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SIMULTANEOUS ESTIMATION OF CEFTAZIDINE AND AVIBACTUM IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple and selective LC method is described for the determination of Ceftazidine and avibactum in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column Inertsil ODS, (250×4.6× 5µ) using mobile phase consisting of a mixture of Phosphate buffer and Acetonitrile(60:40), P^H -4 ,with detection of 231nm.The retention times were 2.523mins and 4.410mins for Ceftazidine and Avibactum respectively. Linearity was observed in the range 6-14 µg/ml for Ceftazidine (r^2 =0.995) and 6-14 µg /ml for Avibactum (r^2 =0.999).

The proposed method was validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: RPHPLC, CEFTAZIDINE, AVIBACTUM.

INTRODUCTION

Ceftazidime chemically is 1-{ [(6R,7R)-7-[(2Z)-2-(2-amino-1, 3-thiazol-4-yl) -2- [(1-carboxy-1-methylethoxy) imino] acetamido] -2- carboxylato-8-oxo-5-thia-1-azabicyclo [4. 2. 0] oct – 2 – en – 3 -yl] methyl} pyridin-1-ium the bactericidal activity of ceftazidime results from the inhibition of cell wall synthesis via affinity for penicillin-binding proteins (PBPs) [1-2]. Avibactum chemically is sodium (2S, 5R)-2-carbamoyl-7-oxo-1, 6-diazabicyclo [3.2.1] octan-6-yl sulfate. Avibactam is a non-β lactam β-lactamase inhibitor that inactivates some β-lactamases (Ambler class A β-lactamases, including Klebsiella pneumoniae carbapenemases. Ambler class C and some Ambler class D β-lactamases) by a unique covalent and reversible mechanism, and protects ceftazidime from degradation by certain β-lactamases [3-4].

Literature survey reveals there are analytical methods developed for simultaneous estimation of caftazimide either individually [5-6] or in combination with other drugs in tablet dosage form [7-9] or in plasma [10] but not in combination with Avibactum. Hence an attempt is made in order to develop a new method for simultaneous estimation of Ceftazidime and Avibactum in tablet dosage form. The proposed method was validated according to ICH guidelines [11-12].

EXPERIMENTAL SECTION: The instrument employed for present study is as follows

Table No 1: INSTRUENT EMPLOYED

UV-Visible Spectrophotometer	**Nicolet evolution 100**
UV-Visible Spectrophotometer software	Vision Pro
HPLC software	Spin chrome (LC SOLUTIONS)
HPLC	Shimadzu(LC 20 AT VP)
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	Inertsil ODS 3V(250x4.6mm) 5µm

The reagents used in the present study are listed in table 2.

Table No 2: REAGENTS USED

Water	**HPLC Grade**
Potassium Phosphate	AR Grade
Acetonitrile	HPLC Grade
Ammonium acetate	AR Grade
Disodium hydrogen phosphate	AR Grade

Drugs used in the present study are listed in table 3

Table No 3: Drugs used in the present study

Ceftazidime and Avibactum standards	Gift Samples obtained from Chandra labs, Hyd.
Ceftazidime(2gm) & Avibactum (0.5gm) (label claims).	Obtained from local pharmacy

CHROMATOGRAPHIC CONDITIONS:

Mobile Phase:

The mobile phase used was a mixture of Phosphate buffer and acetonitrile pH-4.0 in the ratio of 60:40 v/v; it was filtered before use through a 0.45 µm membrane filter and degassed for 30 min. The elution was carried out isocratically at the flow rate of 1.0 ml/min. Detection was carried out at 231 nm at ambient temperature.

Preparation of buffer:

28.8 gm of potassium di hydrogen phosphate (KH_2PO_4) was weighed and dissolved in 100ml of water and volume was made up to 1000ml with water. Adjust the pH to 6.8 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of standard stock solution of CEFTAZIDIME

10 mg of CEFTAZIDIME was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of AVIBACTAM

10 mg of AVIBACTAM was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

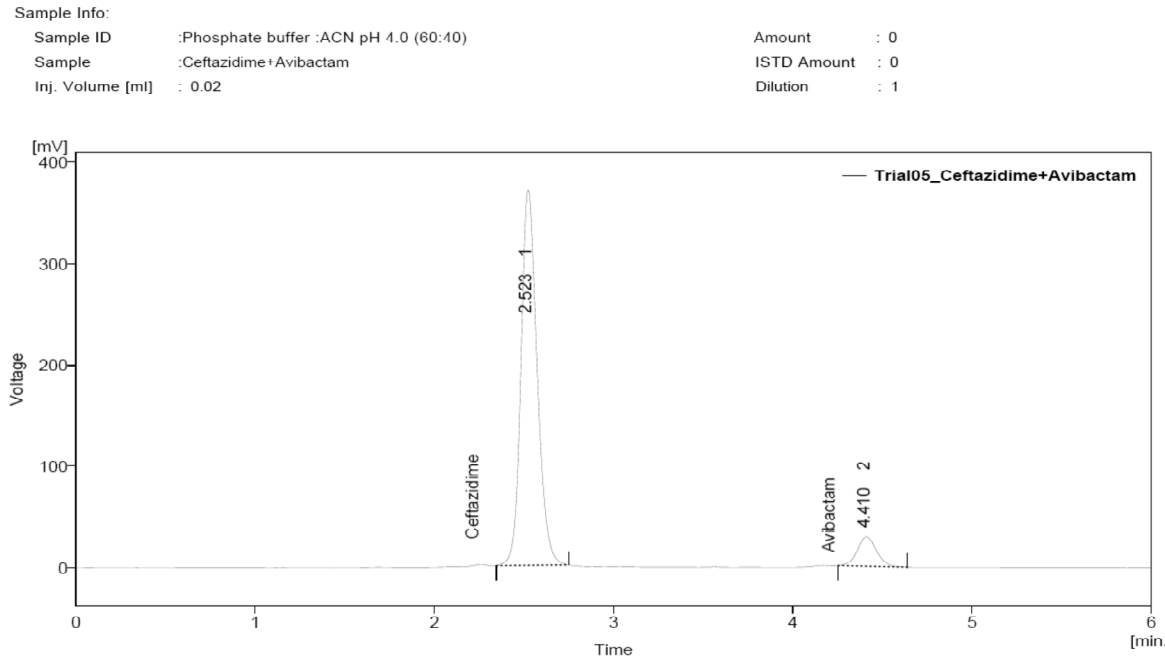
ISOBESTIC POINT OF CEFTAZIDIME AND AVIBATAM:

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The isobestic point was found to be 231 nm for the combination.

OPTIMISATION OF CHROMATOGRAPHIC CONDITIONS:

Preparation of mixed standard solution

Weigh accurately 2.0 gms of CEFTAZIDIME and 0.5gms of AVIBACTAM in 100 ml of volumetric flask and dissolve in 100ml of mobile phase and make up the volume with mobile phase. From above stock solution 25000 µg/ml of CEFTAZIDIME and AVIBACTAM is prepared by diluting 5 ml to 50ml with mobile phase.



Result Table (Uncal - Trial05_Ceftazidime+Avibactam)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.523	2325.117	369.999	91.2	92.6	0.10
2	4.410	224.128	29.732	8.8	7.4	0.12
	Total	2549.245	399.731	100.0	100.0	

Column Performance Table (From 50% - Trial05_Ceftazidime+Avibactam)

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.523	0.097	1.375	0.00	3775	75498	-
2	4.410	0.120	1.161	0.00	7482	149642	10.248

Figure No 1: Chromatogram of AVIBACTAM and CEFTAZIDIME

TABLE No 4: ASSAY RESULTS

	CEFTAZIDIME		**AVIBACTAM**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	2334.362	2344.463	207.967	212.684
Injection-2	2323.199	2351.614	199.698	209.655
Injection-3	2337.863	2337.863	207.039	207.039
Injection-4	2331.502	2334.732	207.632	210.092
Injection-5	2328.483	2341.801	198.197	210.080
Average Area	2336.588	2342.095	205.1066	209.91
Standard deviatuion	6.489006		2.004044	
%RSD	0.276506		0.952806	
Assay(%purity)	**100.23**		**102.21**	

The amount of CEFTAZIDIME and AVIBACTAM present in the taken dosage form was found to be 100.23 % and 102.21% respectively.

Table No 5: Results for system suitability of CEFTAZIDINE

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.523	2334.362	3304	1.308
2	2.523	2323.199	3304	1.308
3	2.520	2337.863	3295	1.400
4	2.523	2331.502	3304	1.308
5	2.520	2328.583	3295	1.400
Mean	2.5218	2331.102	-	-
SD	0.001643	5.596907	-	-
%RSD	0.065028	0.239617	-	-

Table No 6: Results for system suitability of AVIBACTUM

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	4.417	207.967	3304	1.308
2	4.417	199.698	7105	1.161
3	4.403	207.039	7460	1.156
4	4.417	207.632	7505	1.156
5	4.403	198.197	7406	1.194
Mean	4.4114	204.1066	-	-
SD	0.007668	4.751032	-	-
%RSD	0.173477	2.323066	-	-

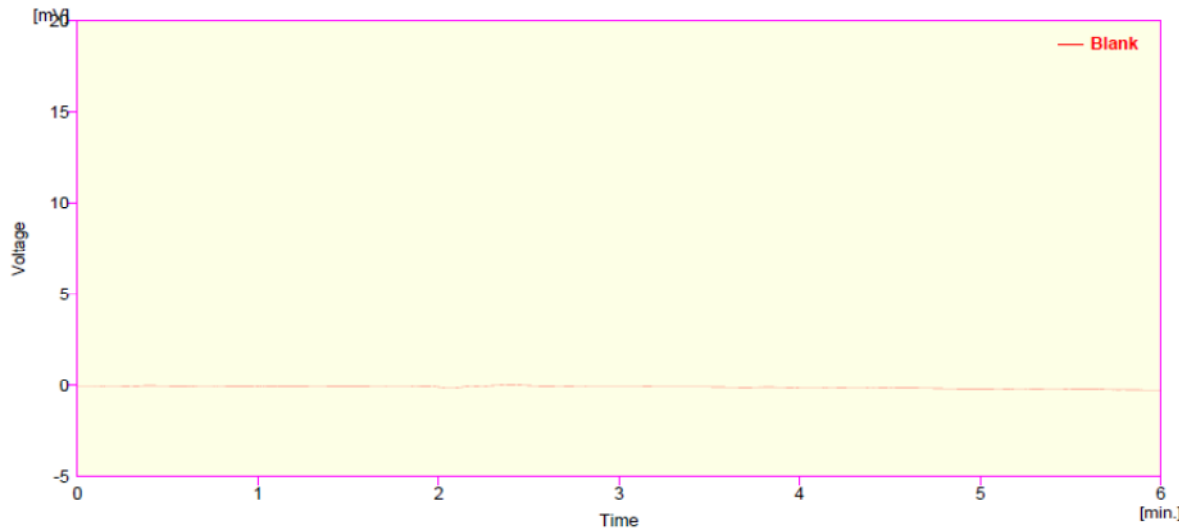


Figure No 2: Blank chromatogram for specificity by using mobile phase

Table No 7: Linearity Preparations

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
			CEFTAZIDINE	AVIBACTUM
Preparation 1	0.6	10	60	15
Preparation 2	0.8	10	80	20
Preparation 3	1.0	10	100	25
Preparation 4	1.2	10	120	30
Preparation 5	1.4	10	140	35

From the above stock solution 1000µg/ml of CEFTAZIDIME and AVIBACTUM is prepared by diluting 1ml to 10 ml with mobile phase (100µg/ml). This solution is used for recording chromatogram.

ASSAY:

Preparation of mixed standard solution: Weigh accurately 2.0 gm of CEFTAZIDIME and 0.5 gms of AVIBACTAM in 100 ml of volumetric flask and dissolve in 100ml of mobile phase and make up the volume with mobile phase. From above stock solution 25000 µg/ml of CEFTAZIDIME and AVIBACTAM is prepared by diluting 5ml to 50ml with mobile phase. The above stock solution 1000µg/ml of CEFTAZIDIME and AVIBACTUM is prepared by diluting 1ml to 10 ml with mobile phase (10µg/ml). This solution is used for recording chromatogram.

Tablet sample: Weigh accurately 2.0 gms of CEFTAZIDIME and 0.5 gms of AVIBACTUM were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of AVIBACTAM and CEFTAZIDIME (25000µg/ml) were prepared by using mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of AVIBACTAM and CEFTAZIDIME was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Linearity and range: Preparation of standard stock solution

Standard stock solutions of CEFTAZIDINE and AVIBACTUM (microgram/ml) were prepared by AVIBACTUM dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase. From the above concentration pipette out 5ml to 50ml and make up to the mark with mobile phase (1000µg/ml).Pipette out 1ml to 10 ml from the above concentration and dilute with mobile phase up to the mark (100µg/ml). Further dilutions were given in the table 7.

Table No 8: linearity of CEFTAZIDINE

S.No.	Conc.(µg/ml)	Area
1	60	1344.606
2	80	1849.853
3	100	2338.421
4	120	2563.186
5	140	3106.591

Table No 9: linearity of AVIBACTUM

S.No.	Conc.(µg/ml)	Area
1	60	108.783
2	80	159.306
3	100	204.849
4	120	222.682
5	140	243.873

Accuracy: Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%. Recovery results for AVIBACTUM

Observation

The percentage mean recovery of CEFTAZIDINE and AVIBACTUM is 100.10% and 99.75% respectively.

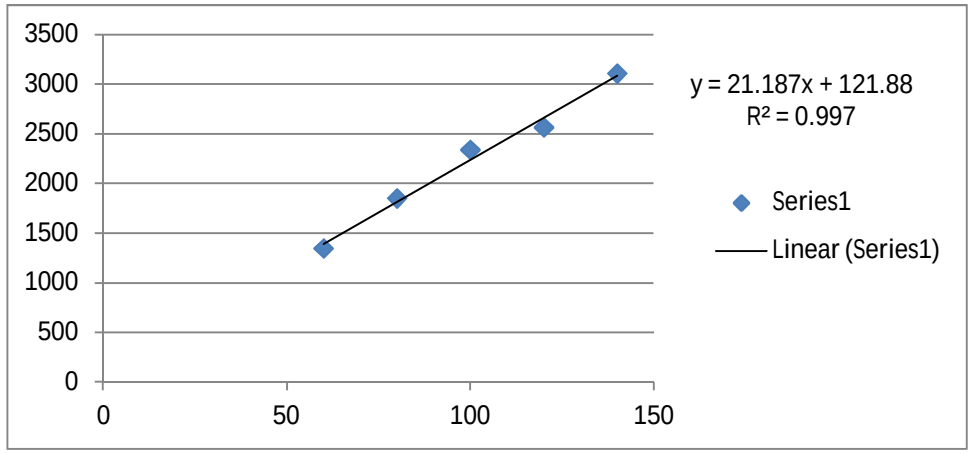


Figure No 3: Linearity graph of CEFTAZIDINE

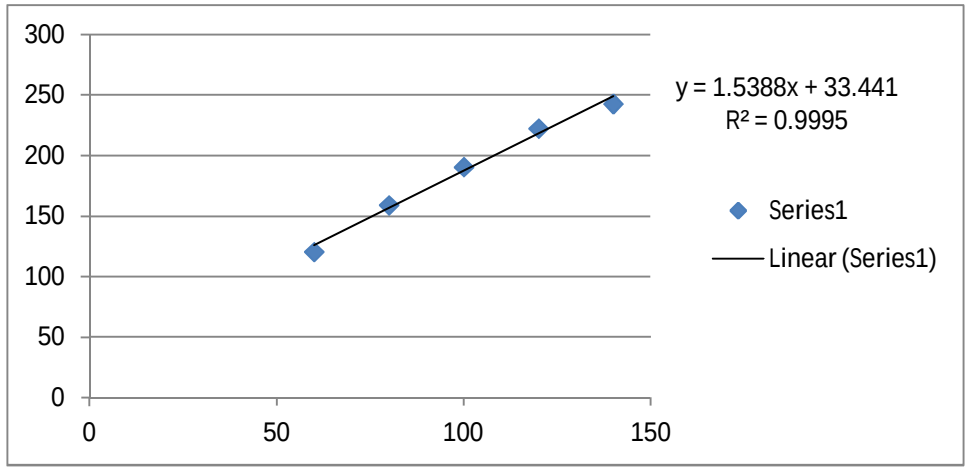


Figure No 4: Linearity graph of Avibactum

Table No 8: Recovery results for Avibactum

Recovery level	Accuracy AVIBACTUM					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
50%	10	236.388	237.6637	9,56	92.65	
	10	234.158				
	10	242.445				99.75
100%	15	224.953	254.704	15.03	99.34	
	15	269.243				
	15	269.916				
150%	20	280.051	275.7967	21.07	107.35	
	20	280.286				
	20	267.053				

Table No 9: Recovery results for CEFTAZIDINE

Recovery level	Accuracy CEFTAZIDINE					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recoverd	%Recovery	
50%	10	2608.241	2607.639	10.8	111.3	
	10	2609.160				
	10	2605.517				
100%	15	2194.643	2244.257	13.25	95.85	100.10
	15	2211.816				
	15	2326.313				
150%	20	3109.681	3109.702	22.5	93.16	
	20	3112.744				
	20	3106.682				

Table No 10: Results for Method precision of CEFTAZIDINE and AVIBACTUM

	CEFTAZIDINE			AVIBACTUM	
S.No.	Rt	Area	S.No.	Rt	Area
1	2.510	2192.417	1	4.397	267.545
2	2.523	2322.573	2	4.410	211.442
3	2.523	2321.138	3	4.413	202.102
4	2.523	2333.196	4	4.413	200.853
5	2.507	2350.119	5	4.397	202.888
6	2.497	2341.355	6	4.390	198.551
avg	2.513833	2310.133	avg	4.403333	213.8968
stdev	0.010926	-	stdev	0.009893	-
%RSD	0.433746	-	**%RSD**	0.224216	-

Table No 11: Result of Robustness study

Parameter	CEFTAZIDINE		AVIBACTUM	
	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

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

Table No 12: Ruggedness

CEFTAZIDINE	%Assay	AVIBACTUM	%Assay
Analyst 01	99.36	**Analyst 01**	96.28
Anaylst 02	99.30	**Anaylst 02**	95.97

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

Precision

Method precision

Prepared sample preparations of AVIBACTUM and CEFTAZIDINE as per test method and injected 6 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of AVIBACTUM and CEFTAZIDINE should be not more than 2.0%.

Observation

Test results for AVIBACTUM and CEFTAZIDINE are showing that the %RSD of Assay results are within limits.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts Results for Ruggedness

CONCLUSION

An attempt is made to develop a simple, cost effective, robust, Accurate and Precise analytical method for simultaneous estimation of Ceftazidine and Avibactum in tablet dosage form. The method was accurate and precise as RSD obtained was less than 2%.The proposed method was estimated for its linearitry and range and Regression coefficient was 0.999 for both the drugs. The method was validated for all validative parameters according to ICH guidelines including ruggestness and robustness and the results were satisfactory and within the limits. Hence the proposed method can be used for routine analysis of Ceftazidine and Avibactum in pharmaceutical preparations.

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