Method Development and Validation for the Simultaneous Estimation of Ceftazidime and Avibactam in Bulk and Pharmaceutical Dosage forms by RP-HPLC Method

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Abstract— **A rapid, sensitive, and highly precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the simultaneous quantification of Ceftazidime and Avibactam using a Waters HPLC system. Chromatographic separation was achieved on an Inertsil ODS C18 column (250 × 4.6 mm, 5 µm particle size) maintained at ambient temperature. The mobile phase consisted of acetonitrile and water (85:15, v/v) and was filtered through a 0.45 µm membrane filter prior to use. The flow rate was set at 1.0 mL/min, and detection was performed at 260 nm using a photodiode array (PDA) detector.**

Index Terms—**Ceftazidime, Avibactam, RP-HPLC**

I. INTRODUCTION

Avycaz® (ceftazidime and avibactam) is a combination antibiotic medication used to treat serious, multidrug-resistant Gram-negative bacterial infections, particularly when other treatment options are limited. It is administered intravenously.

Ceftazidime (5-6): A third-generation cephalosporin antibiotic that kills bacteria by inhibiting cell wall synthesis through binding to penicillin-binding proteins.

Avibactam (7-8): A novel, non-beta-lactam beta-lactamase inhibitor. Avibactam protects ceftazidime from degradation by certain beta-lactamase enzymes produced by resistant bacteria, thereby expanding ceftazidime's spectrum of activity.

II. MATERIALS AND METHODS

Preparation of Stock solution: 100 mg of Avibactam and 100 mg of Avibactam API standards were accurately weighed and are transferred into two separate 100 ml volumetric flasks and dissolved in 100ml of mobile phase. The mixture was then sonicated for 20 minutes to obtain 1000ppm.

Preparation of working standard solution: From the stock solutions of both standards, each 4 ml was pipetted out and transferred into 100ml volumetric flasks, made up to 100 ml with mobile phase and sonicated for 10 minutes, to get 40ppm Ceftazidime and Avibactam.

III. RESULTS AND DISCUSSION

Method validation: Validation parameters include specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness, and assay (1-4).

Specificity: Specificity is the ability to assessing equivocally the analyte in the presence of components which may be expected to be present. Typically, these components include impurities, degradants, matrix, etc. Blank solution and standard solutions of Ceftazidime (40µg/ml) and Avibactam (40µg/ ml) were injected into the HPLC system. The peak purity data of Ceftazidime and Avibactam were compared. There should not be any interference at the retention time of the main peaks.

Linearity: Linearity for the drugs Ceftazidime and Avibactam (8-16) was determined by preparing the standard solutions at six concentrations levels in six replicates in the range of 20-70µg/ml Ceftazidime and 20-70µg/ml for and Avibactam from stock solution. The linearity charts of Ceftazidime and Avibactam was shown in the figure no 2&3. The correlation

coefficient was found to be 0.999 for both the drugs. Linearity results were tabulated in table 2.

Accuracy: Accuracy was performed by spiking known amounts of standard solution to sample solution at three different concentrations levels (50%, 100%, 150%) and there by analyzed for %RSD which should not be more than 2.0. The % recovery was calculated and the results were reported in table no. 3 & 4.

Precision: The precision of the analytical method was studied by injecting six replicates of standard containing 40µg/ml of Ceftazidime and 40µg/ml of Avibactam which were injected into the HPLC system. The % RSD was calculated and the results were reported in the table no.5 & 6.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection was defined as the concentration which yields a signal - to – noise ratio 3:1 where as the limit of quantification was calculated to be the lowest concentration that could be measured with signal - to – noise ratio10:1. LOD and LOQ were calculated from slope and standard deviation. The results were tabulated in table no. 7.

Robustness: The smallest deliberate changes in method like change in flow rate are made but there were no predictable changes in the results and are in the range as per ICH guidelines. Conditions like decrease in flow rate (0.8 ml/min), increase in flow rate (1.2 ml/min) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was found to be within the limits and results were tabulated in table no. 8.

Assay: Assay was conducted on marketed formulation and mean % assay was found. The results were tabulated in table no. 9.

Table 1: Optimised Chromatographic Conditions

Parameter	Method
Stationary Phase (column)	Inertsil -ODS C18 (250 x 4.6 mm, 5 µ)
Mobile Phase	Acetonitrile: Water (85:15)
Flow rate (ml/min)	1.0 ml/min
Duration of operation	12 min
Injection volume (ml)	20
Detection wavelength (nm)	260nm
Drug RT (min)	5.487minfor Ceftazidime and 8.320 for Avibactam

Figure 1: Optimised Chromatogram

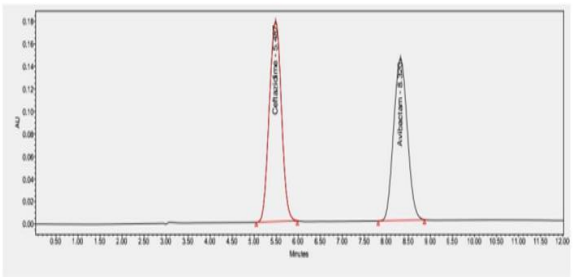

Table 2: Linearity data of Ceftazidime and Avibactam

Ceftazidime		Avibactam	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
20	1689893	20	1342642
30	2561950	30	2024703
40	3405421	40	2712395
50	4210642	50	3338671
60	5022250	60	4016478
70	5815852	70	4671575

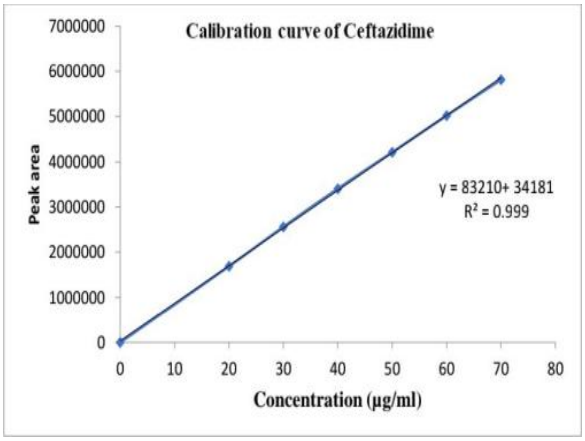

Figure 2: Calibration curve of Ceftazidime

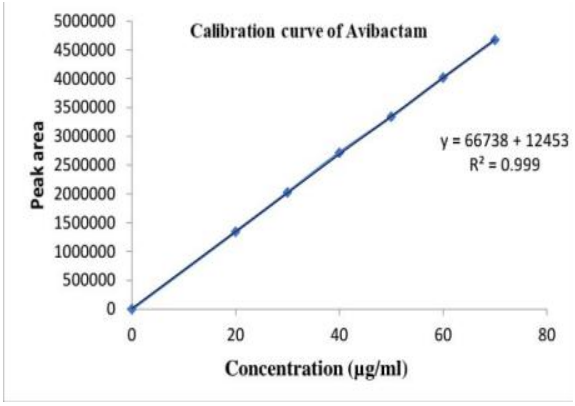

Figure 3: Calibration curve of Avibactam

Table 3: Accuracy Data of Ceftazidime

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% - 1	20	20.16	100.55	MEAN	99.98
50% - 2	20	20.16	100.31		
50% - 3	20	19.82	99.05	%RSD	0.84
100 % - 1	40	39.92	99.82	MEAN	99.91
100 % - 2	40	40.15	100.22		
100% - 3	40	39.81	99.52	%RSD	0.52
150% - 1	60	60.12	100.21	MEAN	99.99
150% - 2	60	59.88	99.84		
150% - 3	60	60.14	100.14	%RSD	0.54

Table 4: Accuracy Data for Avibactam

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% - 1	20	20.15	100.51	MEAN	99.98
50% - 2	20	20.12	100.5		
50% - 3	20	19.91	99.42	%RSD	0.98
100 % - 1	40	39.99	99.98	MEAN	100.04
100 % - 2	40	40.15	100.4		
100% - 3	40	40.05	100.05	%RSD	0.376
150% - 1	60	60.13	99.21	MEAN	100.14
150% - 2	60	60.83	101.27		
150% - 3	60	60.11	100.15	%RSD	0.93

Table 5: System Precision data of Ceftazidime and Avibactam

S. No	Ceftazidime	Avibactam
1	3406244	2713524
2	3401137	2706312
3	3402371	2703412
4	3406712	2706784
5	3403124	2706345
Mean	3403918	2707275
SD	2448.179	3741.862
% RSD	0.071922	0.138215

Table 6: Method Precision data of Ceftazidime and Avibactam

S. No	Ceftazidime	Avibactam
1	3406321	2715432
2	3402262	2703642
3	3401712	2712412
4	3406721	2710567
5	3403441	2712323
6	3401217	2710046
Mean	3403612	2712737
SD	2374.966	3954.983
% RSD	0.069778	0.145901

Table 7: LOD and LOQ data of Ceftazidime and Avibactam

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
Ceftazidime	0.094	0.286
Avibactam	0.189	0.571

Table 8: Robustness data of Ceftazidime and Avibactam

S. No	Drug name	Condition	Peak area	% RSD
1	Ceftazidime	Decreased Flow rate of 0.8 ml/min	3395690	0.047
2		Increased Flow rate of 1.2 ml/min	3413640	0.072
3	Avibactam	Decreased Flow rate of 0.8 ml/min	2646749	1.332
4		Increased Flow rate of 1.2 ml/min	2804218	0.062

Table 9: Assay data Ceftazidime and Avibactam

S. No	Peak area of Ceftazidime	% Assay	Peak area of Avibactam	% Assay
1	3403451	101.26	2715343	101.28
2	3407112		2717024	
3	3402143		2713451	
4	3402234		2713372	
5	3406341		2710842	
6	3401218		2715542	

IV. CONCLUSION

The developed RP-HPLC method was validated as per ICH guidelines. All the system suitability parameters were within the range as stated by ICH guidelines. Interference peaks were not observed in blank, standard and sample chromatogram. Hence simple, precise and accurate, sensitive, specific and robust method was developed and validated. This can be used in quality control department with respect to routine analysis.

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