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R E S E A R C H A R T I C L E

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

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A B S T R A C T

The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of active pharmaceutical ingredients of Ceftazidime and Sulbactam and their related substances. A simple, selective, validated and well-defined stability that shows gradient RP-HPLC methodology for the quantitative determination of Ceftazidime and Sulbactam. The chromatographic strategy utilized Column of C18 (150 mm x 4.6 mm, 5 µm), using isocratic elution with a mobile phase of 0.01N Na_2HPO_4 and Acetonitrile (55:45 %v/v). A flow rate of 1 ml/min and a detector wavelength of 270 nm utilizing the 2487 UV detector were given in the instrumental settings. Using the impurity-spiked solution, the chromatographic approach was streamlined. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines. LOD and LOQ for the two active ingredients and their impurities were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of 0.999, which means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness was determined as a part of method validation and the results were found to be within the acceptable range. The proposed method to be fast, simple, feasible and affordable. During stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

Keywords: Ceftazidime, Sulbactam, Method development, RP-HPLC, Validation

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Ceftazidine is chemically 7-(2-(2-aminothiazol-4-yl)-2-(2-carboxypropan-2-yloxyimino)-acetamido)-8-oxo-3-(pyridinium-1-ylmethyl)-5-thia-1-aza-bicyclo [4.2.0]-oct-2-ene-2-carboxylate. It is a β-lactam antibiotic, belongs to cephalosporin, is a penicillin binding proton (PBP) inhibitor, through inhibition of essential PBPs, result in impaired cell wall homeostasis, loss of cell integrity, and ultimately bacterial cell death. Ceftazidime has an elimination half-life of 1.5-2.8 hours in healthy subjects. Ceftazidime is a third-generation cephalosporin with broad-spectrum antibacterial activity, against some treatment-resistant bacteria such as *Pseudomonas aeruginosa*, indicated for the treatment of lower respiratory tract infections, skin and skin structure infections, urinary tract infections, bacterial septicemia, bone and joint infections, gynecologic infections, intra-abdominal infections (including peritonitis), and central nervous system infections (including meningitis) caused by susceptible bacteria.[1-4]

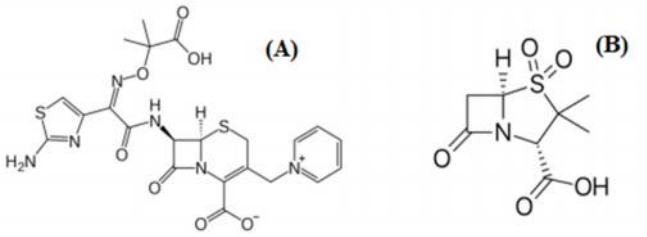

Figure 1: Molecular Structure of Ceftazidime (A) and Sulbactam (B)

Sulbactam is chemically (2S,5R)-3,3-Dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide. It is a β-lactamase inhibitor given in combination with β-lactam antibiotics to inhibit β-lactamase, an enzyme produced by bacteria that destroys antibiotic activity. Sulbactam is currently available in combination products with ampicillin. Within this formulation it is indicated for the treatment of infections due to susceptible strains of the designated microorganisms in the conditions listed below. Skin and Skin Structure Infections caused by beta-lactamase producing strains of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp*. (including *K. pneumoniae*), *Proteus mirabilis, Bacteroides fragilis, Enterobacter spp*., and *Acinetobacter calcoaceticus*. Gynecological infections caused by beta-lactamase producing strains of *Escherichia coli*, and *Bacteroides spp*. (including *B. fragilis*).[5-10]

2. Materials and Methods

Chemicals and Reagents

Ceftazidime and Sulbactum were procured from Yarrow Chem Products, Mumbai. HPLC-grade acetonitrile, ortho phosphoric acid, methanol, potassium dihydrogen ortho phosphate was purchased from Merck India Ltd, Mumbai. Milli-Q System double-distilled water was utilised in all studies (Millipore).

Instrumentation

The absorbance of solutions was determined using a double beam Schimadzu UV-Visible spectrophotometer, with a spectral bandwidth of 2 nm and wavelength accuracy of 0.5 nm, and a set of matching quartz cells of 1 cm in diameter. The RP-HPLC technique was carried out utilising a binary gradient pump HPLC system Waters 2695 and a UV detector 2487 for analysis. Lab solutions software was used to collect chromatographic data. We used a Thermosil C18 column (150 mm x 4.6 mm, 5 µm) as our stationary phase to accomplish this separation. To isocratically elute Ceftazidime and Sulbactam, a mobile phase of 0.01N Na_2HPO_4: Acetonitrile (55:45 %v/v) was used at a flow rate of 1.0 mL/min. UV spectrum wavelength selected as 270 nm. At this wavelength both the drugs show good absorbance. The developed HPLC method was utilized for the estimation of both the drugs in bulk and pharmaceutical dosage. [11-14]

Validation

The analytical parameters such as system suitability parameters, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2-R1 guidelines.[15-25]

Preparation of buffer (0.1% OPA buffer):

1 mL of ortho phosphoric acid was diluted to 1000 mL with HPLC grade water. Accurately weighed 1.36 gm of Potassium dihyrogen Ortho phosphate in a 1000 mL of Volumetric flask add about 900 mL of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1 mL of triethylamine then pH adjusted to 3.8 with dil. ortho phosphoric acid solution.[26]

Preparation of mobile phase:

Mobile phase was prepared by mixing 0.01 N Na_2HPO_4 and acetonitrile taken in ratio 55:45 %v/v. Those were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ membrane filter under vacuum filtration to remove the impurities which may interfere in the final chromatogram.[27]

Diluent: Based up on the solubility of the drugs, diluent was selected, acetonitrile and water taken in the ratio of 50:50 %v/v.[28]

Preparation of standard solutions: Accurately weighed 10 mg of Ceftazidime, 4 mg of Sulbactam and transferred to 50 mL volumetric flasks and 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (200 µg/mL of Ceftazidime and 80 µg/mL Sulbactam). 1 mL from each stock solution was pipetted out and taken into a 10 mL volumetric flask and made up with diluent. (20 µg/mL of Ceftazidime and 8 µg/mL of Sulbactam).[29]

Preparation of sample solutions:

10 Tablets were accurately weighed and average weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 50 mL of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (200 µg/mL of Ceftazidime and 80 µg/mL of Sulbactam). 1 mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up with diluent. (20 µg/mL of Ceftazidime and 8 µg/mL of Sulbactam).[30]

System suitability parameters: The system suitability parameters were determined by preparing standard

solutions of Ceftazidime and Sulbactam, the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2 %.[31-33]

Results and Discussion

Method development was done by changing various, mobile phase ratios, buffers etc. Ceftazidime and Avibatam were eluted at 2.399 min and 2.984 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated. In order to provide a good performance, the chromatographic conditions

System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines. According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits

3. Results and Discussion

Table-1: System suitability parameters for Ceftazidime and Sulbactam

S. No.	Ceftazidime			Sulbactum		
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing
1	2.364	3490	1.33	2.953	4985	1.35
2	2.380	3544	1.35	2.976	4870	1.36
3	2.389	3444	1.36	2.994	4874	1.34
4	2.394	3457	1.36	2.999	5035	1.34
5	2.398	3571	1.34	3.000	5069	1.36
6	2.405	3577	1.33	3.009	4908	1.35

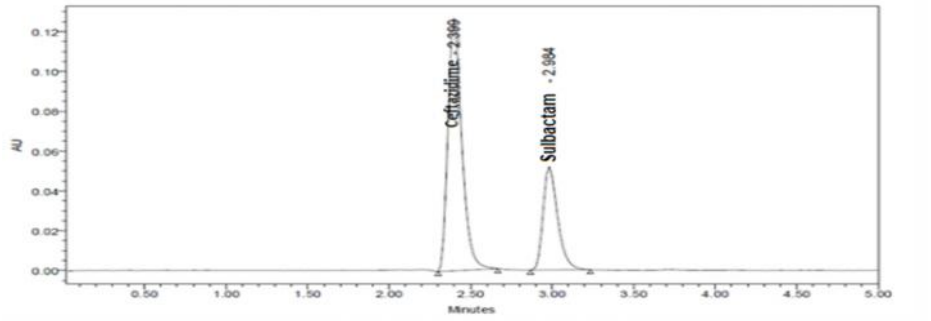

Figure 2: Optimized Chromatogram

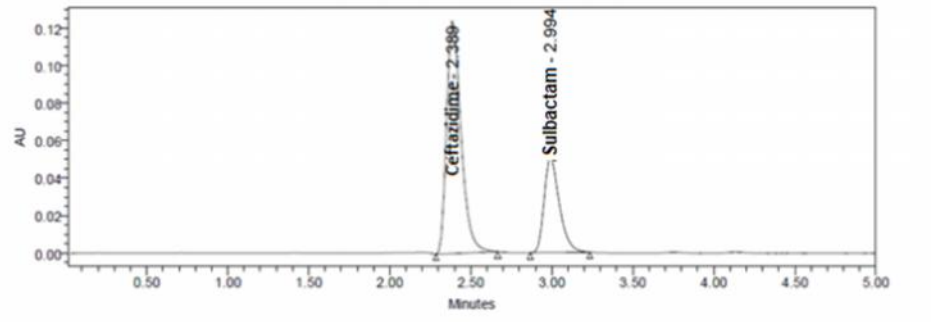

Figure 3: System suitability Chromatogram

Table-2: Linearity table for Ceftazidime and Sulbactam

S. No.	Ceftazidime		Sulbactam	
	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area
1	0	0	0	0
2	5	194360	2	84748
3	10	391770	4	169442
4	15	585953	6	252059

5	20	767884	8	327739
6	25	954354	10	413524
7	30	1149367	12	489342
Regression equation	y = 38173x + 5076		y = 40783x + 3421.2	
Slope	38173		40783	
Intercept	5076		3421.2	
R^2	0.999		0.999	

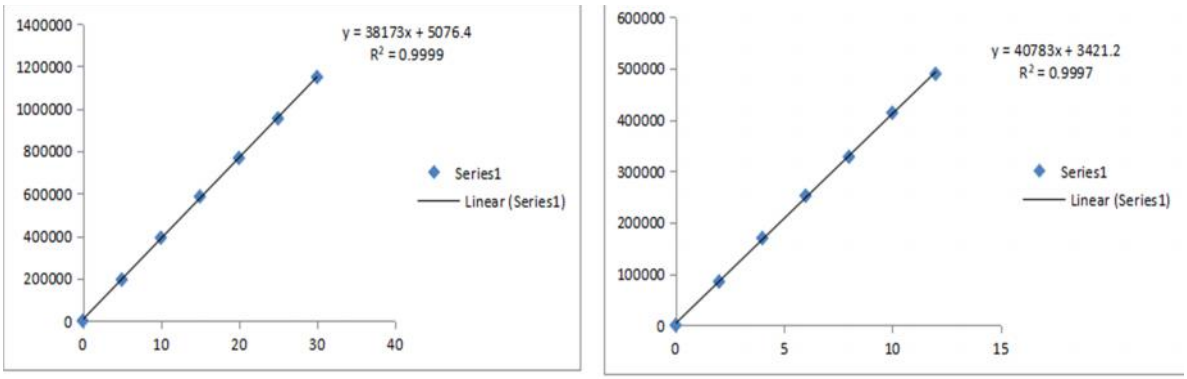

Figure 4: Calibration curve of Ceftazidime and Sulbactum

Six linear concentrations of Ceftazidime (5-30 µg/mL) and Sulbactam (2-12 µg/mL) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Ceftazidime was y=38173x + 5076and of Sulbactam was y =40783x + 3421 Correlation coefficient obtained was 0.999 for the two drugs.

Precision:

System Precision

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.5% and 0.6% respectively for Ceftazidime and Sulbactam. As the limit of precision was less than "2" the system precision was passed in this method.

Table-3: System precision table of Ceftazidime and Sulbactam

S. No	Area of Ceftazidime	Area of Sulbactam
1.	758657	326724
2.	762233	327817
3.	770301	331051
4.	764507	328077
5.	764751	331367
6.	766195	331194
Mean	764441	329372
S.D	3894.8	2060.4
%RSD	0.5	0.6

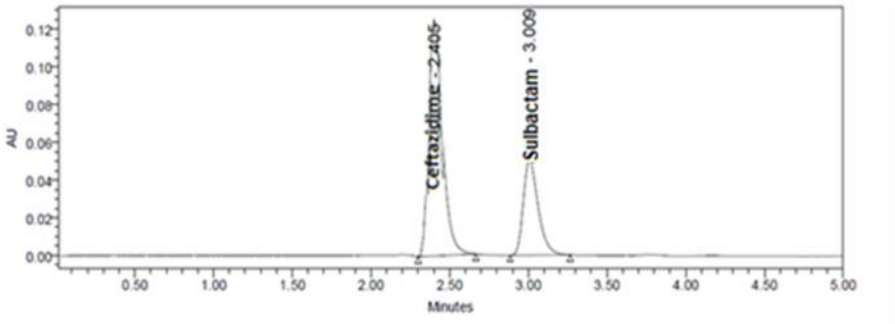

Figure 5: System precision chromatogram

Accuracy:

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.53% and 100.84% for Ceftazidime and Sulbactam respectively.

Table-4: Ceftazidime Accuracy table

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	%Recovery	Mean %Recovery
50%	10	10.2	101.5	101.53%
	10	10.3	103.0	
	10	10.2	102.4	
100%	20	20.4	101.9	
	20	20.0	100.2	
	20	20.3	101.6	
150%	30	30.0	100.1	
	30	30.4	101.3	
	30	30.6	101.8	

Table-5: Sulbactam Accuracy table

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	4	4.05	101.27	100.84%
	4	4.02	100.38	
	4	3.98	99.40	
100%	8	8.14	101.71	
	8	8.06	100.80	
	8	8.15	101.87	
150%	12	12.00	99.97	
	12	12.15	101.29	
	12	12.10	100.87	

Table-6: Ceftazidime and Sulbactam Sensitivity table

Molecule	LOD	LOQ
Ceftazidime	0.18	0.56
Sulbactam	0.08	0.25

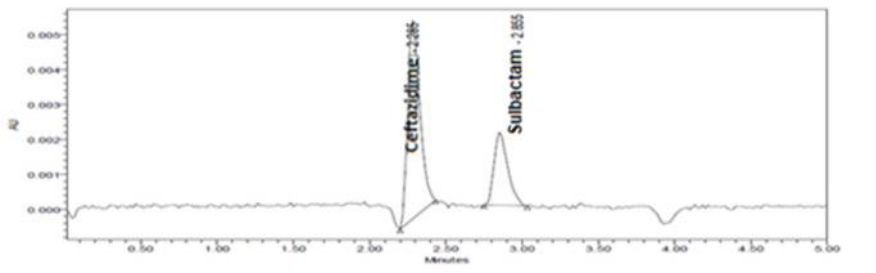

Figure 6: LOD Chromatogram of Standard

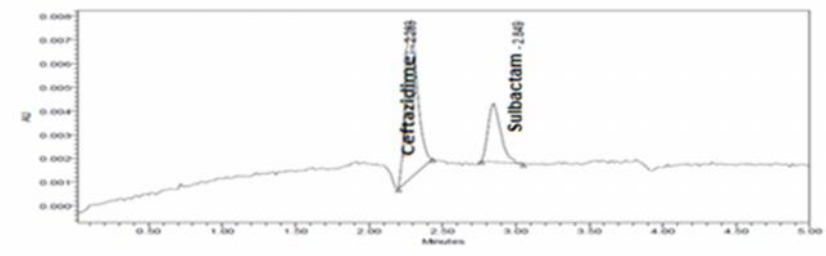

Figure 7: LOQ Chromatogram of Standard

Robustness:

Robustness conditions like Flow minus (0.9 mL/min), Flow plus (1.1 mL/min), mobile phase minus (60B:40A), mobile phase plus (50B:50A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table-7: Robustness data for Ceftazidime and Sulbactam

S.No.	Condition	%RSD of Ceftazidime	%RSD of Sulbactam
1	Flow rate (-) 0.9mL/min	0.5	0.2
2	Flow rate (+) 1.1mL/min	0.8	1.3
3	Mobile phase (-) 60B:40A	0.4	0.2
4	Mobile phase (+) 50B:50A	0.2	0.5
5	Temperature (-) 25°C	0.6	0.8
6	Temperature (+) 35°C	1.2	0.2

Table-8: Ceftazidime Assay Data

S.No.	Standard Area	Sample area	% Assay	Standard Area	Sample area	% Assay
1	758657	751061	98.15	326724	329028	99.80
2	762233	751074	98.15	327817	327696	99.39
3	770301	759533	99.26	331051	326706	99.09
4	764507	759448	99.25	328077	331505	100.55
5	764751	763108	99.73	331367	327461	99.32
6	766195	762888	99.70	331194	331303	100.49
Avg	764441	757852	99.04	329372	328950	99.77
SD	3894.8	5484.9	0.72	2060.4	2044.3	0.62
%RSD	0.5	0.7	0.7	0.6	0.6	0.62

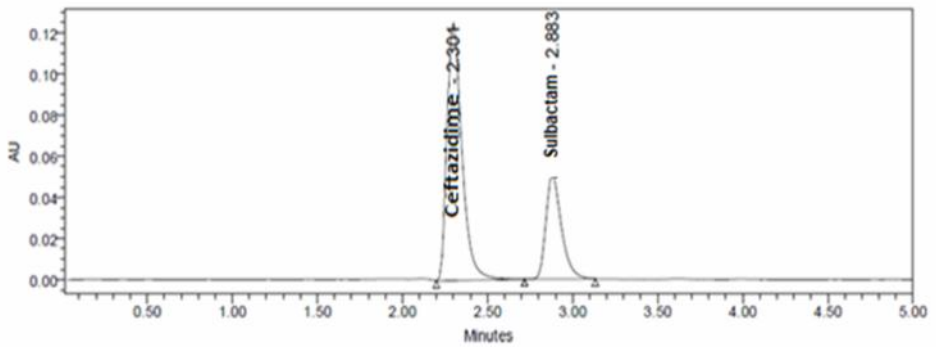

Figure 8: Assay Chromatogram

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. Regarding the pH adjustment in mobile phase for the acid and base degradation studies have movement in retention time of drugs. But due to neutralized acid sample with 2N base solution and base sample with 2N acid solution there will be no change in retention time**.**

Table-9: Ceftazidime and Sulbactam Degradation Data

Degradation Condition	Ceftazidime		Sulbactum	
	% Drug Degraded	%drug undregraded	% Drug Degraded	%drug undregraded
Acid	5.41	94.59	4.49	95.51
Alkali	4.04	95.96	3.38	96.62
Oxidation	4.76	95.24	3.59	96.41
Thermal	3.01	96.99	2.98	97.02
UV	1.69	98.31	1.83	98.17
Water	0.27	99.73	0.32	99.68

Parameters		Ceftazidime	Sulbactam	Limit
Linearity Range (µg/mL)		5-30	2-12	R< 1
Regression coefficient		0.999	0.999	
Slope (m)		38119	40783	
Intercept(c)		5076	3421.2	
Regression equation (y=mx+c)		y=38173x+5076	y =40783x+3421.2	
Assay (% Recovery)		99.04%	101.53%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision (%RSD)		0.5	0.5	NMT 2.0%
Method precision (%RSD)		0.7	0.6	NMT 2.0%
Accuracy (%Recovery)		101.53%	100.84%	98-102%
LOD (µg/mL)		0.18	0.08	NMT 3
LOQ (µg/mL)		0.56	0.25	NMT 1
Robustness	**FM**	0.5	0.2	%RSD NMT 2.0
	FP	0.8	1.3	
	MM	0.4	0.2	
	MP	0.2	0.5	
	TM	0.6	0.8	
	TP	1.2	0.2	

4. Conclusion

The developed HPLC method for the estimation of selected drugs is simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming. The sample recoveries were in good agreement with their respective label claims and they suggested no interference of formulation recipients in the estimation and can be used in laboratories for the routine analysis of selected drugs. Since the system validation parameters of HPLC method used for estimation of selected drugs in pure and have shown satisfactory, accurate and reproducible results (without any interference of recipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose. The present work concluded that stability indicating assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the placebo and degradation products. Hence these can be used for routine analysis of Ceftazidime and Sulbactam in bulk and Pharmaceutical formulations.

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