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Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Avibactam and Ceftazidime in Bulk drug and injection dosage Form

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Abstract:

The aim of the present work was to develop simple, shorter and effective HPLC method with UV detection (255 nm) and subsequent validation for the simultaneous determination of Avibactam and Ceftazidime in marketed tablet samples. The mobile phase consisted of 1% OPA: Acetonitrile 55:45 (v/v) [pH 5.5], an isocratic elution at a flow rate of 1.5 ml/min at ambient temperature. The detection was carried out at 255nm. Retention times were found to be 3.00 min for Avibactam and 5.05 min for Ceftazidime. Good linear relation was observed with in a concentration range of 5-30µg/ml for Avibactam and 10-60µg/ml for Ceftazidime with high r^2 value. The developed method was validated as per ICH guide lines. The developed methods were found to be precise and accurate for the estimation of Avibactam and Ceftazidime in pharmaceutical dosage forms.

Key Words: Avibactam, Ceftazidime analytical method validation, HPLC

Introduction:

Avibactam is a non-β-lactam β-lactamase inhibitor antibiotic drug. Ceftazidime is an antibiotic useful for the treatment of a number of bacterial infections. It is a third-generation cephalosporin.

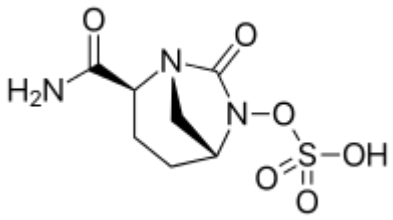


Figure.1 Structure of Avibactam

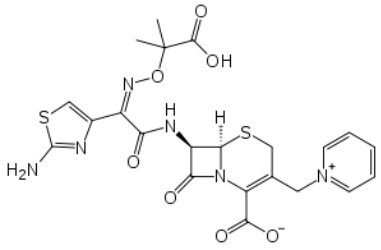


Figure.1 Structure of Ceftazidime

Materials and Methods:

Instrumentation:

Chromatographic separation was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump; Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L) Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver

electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

Chemicals and Solvents:

The drug samples, Avibactam and Ceftazidime obtained as gift sample from Mars Pharma, Himachal Pradesh. The pharmaceutical formulation was procured from local market.Methanol, Acetonitrile and water used were HPLC grade and were purchased from Merck Specialties Private Limited, Mumbai, India. Orthophosphoric acid and reaming buffer solutions used were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.

Preparation of standard stock solution:

Standard stock solution of Avibactam and Ceftazidime drug (1mg/ml) was prepared by accurately weighing about 10 mg of each drug in 10 ml volumetric flask. The drugs were dissolved with few ml of methanol, and sonicated to dissolve it completely and made up to the mark with the same solvent. The contents were mixed well and filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. From this stock solution selected concentrations were prepared by selected dilutions. 1ml from the selected concentrations of both the drugs were mixed and used as a combined standard solution for the estimation in pharmaceutical formulations.

Preparation of Formulation Solution:

Marketed formulation of Avibactam and Ceftazidime were purchased (AVYCAZ Injection) and mixed with mobile phase. It was filtered through 0.45µm nylon membrane filter paper. This was further diluted with mobile phase to get linearity range concentrations.

Development and Validation:

Method was developed with optimization HPLC conditions i.e Mobile phase, Flow rate, PH of Mobile phase, Detector wavelength. Finally the conditions are developed and validated to analysis of Avibactam and Ceftazidime in formulations and Bulk drug. The conditions are showed in Table.1

S.NO	Parameter	Results
1	MP	1% OPA: Acetonitrile 55:45 (v/v)
2	Wavelength	245nm
3	Stationary Phase	RP- C8 Column
4	pH of MP	5.2
5	Flow Rate	1.5 ml/min
6	Pump Mode	Isocratic
7	Pump Pressure	15.0±5MPa
8	API Concentration	Avibactam:20 µg/ml Ceftazidime:30 µg/ml
9	RT	Avibactam:3.0 Min Ceftazidime: 5.05 Min
10	Resolution	Avibactam:0.0 Ceftazidime: 15.51
11	Area	Avibactam :701665 Ceftazidime: 758553

12	Theoretical Plates	Avibactam:8125 Ceftazidime:23452
13	Tailing Factor	Metronidazole– 1.47 DiloxanideFumarate- 1.37

Table.1 HPLC conditions for analysis of Avibactam and Ceftazidime

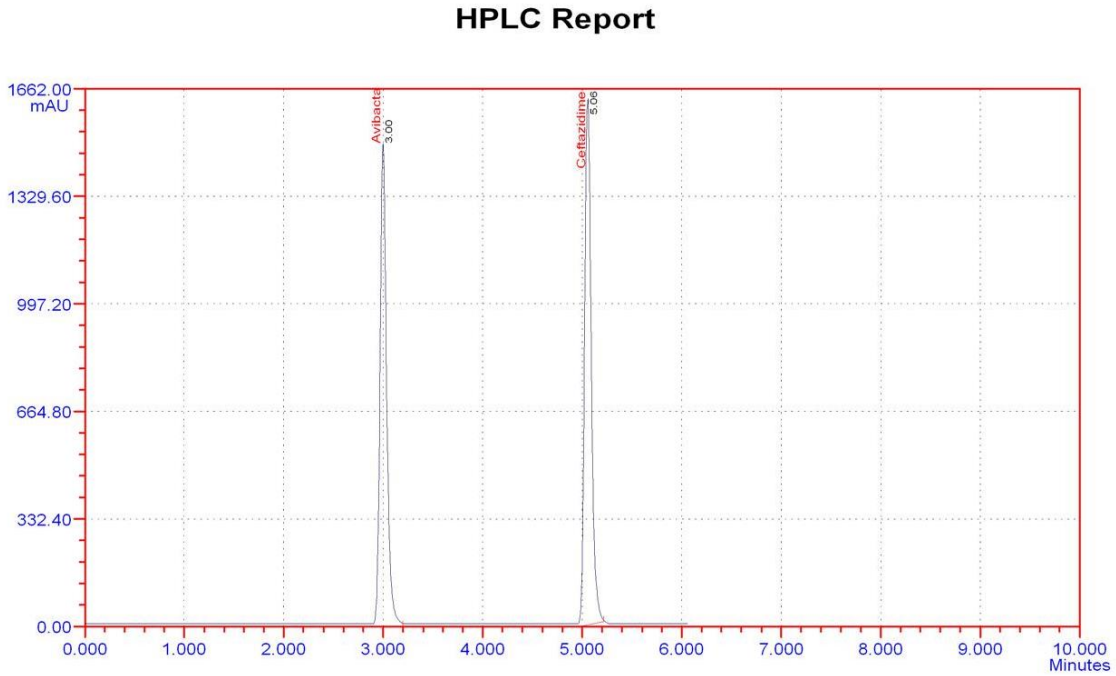
Validation Parameters:

Parameters	Avibactam	Ceftazidime
Linearity range	5-30 µg/ml	10-60 µg/ml
% Accuracy	99.85	100.02
Precession % RSD	0.25	0.41
Robustness % RSD	0.58	0.93
Ruggedness % RSD	1.25	0.85
LOD	0.2 µg/ml	1.0 µg/ml
LOQ	1.5 µg/ml	3 µg/ml
% Assay of Formulation	99.8	99.92

Table.2 Validation parameters of developed method

Results and Discussion:

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drug products. The objective of this study was to develop a rapid and sensitive HPLC method for estimation of Avibactam and Ceftazidime in tablet formulations using the most commonly employed RP C-8 column with UV detection. The mobile phase was optimized with 1% OPA: Acetonitrile 55:45 (v/v) at pH 5.2. From the overlain spectrum of Avibactam and Ceftazidime wavelength was selected, at 255nm, iso-absorptive point for both the drugs. Good resolution was carried out at 255nm and both drugs showed good absorbance at this wavelength with minimum interference of the other drug. Optimized chromatographic conditions were shown in table 1. Standard and blank and formulation chromatograms were shown in figures 3. All parameters of these proposed method was validated as per the ICH guidelines.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate	Res
1	Avibactam	3.000	149278	701665.2	48.052	1.47	8120	0.00
2	Ceftazidime	5.057	162721	758553.5	51.948	1.37	23452	15.51
	Sum:		311999	1460218.7	100.0000			

Figure 1.C:Standard chromatogram of Avibactam and Ceftazidime

Conclusion:

The method developed in the present investigation is simple, sensitive, accurate, rugged, robust, rapid and precise. The absence of additional peaks in the chromatogram indicated that there is no interference of the common excipients used in the tablets. Hence, the above said method can be successfully applied for the simultaneous estimation of Avibactam and Ceftazidime in tablet dosage forms.

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