (OPA) buffer pH 3 and methanol in the ratio of (32:68, v/v), respectively at a flow rate 0.7 ml/min. The Diode array detector (DAD) detection was carried out at 256 nm. The suggested method was validated by performing linearity, system suitability, sensitivity, accuracy, precision, LOD, LOQ, and robustness. The method was validated as per ICH guidelines.

Results: The retention time of Carfilzomib was found to be 3.525 min .the calibration curves are linear (r2 = 0.999) over the concentration range of 5-25µg/ml of plasma analytes concentration. Percentage means recovery of Carfilzomib from spiked plasma was98.6%. All the validated parameters were found to be within the limit.

Conclusion: A simple, accurate, precise, linear and rapid RP-HPLC method was developed for quantitative estimation of Carfilzomib inhuman plasma and should be suitable for conducting pharmacokinetics studies and therapeutic drug monitoring.

Keywords: Carfilzomib, Human plasma, RP-HPLC, Validation, DAD Detector

1. INTRODUCTION

Bio-analytical method is essential to employ well-characterized and fully validated to yield reliable results that can be satisfactorily interpreted. It is recognized that bioanalytical methods and techniques are constantly undergoing changes and improvements, and in many instances, they are at the cutting edge of the technology. It is also important to emphasize that each bioanalytical technique has its own characteristics, which will vary from analyte to analyte. In these instances, specific validation criteria may need to be developed for each analyte.[1]Carfilzomibis an anti-cancer medication acting as a selective proteasome inhibitor. Chemically, it is (2S)-4-methyl-N-[(2S)-1-[[(2S)-4-methyl-1-[(2R)-2-methyloxiran-2-yl]-1-oxopentan-2-yl]amino]-1-oxo-3-phenylpropan-2-yl]-2-[[(2S)-2-[(2-morpholin-4-ylacetyl)amino]-4phenylbutanoyl] amino]pentanamide. The proteasome is a multienzyme catalytic complex found in the nucleus and cytoplasm of eukaryotic cells that is responsible for degrading or processing intracellular proteins.[2,3](fig no.1)

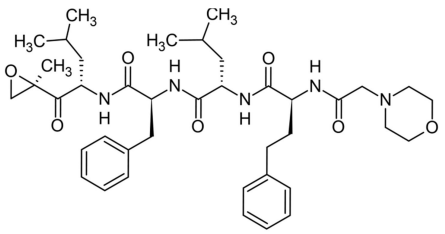

Figure 1: Chemical structure of Carfilzomib

2. EXPERIMENTAL

2.1. Chemicals and reagents

Working standards of pharmaceutical grade Carfilzomib was received as a gift sample from Swapnroop drug and pharmaceutical, Aurangabad Maharashtra, India. A Carfilzomib product containing the injection was purchased from local pharmacy shop. TEA, OPA, HPLC grade Acetonitrile, water and methanol were purchased from Merck Ltd., India.

2.2. Equipment and chromatographic condition

The modular HPLC system used was equipped with Agilent Quaternary Gradient HPLC pump ((G130A) S.NO.DE9180834), An Auto injector, solvent degasser and DAD detector (G13148 S.NO. DE71365875). A Data Ace Chromatography data system was used to record and evaluate the data collected during and following chromatographic analysis. The chromatographic separation was achieved on aC-18 Agilent, (100 mm × 4.6 mm i. d., and 2.5µm particle size) column using a mixture of methanol: 0.05%OPA in the ratio 68:32 v/v (pH 3) as mobile phase at a flow rate of 0.7 ml/min. The eluent was monitored using Diode array detector (DAD) detection at a wavelength of 256 nm. The mobile phase was filtered through a 0.45 µm nylon filter

prior to use and sonicated using an ultrasonic bath (Ultrasonic electronic instrument). Other equipments used were analytical balance (WENSAR™ High Resolution Balance.) and Micro pipettes.

2.3. Preparation of standard and stock solution

A stock solution of Carfilzomib (500µg/ml) was prepared by accurately weighing approximately 5 mg into a 200 ml A-grade volumetric flask and making up the volume with HPLC grade Methanol. The stock solution was protected from light using aluminium foil. Aliquots of the standard stock solution of marketed formulation were transferred using A-grade bulb pipette into 10 ml volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations of 5,10,15,20, and 25µg/ml.

2.4. Preparation of sample

Protein precipitation technique

Frozen human plasma was thawed to ambient temperature and aliquots of 200 ml plasma were taken in centrifugation tubes of 10 ml capacity with the help of micropipette and 500 ml of stock solution was added and the plasma proteins were precipitated by using methanol. The tube was vortexed for 30 sec. Then the solution was centrifuged at 5000 RPM for 1 hr. below the temperature 10 oC. The supernatant liquid was taken and transfer to HPLC vials.

2.5. Method validation

The method performance was evaluated for accuracy, precision, linearity which include freeze thaw stability, stock solution and formulation.

2.6. Linearity and Range

Working solution of various concentrations was injected under the operating chromatographic condition and peak areas of each drug were calculated at 256 nm. The calibration curves were constructed using simple linear regression between peak area and concentrations (Fig. 4). The range of solution has been decided according to correlation coefficient of regression equation.

2.7. Accuracy

The accuracy of the method was performed by calculating % recovery for the different concentration levels of drug. The samples of three concentration levels prepared as 80%, 100% and 120% by standard addition method.

2.8. Precision

The precision of this method was evaluated by the % RSD at different concentration levels. Intraday and interday precision was evaluated in 2 replicate batches of different concentrations (5, 15 and 25 µg/ml).

2.9 Limit of detection and limit of Quantitation

The LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively; where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve.

2.10 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

3. RESULTS AND DISCUSSION:

3.1. Optimization of Chromatographic conditions

The chromatographic conditions were optimized in order to provide good system suitability parameters. The mobile phase was selected on the basis of its polarity and different trials were taken. Methanol was selected as an organic modifier finally, a mobile phase consisting Methanol: 0.05% OPA in water (PH3) (68:32v/v) at a flow rate of 0.7ml/min was selected. The retention time of Carfilzomib was found to be 3.5 min. The chromatogram of Carfilzomib obtained by optimized conditions is shown in fig. 3. The optimized chromatographic conditions and system suitability parameters are listed in table 1.

Chromatograms of drug free human plasma and spiked drug-plasma of Carfilzomib are shown in fig.2 and 3, respectively. The retention time of Carfilzomib was found to be 3.5 min indicating this method is faster than other methods. The typical column efficiency expressed as the number of theoretical plates was found to be 5900 for Carfilzomib.

3.2. Linearity and Range

The calibration curve was found to be linear in the range 5-25 µg/ml (R^2= 0.999) and equation is y = mx-c, where y represents the area of Carfilzomib and x represents concentration of Carfilzomib in µg/ml (fig. 4).

3.3 Accuracy

The mean % recovery of calculated concentrations for all quality control samples at 80%, 100% and 120% concentration levels are ranged from 98.2-102.88 %, which are within the acceptance criteria 98-102 % (Table 2).

3.4. Precision

The % RSD of calculated concentrations for all quality control samples at 5, 15 and 15µg/ml concentration levels are ranged from 0.13-0.55 % for intraday and 0.03-1.26 % for interday precision, which is within acceptance criteria >2 % (Table 3).

3.5 Limits of Detection and Quantitation

The limits of detection and quantitation were found to be 1.265µg/mL and 3.833 µg/mL respectively. This indicates the method is sufficiently sensitive.

3.6 Robustness:

There were no significant changes in the retention times of Carfilzomib,when the flow rate (± 1 mL/min.) and mobile phase (± 1) and wavelength (± 1) were changed. The low values of the % RSD indicate the robustness of the method, as shown in Table 4.

3.6 Analysis of Carfilzomib from marketed formulation

The percentage assay of injection was found to be 100.99 for Carfilzomib, as shown in Table 5.

Table 1: Optimized chromatographic conditions and system suitability parameters

Sr.no	Parameter/condition	Details
1	Column	Agilent C18 2.5 (100mm X 4.6)
2	Mobile Phase	Methanol:0.05% OPA water (68:32)
3	Flow Rate	0.7ml/min
4	Column temperature	25^0c
5	Volume of injection	20
6	Wavelength	256nm
7	Theoretical Plate	5900
8	Retection Time	3.257
9	Railing Factor	0.77

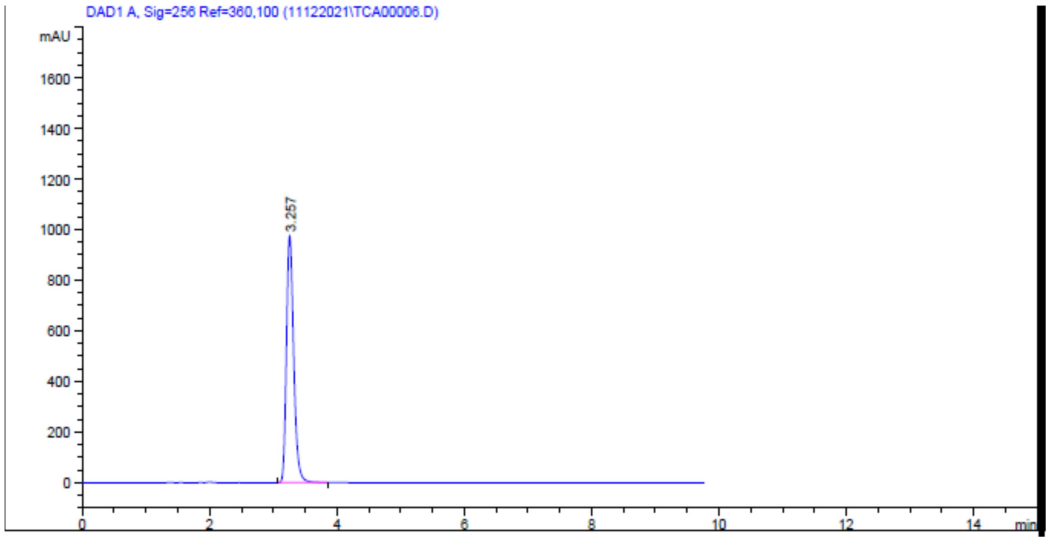

Fig. 2. Chromatogram of drug free human plasma

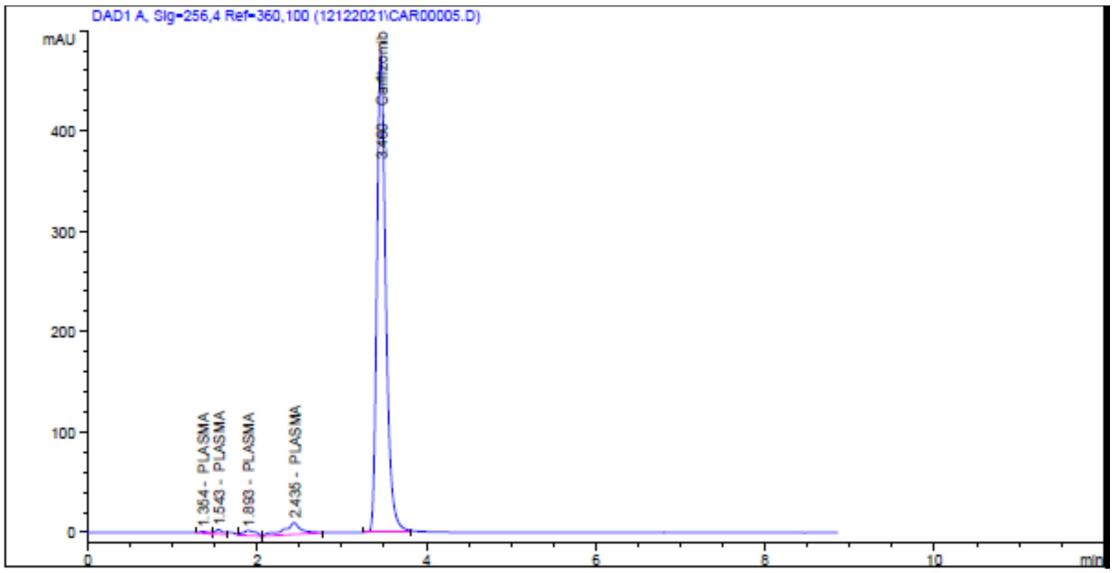

Fig. 3. Chromatogram of spiked drug-human plasma

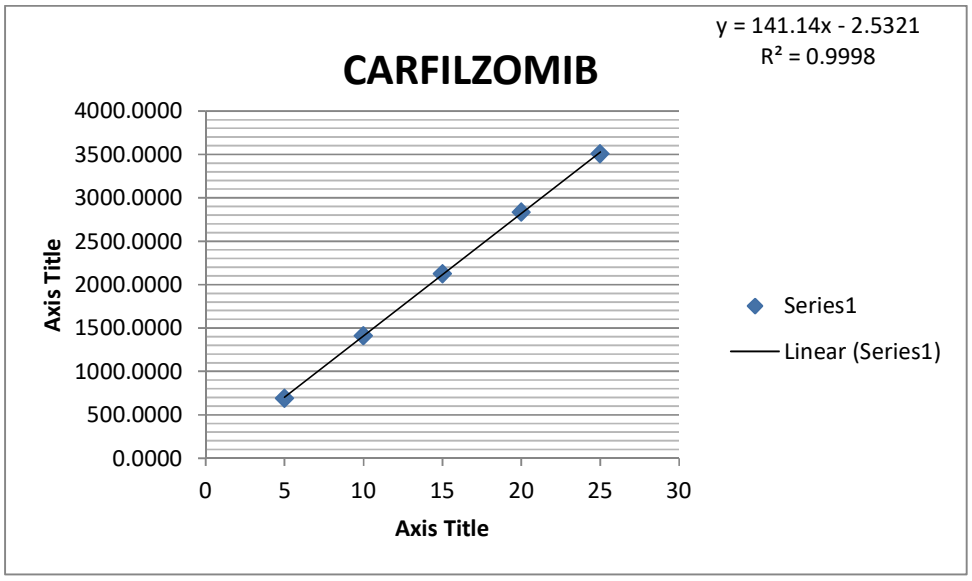

Fig. 4. Calibration curve of Carfilzomib

Table 2: Results of Accuracy

Level of Recovery (%)	Mean % Recovery	Standard Deviation*	% RSD
80%	99.3	0.22	0.23
100%	102.33	0.2	0.20
120%	98.6	0.18	0.18

(N=2 and Concentrations are given in µg/ml)

Table 3: Results of intraday and interday precision

Conc^{n} (µg/ml)	Intraday Precision			Interday Precision		
	Mean± SD	%Amt Found	%RSD	Mean± SD	%Amt Found	%RSD
5	695.20±2.76	98.54	0.52	683.46±8.60	97.23	1.26
15	2133.19±1.97	100.79	0.13	2108.76.±0.63	99.75	0.03
25	3514.66±2.20	99.64	0.27	3531.32±1.66	100.18	0.05

(N=2 and Concentrations are given in µg/ml)

Table 4: Results of Robustness (N=2 and Concentrations are given in µg/ml)

Parameters	Conc.(µg/ml)	Amount of detected(mean ±SD)	%RSD
Mob-phase composition(69ml+31ml) Methanol + 0.05% (OPA)water	20	2653.8±3.31	0.12
Mob-phase composition (67ml+33ml) Methanol + 0.05% (OPA)water	20	3042.91±3.73	0.12
Wavelength change255nm	20	3077.8±3.52	0.11
Wavelength Change 257nm	20	2642.84±3.51	0.13
Flow rate change(0.6ml)	20	3263.51±3.40	0.10
Flow rate change(0.8ml)	20	2493.8±3.51	0.14

Table 5: Results of Robustness

Sample	Drug	%Label claimed± SD	%RSD
carfilnat	Carfilzomib	100.99 ± 0.11	0.115

4. CONCLUSION:

The work describe in this paper deals with analysis of Carfilzomib using RP-HPLC method in human plasma. The precision and accuracy of the method met the acceptance criteria laid down in guideline for industry, Bioanalytical method validation. From the results, we can conclude that developed method is simple, accurate, rapid and precise. Thus, it can be used for routine analysis of Carfilzomib in human plasma.

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