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Stability indicating analytical method development and validation for estimation of Ceftazidime and Avibactam in bulk and pharmaceutical dosage form using RP-HPLC

Syeda Saniya Fatima[1*], R. Vani[2]

Deccan School of Pharmacy, Aghapura, Dar-Us-Salam, Hyderabad, Telangana

Corresponding Author: Syeda Saniya Fatima

Email: ainas_tremendous.queen@yahoo.co.in

ABSTRACT

A new simple, rapid, specific, accurate and precise stability indicatind RP-HPLC method has been developed for the estimation of Ceftazidime and Avibactam in bulk & pharmaceutical dosage form. From UV spectrophotometric method selected wavelength for estimation of drugs were 231 nm as isobestic point and 256 nm, 210 nm as λ max of CAZ and AVY respectively by using methanol as a solvent. RP-HPLC method was developed by using Hypersil ODS (150×4.6mm) 5µ. The samples were analyzed by using mixed phosphate buffer (P^H adjusted to 4 using orthophosphoric acid): Acetonitrile (60:40 % v/v) as the mobile phase at the flow rate of 1.0 ml/min and detection wavelength is 231 nm. Both the drugs were eluted within 5 minutes and gave sharp peaks with high theoretical plate count and low tailing factor. The retention time for Ceftazidime and Avibactam was found to be 2.523 and 4.410 min respectively. Calibration curve was linear with correlation coefiicient of 0.996 and 0.999 over a concentration range of 240-560 µg/ml and 60-120 µg/ml for Ceftazidime and Avibactam respectively. The percent recovery was 100.10 and 99.75 for Ceftazidime and Avibactam respectively indicating accuracy and reliability of the method. Forced degradation studies were carried out and drug peaks were well resolved without any significant degradation products when subjected to stress conditions. So the developed stability indicating method could be utilized for routine analysis of Ceftazidime and Avibactam in bulk and pharmaceutical dosage form.

Keywords: Ceftazidime, Avibactam, Stability indicating, UV, RP-HPLC.

INTRODUCTION

Avibactam (AVIB) is chemically named as (1R,2S,5R)-2-Carbamoyl-5-methyl-7- oxo-1,6-diazabicyclo [3.2.1]octan-6-yl hydrogen sulphate. Avibactam is a non β lactam β-mlactamase inhibitor antibiotic used for treating complicated urinary tract and complicated intraabdominal infections caused by antibiotic resistant-pathogens, includind those caused by multi-drug resistant gram negative bacterial pathogens.[4,5] Ceftazidime (CEF) is chemically named as (7R,Z)-7-(2-(2-aminothizol-4yl)-2- (2-carboxypropan-2-yloxyimino) acetamido)-8-oxo-3-(pyridinium-1-ylmethyl)-5-thia-1aza bicycle[4.2.0]oct-2-ene-2-carboxylate. [1-3]

Ceftazidime is a semi synthetic broad spectrum beta lactam antibiotic for parentral administration. Ceftazidime is bactericidal in action exerting its effect by inhibition of enzymes responsible for cell-wall synthesis, primarily penicillin binding protein 3 (PBP3). It is a third generation cephalosporin. As a class cephalosporin's have activity against gram-positive and gram-negative bacteria. The balance of activity tips toward gram positive organisms for earlier generations; later generations of cephalosporin's have

more gram-negative coverage. Ceftazidime is one of the few in this class with activity against pseudomonas. It is not active against methicillin-resistant Staphylococcus aureus. [4-6] The review of literature revealed that several analytical methods have been reported for ceftazidime in spectrophotometry, HPLC, HPTLC, LC/MS individually and in the combination with other drugs. [6-12] To date there have been no published reports about the stability indicating method for estimation of avibactam and ceftazidime by HPLC in bulk and in pharmaceutical dosage forms. This present study report for the first time stability indicating simultaneous estimation of avibactam and ceftazidime by RP-HPLC in bulk drug and in pharmaceutical dosage form.

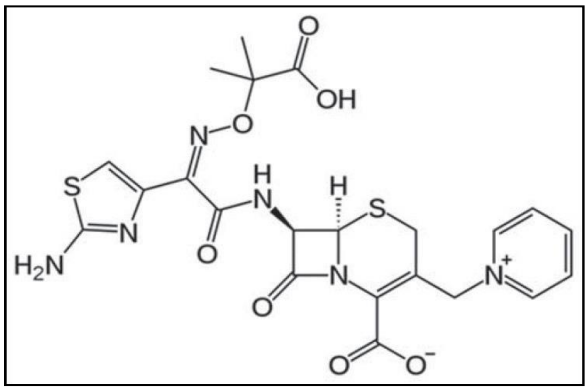

Fig 1: Structure of Ceftazidime

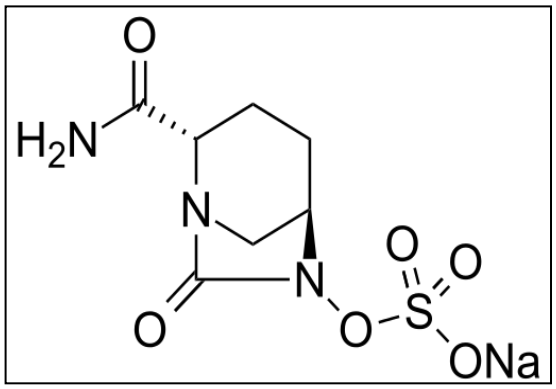

Fig 2: Structure of Avibactam

MATERIALS AND METHODOLOGY

Chemicals

Avibactam and ceftazidime were obtained as gift sample from Chandra Pharma Research Laboratory in Hyderabad and marketed formulation was purchased from local market.

Instrument and chromatographic condition

All solvent used in this work are HPLC & AR grade. Instrument and chromatographic condition RP-HPLC Agilent 1220 infinity series separation model equipped with UV Detector was employed in this method. The EZchrom software was used for LC peak integration along data acquisition and data processing. The column used for separation of analyte is Hypersil ODS (150×4.6mm) 5µ. Mobile phase consisting of phosphate buffer: Acetonitrile in the ratio of 60:40 % v/v at flow rate 1 ml/min. it was filter through 0.45 µm nylon filter and sonicated for 5 min in ultrasonic bath. Sample was analyzed at 231 nm at an injection volume of 20µl.

Preparation of phosphate buffer

1.625 gm of Potassium Di Hydrogen ortho phosphate and 0.3 gms of Di Potassium Hydrogen ortho phosphate was weighed and dissolve in 100 ml of water and volume was made up to 550 ml with water. Adjust the P^H using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of Solutions

Preparation of Mixed Standard Solution: (400µg/ml & 100µg/ml)

Weigh accurately 400 mg of Ceftazidime & 100 mg of Avibactam and dissolve in 100 ml of diluents. From the above stock solution 400 µg/ml of Ceftazidime and 100 µg/ml of Avibactam is prepared by diluting 1 ml to 10 ml with mobile phase. This solution (400 µg/ml of Ceftazidime & 100 µg/ml of Avibactam) is used as working standard concentration for estimation of IV infusion.

Preparation of Sample Solution

5 bottles were weighed & calculate the average weight of each bottle then the weight equivalent to 1 bottle was transferred into a 500 ml of volumetric flask. Add sufficient quantity of diluents & sonicated for 25 minutes further the volume is made up with diluents and filtered. From the filtered solution 1 ml was pipette out into a 10 ml with diluents.

Table 1: Summary of Chromatographic conditions

S. No	Parameter	Description
1.	Column	ODS Hypersil C-18 (150×4.6mm) 5µ
2	Mobile Phase & P^H	Phosphate Buffer : Acetonitrile P^H 4, 60:40 v/v
3	Flow rate	1 ml/min
4	Column & sample temperature	Room temperature (20-25^{o}C)
5	Detection Wavelength	231 nm
6	Detector	UV
7	Injection Volume	20 µl
8	Retention time	Ceftazidime – 2.523 Min Avibactam - 4.410 Min
9.	Run time	10 mins

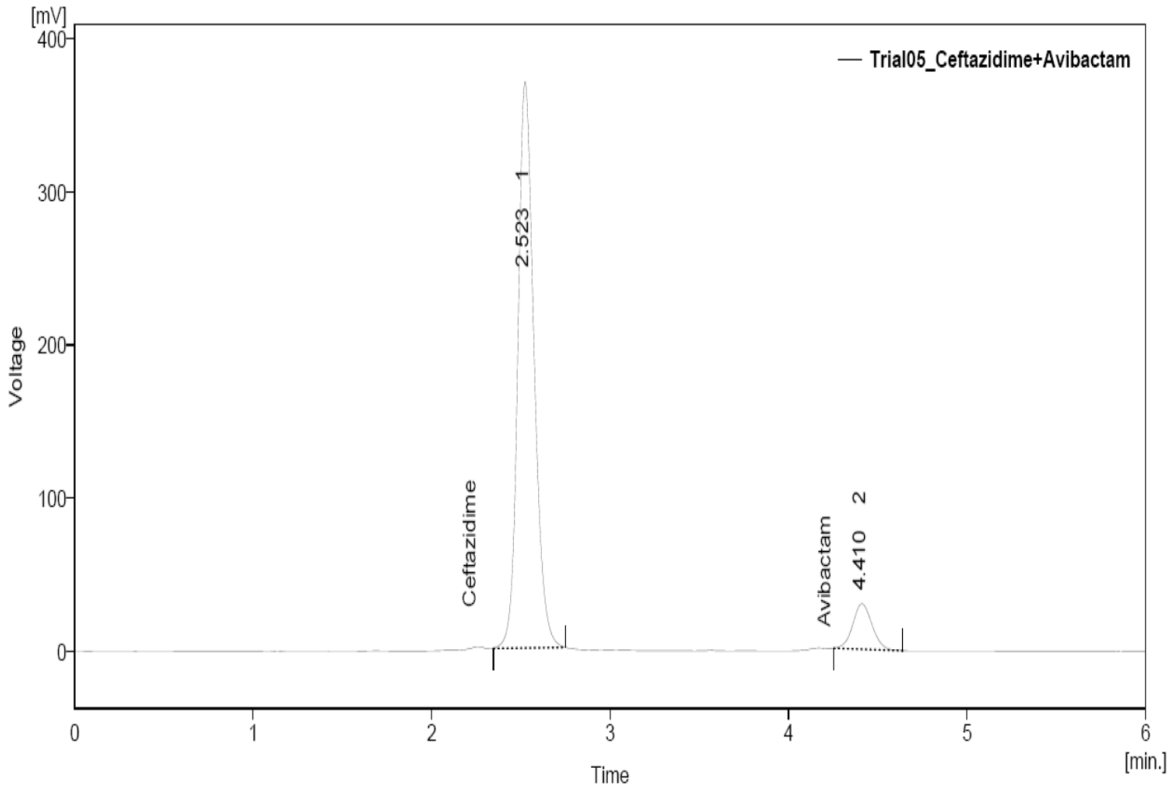

Fig 3: Typical chromatogram of Avibactam and Ceftazidime

Method Validation

The validation of method was carried out as per ICH guideline. The parameters assessed were specificity, linearity, precision, accuracy, stability, LOD and LOQ. Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation product and related substances.

Accuracy

The accuracy was determined by calculating % recoveries of avibactam and ceftazidime. It was

carried out by adding known amount of each analyte corresponding to three conc. Levels (80, 100, and 120) of the label claims to the excipients. At each level, six determinations were performed and the accuracy results were expressed as percent analyte recovered by proposed method.

Precision

Method precision

Precision of an analytical method is usually expressed as the standard deviation. Method precision was demonstrated by preparing six samples as per test method representing single batch and were chromatographed. The precision of the method was evaluated by computing the %RSD of the results. The individual values and the low % RSD observed on the values are within acceptance criteria and indicates that method is precise.

Linearity

The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits linear responses and directly proportional over the relevant conc. Range for the target conc. of the analyte. The linear regression data for the calibration plot is the indicative of a good linear relationship between peak and concentration over wide range. The correlation coefficient was indicative of high significance.

Robustness

Robustness of method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase, flow rate and temperature. This deliberate change in the method has no effect on the peak tailing, peak area and theoretical plates finally the method was found to be robust.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts. % RSD Assay values between two analysts not greater than 2.0%, hence the method was rugged.

Limit of Detection and Limit of Quantitation

The LOD can be defined as the smallest level of analyte that gives a measurable responses and LOQ was determined as the lowest amount of the analyte that was reproducibly quantified. These two parameters were calculated using formula based on standard deviation of the response and slope. LOD and LOQ were calculated by equation, LOD=3.3 x σ/s and LOQ= 10 x σ/s, where s = standard deviation, S = slope of calibration curve.

Assay of avibactam and ceftazidime in pharmaceutical dosage form

Assay of marketed product was carried out by using the developed method. Sample solutions were prepared and injected into RP –HPLC system. The sample solution was scanned at 231 nm. The percentage drug estimated was found to be 100.23% and 101.21% respectively as ceftazidie avibactam. The chromatogram showed two single peaks of ceftazidime and avibactam was observed with retention time 2.523 and 4.410 min respectively.

Forced degradation studies

Stress studies are performed according to ICH guidelines under following conditions.

Acid degradation

To 5 ml of sample solution add 1 ml of 0.1 N HCL and sonicate place it aside for 3 hrs, then neutralize the solution with 1ml of base and then transfer above solution into 10 ml volumetric flask dilute with mobile phase and record the chromatogram.

Alkaline degradation

To 5 ml of sample solution add 1 ml of 0.1 N NaOH place it aside for 3 hrs, then neutralize the solution with 1ml of acid and then transfer above solution into 10 ml volumetric flask dilute with mobile phase and record the chromatogram.

Peroxide degradation

To 5 ml of sample solution add 1 ml of 3% H_20_2 and sonicate place it aside for 3 hrs, then transfer above solution into 10 ml volumetric flask dilute with mobile phase and record the chromatogram.

Photolytic degradation

Expose about 100 mg of sample in UV light chamber at 231 nm for 3 hrs. weigh accurately this

power equivalent to 40 mg of Ceftazidime and 10 mg of Avibactam into a 100 ml volumetric flask and make up the volume and sonicate for 30 minutes with intermittent shaking and volume is made up to the mark with mobile phase and record the chromatogram.

Thermal degradation

Expose about 100 mg of sample in to dry heat at 80^0C for 3 hrs. weigh accurately this power equivalent to 40 mg of Ceftazidime and 10 mg of Avibactam into a 100 ml volumetric flask and make up the volume and sonicate for 30 minutes with intermittent shaking and volume is made up to the mark with mobile phase and record the chromatogram.

Records the peak area of stressed samples then compare it with peak area of unstressed sample to determine the % degradation.

$$\% \text{ degradation} = \frac{\text{Response of unstressed sample} - \text{response of stressed sample}}{\text{Response of unstressed sample}} \times 100$$

RESULTS & DISCUSSION

Ceftazidime/avibactam (AVY-CAZ) is a recent combination in the market used in the treatment of complicated intra-abdominal infections (cIAI), complicated urinary tract infections (cUTI), acute pyelonephritis (AP). Literature survey reveals that various methods for the estimation of Ceftazidime individually and in combination with other drug is reported, but no method has been reported for the estimation of the Ceftazidime and Avibactam in combine dosage form by RP-HPLC method. So here an attempt has been made to develop simple, accurate, sensitive, rapid and economic method for estimation of Ceftazidime and Avibactam from combine dosage form using HPLC. Specific objectives includes

The conditions in HPLC were optimized in order to obtain the drugs separation of eluted compounds. Initially various composition of mobile phase & PH range were tried in order to have a good separation of the titled ingredients. The composition of Mobile phase & PH selections were based on peak parameters like height, tailing, capacity factor, symmetric factor, theoretical plates, run plates, run time & resolution. The system with phosphate buffer: acetonitrile of 70:40 was found to be robust with PH 4. The optimum wavelength for detection was 231 nm at which both drugs have good response. CAZ was eluted at 2.523 min and AVI was eluted at 4.410 min.

System suitability test were carried out on a freshly prepared stock solution % RSD of peak area of five replicated injections of standards were taken & was found to be less than 2%. Specificity of the method developed was performed & it was found that there is no interference of excipients with the analyte which indicate that the method is specific for the analysis of the analytes in their dosage form. Assay was determined for both Standard & sample solution & the % assay was found to be 100.23% & 101.21% and respectively.

A calibration curve was found to be linear in the concentration range of 240-560 µg/ml and 60-140 µg/ml for CAZ & AVI. Accuracy studies of the method developed wad determined by spiking the known amount of analyte to sample solution and the percentage mean recovery was found to be 100.10% and 99.75% for CAZ & AVI respectively. Precision of the method & system were found to be within limits. Limit of detection was found to be 11.2 and 2.5 µg/ml for CAZ & AVI respectively and Limit of Quantification was found is to be 33.8 and 7.69 µg/ml for CAZ & AVI respectively. Robustness of the method was determined by varying flow rate and wavelength & Ruggedness of the method was determined by carrying out the determination by two different analyst.

Samples containing Ceftazidime and Avibactam were subjected to various stress conditions and it was found that overall net degradation was within the limits without any significant degradation products.

A RP-HPLC method developed for Ceftazidime and Avibactam shows that the results obtained for RP-HPLC are promising with better resolution in set chromatographic conditions. The developed methods were statistically validated which suggested that the methods were within the acceptable limits hence these methods can be used for the routine determination of Ceftazidime and Avibactam in bulk drug and pharmaceutical formulation.

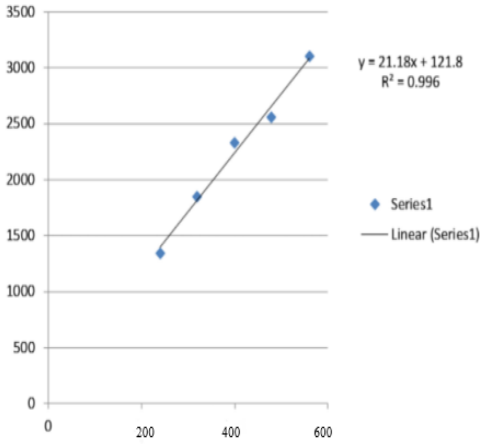

Fig 4: Linearity curve of Ceftazidime

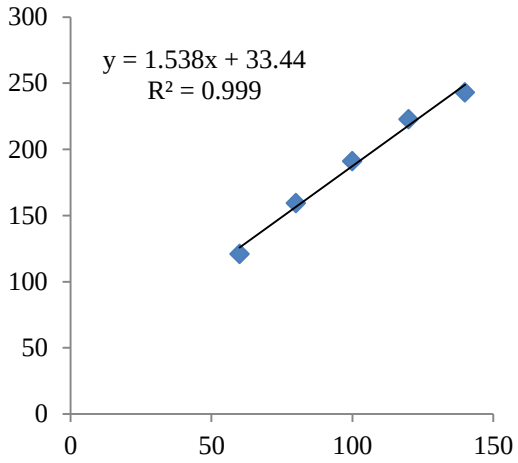

Fig 5: Linearity curve of Avibactam

Table: 2 Results of Linearity

CEFTAZIDIME			AVIBACTAM	
S.No.	**Conc.(µg/ml)**	**Area**	**Conc.(µg/ml)**	**Area**
1	240	1344.606	60	108.783
2	320	1849.853	80	159.306
3	400	2338.421	100	204.849
4	480	2563.186	120	222.682
5	560	3106.591	140	243.873

Table: 3 Precision method of proposed RP- HPLC method

CEFTAZIDIME			AVIBACTAM	
S.No.	**Rt**	**Area**	**Rt**	**Area**
1	2.510	2192.417	4.397	267.545
2	2.523	2322.573	4.410	211.442
3	2.523	2321.138	4.413	202.102
4	2.523	2333.196	4.413	200.853
5	2.507	2350.119	4.397	202.888
6	2.497	2341.355	4.390	198.551
Avg	2.513833	2310.133	4.403333	213.8968
Stdev	0.010926	6.248003	0.009893	2.001001
%RSD	0.433746	0.270460	0.224216	0.935498

Table: 4 Recovery data for Ceftazidime

Recovery level	**Amount taken (mcg/ml)**	**Accuracy of Ceftazidime**				
		Area	Average area	Amount recoverd	%Recovery	Average % Recovery
80%	80+320=400	2608.241 2609.160 2605.517	2607.639	10.8	111.3	
100%	80+400=480	2194.643	2244.257	13.25	95.85	

		2211.816				100.10
		2326.313				
120%	80+480=560	3109.681	3109.702	22.5	93.16	
		3112.744				
		3106.682				

Table: 5 Recovery data for Avibactam

Recovery level	**Amount taken (mcg/ml)**	**Accuracy of Avibactam**				
		Area	Average area	Amount recoverd	%Recovery	Average % Recovery
80%	20+80=100	236.388	237.6637	9,56	92.65	
		234.158				
		242.445				
100%	20+100=120	224.953	254.704	15.03	99.34	
		269.243				99.75
		269.916				
120%	20+120=140	280.051	275.7967	21.07	107.35	
		280.286				
		267.053				

Table: 6 Robustness data

Parameter	**CEFTAZIDIME**		**AVIBACTAM**	
	Retention time(min)	**Tailing factor**	**Retention time(min)**	**Tailing factor**
Flow Rate				
0.8 ml/min	3.130	1.258	5.443	1.167
1.2 ml/min	2.090	1.036	3.663	0.943
Wavelength				
229nm	2.513	1.222	4.380	1.088
233nm	2.517	1.179	4.380	1.125

Table: 7 Result of Ruggedness

Ceftazidime	**% Assay**	**Avibactam**	**% Assay**
Analyst 01	99.3	Analyst 01	96.28
Analyst 02	99.30	Analyst 02	95.97
% RSD	1.82%	% RSD	1.67%

Table: 8 Result of LOD and LOQ

DRUG	**LOD(µg/ml)**	**LOQ(µg/ml)**
Ceftazidime	11.2	33.8
Avibactam	2.5	7.69

Table: 9 Results of Forced Degradation studies

STRESS CONDITION	PEAK AREA		% DEGRADATION	
	CAZ	AVI	CAZ	AVI
Unstressed sample	2192.417	267.545	0	0
Acid	3981.699	391.641	0.81	0.46
Alkaline	3934.512	396.009	0.79	0.48
Oxidation	3943.709	391.379	0.79	0.46
Photolytic	3951.770	397.622	0.80	0.49
Thermal	3971.642	385.227	0.81	0.43

CONCLUSION

Ceftazidime and Avibactam are essential for treatment of complicated intra-abdominal infections (CIAI), complicated urinary tract infections (cUTI), acute pyelonephritis (AP) so it is therefore necessary to know the quality of these drugs which is possible through the use of simple, sensitive and cost effective analytical methods so that the compounds can be rapidly, routinely and consistently assessed. Literature survey reveals that there are methods to estimate the ceftazidime individually and with other combinations, but not a single method is reported for estimation of Ceftazidime and Avibactam in bulk and multicomponent formulation. So, an attempt was made to develop RP-HPLC method. A RP-HPLC method proposed as a suitable method for the simultaneous estimation of Ceftazidime and Avibactam. The chromatographic conditions included mixed phosphate buffer (P^H 4): Acetonitrile in the ratio of 60:40 % v/v as mobile phase. The optimum wavelength for detection was 231 nm where as CAZ was eluted at 2.523 Min and AVY was eluted at 4.410 min. The calibration curve of Ceftazidime and Avibactam is linear in the range of 240-560 µg/ml and 60-140 µg/ml respectively.

Accuracy study showed the percentage mean recovery of Ceftazidime and Avibactam is 100.10% and 99.75% respectively. The amount of Ceftazidime and Avibactam present in the taken formulation was found to be 100.23% and 101.21% respectively. Forced degradation studies were carried under various stress conditions. It was found to be stable and the net degradation was within the limits without any significant degradation products. From the above experimental results and parameters it was concluded that, this newly developed methods for the estimation of Ceftazidime and Avibactam was found to be simple, rapid, economic, precise, accurate and reproducible. Forced degradation studies carried out are helpful to determine stability of this drug in combination. The analytical technique showed reliable method hence it can be effectively applied for routine analysis in research institutions & quality control department in industries.

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