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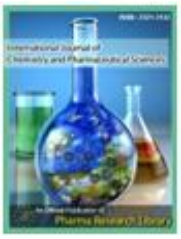

Research Article **Open Access**

Stability Indicating RP-HPLC Method for the Simultaneous Determination of Anti-Infectious Vials Containing Sulbactam and Ceftazidime in Pharmaceutical Dosage

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A B S T R A C T

A simple, accurate, rapid, and stability-indicating RP-HPLC method for a combination of Sulbactam and Ceftazidime has been developed and subsequently validated in commercial formulation vials. The proposed HPLC method utilizes Waters C-18 (250mm x 4.6mm, 5µm) column and mobile phase consisting of Water, Methanol and Acetate Buffer 50:30:10 (v/v) with flow rate of 1.0 ml/min. Quantization was achieved with UV detection at 236 nm. The method is validated in terms of accuracy, precision, linearity, limits of detection, limits of quantization, and robustness. This optimized method has been successively applied to pharmaceutical formulation. Sulbactam and Ceftazidime combination drug product were subjected to acid, base, neutral hydrolysis, oxidation, dry heat, and photolytic stress conditions and the stressed samples were analyzed by the proposed method. As the proposed LC method could effectively separate the drugs from its degradation products, it can be employed as stability-indicating method for the determination of instability of these drugs in bulk and commercial formulations.

Keywords: Ceftazidime (CFZ), Sulbactam (SBT), RP-HPLC, Validation, Forced Degradation

A R T I C L E I N F O

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1. Introduction

Ceftazidime is a semi-synthetic, third generation cephalosporin antibiotic used to treat lower respiratory tract, skin, urinary tract, blood stream, joint, abdominal infections and meningitis (1). The drug is given intravenously (IV) or intramuscularly (IM). It is a broad-spectrum β-lactam antibiotic used as first line treatment for the tropical infection, melioidosis, an important cause of sepsis in Asia and Australia (2, 3). Ceftazidime belongs to cephalosporins class and it has good activity against both gram positive and negative bacteria. Specifically this drug is used for joint infections, meningitis, pneumonia, sepsis, urinary tract infections, malignant otitis externa, *pseudomonas aeruginosa* infection, and vibrio infection. It is given as injection into a vein or muscle (4, 5). Ceftazidime was commercially available from 1984 (6) and it is listed as Essential Medicines by World Health Organization (7) available as a generic medication. This drug is available as injection and works by inhibition of cell wall synthesis via affinity for penicillin-binding proteins (PBPs). The IUPAC name of Ceftazidim is (6R, 7R,Z)-7-(2-(2-aminothiazol-4-yl)-2-(2-carboxypropan-2yloxyimino) acetamido) -8-oxo-3-(pyridinium-1-ylmethyl)-5-thia-1-aza-bicyclo [4.2.0] octa-2-ene-2-carboxylate with molecular formula $C_{22}H_{22}N_6O_7S_2$ and molecular mass 546.58.

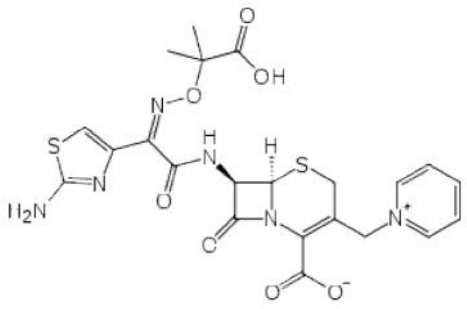

Figure I: Chemical structures of Ceftazidime (CFZ)

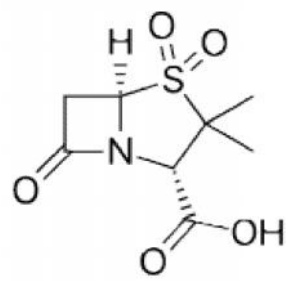

Figure 2: Chemical structures of Sulbactam (SBT)

Sulbactam 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 (8). It does possess some antibacterial activity when administered alone, but it is too weak to have any clinical importance. Sulbactam is an irreversible inhibitor of β-lactamase; it binds to the enzyme and does not allow it to degrade the antibiotic. In United States, Sulbactam is used in combined form. The presence of Sulbactam in formulations with ampicillin effectively extends the antibacterial spectrum of ampicillin to include many bacteria normally resistant to it and to other β-lactam antibacterials. Combination of Ceftazidime and Sulbactam is indicated for the treatment of bacterial infections, intra-abdominal infections, gynecological infections, skin or soft tissue infections, surgical infections and other conditions. The IUPAC name of Sulbactam is (2S,5R)-3,3-dimethyl -7-oxo-4-thia-1-azabicyclo[3.2.0]heptanes-2-carboxylic acid 4,4-dioxide with molecular formula $C_8H_{11}NO_5S$ and molecular mass 233.243. Chemical Structures of CFZ & SBT are shown in Figures 1 & 2.

Study of literature provided that very few analytical methods have been reported with Ceftazidime (CFZ) and Sulbactam (SBT) drugs. RP-HPLC methods (10-19) with Ceftazidime and Sulbactam are reported separately and in combination with other drugs. Plasma spiked RP-HPLC method (18) was reported with Ceftazidime and Sulbactam combination. Few spectrophotometric methods are also reported for the analysis of Ceftazidime and Sulbactam in single form and in combined dosage forms. In the present study, it is proposed to develop and validate a new method with RP HPLC, when Ceftazidime and Sulbactam are in combination with each other.

2. Materials and Methods

2.1 Instrumentation

Chromatographic separation was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump; Rheodyne injector with 20 µl fixed volume loop, variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Standard and sample drugs were weighed by Denver electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

2.2 Materials

Analytically pure samples of Ceftazidime (Biochem Pharmaceutical Limited, Mumbai, India), and Sulbactam (Solitaire Pharmacia Private Limited, Chandigarh, India) are used in this study. The pharmaceutical dosage form used in this study is Vitazid-SB procured from the local pharmacy and labeled to contain Sulbactam- 500mg and Ceftazidime - 1000mg per vial. The chemicals used in this method are Methanol HPLC grade, potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, hydrogen peroxide and triethylamine analytical grade reagents purchased from Merck, India. The HPLC grade water was prepared by using Milli-Q Academic, Millipore, Bangalore, India.

2.3 Standard Solutions

Stock solutions of Ceftazidime (CFZ) and Sulbactam (SBT) were prepared in mobile phase. Working standard solutions were freshly obtained by diluting the stock standard solutions with mobile phase during the day of analysis.

2.4 Sample Preparation

Ten vials were mixed and a uniform formulation sample vial was prepared. An amount of solution equivalent to 10 mg of CFZ was accurately pipetted and transferred into a

100 mL volumetric flask. This mixture was subjected to sonication for 10 min for complete extraction of drugs. From the above solution, 4 ml of solution was pipetted out into 10 ml volumetric flask; the solution was made up to the mark with a mobile phase to obtain concentrations of 40 µg/ml of CFZ. As per the label claim of the two drugs a SBT concentration 20 µg/ml was obtained. The resultant solution was used for the simultaneous estimation of SBT and CFZ in combined dosage forms.

2.5 Chromatographic Conditions

The chromatographic separation was achieved on a Waters C-18 (250mm x 4.6mm, 5µm) column using mobile phase composed of Water, Methanol and Acetate Buffer in the ratio of 50:30:10 (v/v) was filtered with 0.45 µm nylon filter. Flow rate is set to 1.0 mL/min with column temperature of 33°C and detection wavelength is fixed at 236 nm. Volume of the sample injected is 20 µL. Mobile phase is used as diluent for the preparation of standards and samples.

2.6 Method Validation

2.6.1. Specificity: The specificity of the developed method was established to prove the absence of interference from placebo peaks (excipients) which is part of required pharmaceutical preparation.

2.6.2 Linearity

Linearity was evaluated by determining six working standard solutions at a concentration range of 10-60 µg/ml for CFZ and 5-30 µg/ml for SBT. Triplicates of such solutions were prepared. Each was analyzed to plot a calibration curve. Slope, intercept and coefficient of determination of the calibration curves were calculated to ascertain linearity of the method.

2.6.3 Precision:

For method repeatability, working standard solution at a concentration 40 µg/ml for CFZ and 20 µg/ml for SBT were repeatedly performed six times on the same day for intra-day precision. For reproducibility, freshly prepared solutions at a mentioned concentration level were analyzed at three different days (inter-day) and results were statistically evaluated in terms of % RSD.

2.6.4 Recovery:

For recovery studies, pre-assayed portions of formulation equivalent to 20 µg/ml for CFZ and 10 µg/ml for SBT were spiked with extra 50%, 100% and 150% concentration levels. The prepared concentrations were analyzed and the % recovery was calculated using calibration curves.

2.6.5 Robustness:

In order to check the robustness, the effect of small but deliberate variations in the chromatographic conditions was evaluated. The conditions studied were mobile phase change (altered by ±5%), detector wavelength (altered by ±5 nm), and pH of Acetate Buffer solution (altered by ±0.1). These chromatographic variations were evaluated for resolution between CFZ and SBT, % change in each of the changed condition was calculated.

2.6.6 Limit of Detection (LOD) and Limit of Quantization (LOQ): The LOD was estimated using signal-to-noise ratio of 3:1 and the LOQ as 10:1, at which accuracy and standard deviation were within 20% as per ICH guidelines.

2.6.7 Stability Studies:

The solution stability studies for CFZ and SBT are studied at different time intervals. Working standard solution at a concentration 40 µg/ml for CFZ and 20 µg/ml for SBT was repeatedly performed three times in different time intervals of 0-24 Hr and the % stability was calculated using standard calibration curve.

2.6.8 Forced Degradation studies:

Degradation studies are performed by subjecting the standard drug to accelerated degradations such as acid, alkaline, oxidation, thermal, aqueous and photolytic conditions to evaluate the interference of degradation impurities. Thermal degradation is performed by keeping the placebo and standard drugs in different petri dishes and then placed them in an oven at 70°C for 2 days. Photolytic study is carried out by placing the standard drugs in separate petri dishes in a photolytic chamber at 1.2 million hour's illumination and 200-watt hours/square meter ultraviolet energy. Acid, base, and oxidation degradations are performed by adding 0.1 N HCl, 0.1N NaOH, and 30% peroxide solution (H_2O_2), respectively to the standard drugs at 80°C for 2 hour. After the stress expose time, the solutions are neutralized and diluted to get a standard concentration 40 µg/ml for CFZ and 20 µg/ml for SBT. The combination solution is analyzed in the developed method and % stability is calculated using standard calibration curve. The ability of the developed method for the separation of degradation products is also determined using the results obtained during the stress studies.

2.6.9 Application of the Method to Dosage Form:

The accuracy of proposed method is ascertained on the basis of average recovery % to determine CFZ and SBT in its pharmaceutical preparations. The formulation solution prepared from Vitazid-SB vials containing 500mg of SBT and 1000mg of CFZ are analyzed using the developed method. The assay is calculated using standard calibration curve.

3. Results and Discussion

3.1 Method Development:

A series of trials was conducted with different columns like C-18 and C-8 columns with different mobile phases to develop a suitable RP-HPLC method for simultaneous estimation of CFZ and SBT in pharmaceutical dosage form and finally a typical chromatogram was obtained with Water, Methanol, Acetate Buffer in the ration of 50:30:10 (v/v) and pH was adjusted to 5.8 with Acetic acid at a flow rate of 1 ml/min. The chromatographic separation was performed on Waters C-18 (250mm x 4.6mm, 5µm) column by injecting 20 µL and analytes are detected with UV detector at 236 nm. The retention time of CFZ and SBT was found to be 2.85 min and 6.13 min, respectively. Very high resolution of 15.45 was observed in between the CFZ and SBT confirms that the method was high resolved. Number of theoretical plates and tail factor was found to be 3232, 1.15 for CFZ and 18995, 1.33 for SBT confirms that the method obeys system suitable acceptance limit. The optimized conditions were given in Table 1 and standard chromatogram was given in Figure 3.

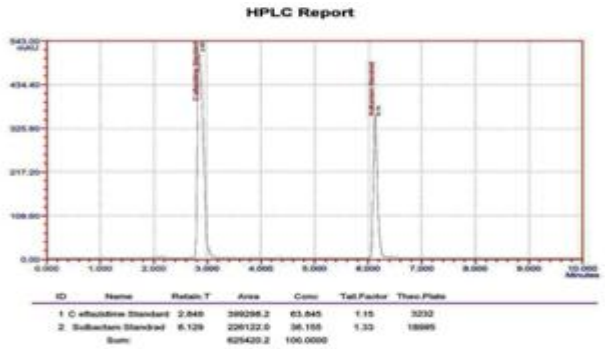

Figure 3: Standard chromatogram for CFZ and SBT

3.2 Method Validation:

3.2.1 Linearity: Linearity was determined by constructing two independent calibration curves for CFZ and SBT, each with six calibration points in the range of 10-60 µg/ml for CFZ and 5-30 µg/ml for SBT. The peak areas of CFZ and SBT against the respective concentrations were used for plotting the graph and the linearity was evaluated by the least square regression analysis. The linearity curve was defined by the following equation y = 9468.x + 28055 (R^2 = 0.998) for CFZ and y = 8513.x + 57253 (R^2 = 0.998) for SBT which indicated the linearity of the calibration curves for the method. Table 2 and Figure III shows the linearity results and linearity graphs respectively obtained in the developed for CFZ and SBT.

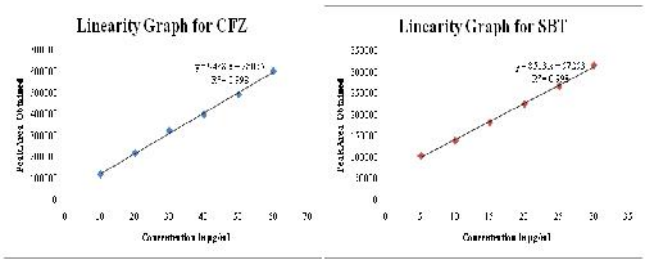

Figure 4: Linearity graphs for CFZ and SBT in the developed method

3.2.2 Precision:

At a concentration 40 µg/ml of Ceftazidime and 20µg/ml Sulbactam was studied for the determination of repeatability of the developed HPLC method. Six replicates analysis was carried for intra-day and inter-day precision. % RSD was found to be 1.099 and 1.333 for intraday precision, 1.213 and 1.534 for inter-day precision for CFZ and SBT respectively. The % RSD was found to be very less confirming that the method was found to be precise.

3.2.3 Accuracy:

Accuracy results were expressed as percent recoveries of the particular components in the samples. The overall results of percent recoveries of drug-matrix solutions are indicating good accuracy of the proposed RP-HPLC method. The % recovery was found to be 99.873, 100.643 and 100.967 for CFZ (Table 3), 100.186, 99.592 and 100.062 for SBT (Table 3) for 50%, 100% and 150% respectively.

3.2.4 Robustness:

Small change in mobile phase ratio by ±5%, detector wavelength by ±5 nm and pH of the mobile phase doesn't affect the separation and detection response of both the drugs CFZ and SBT (Table 3) in the optimized method. This confirms that small variations in the method conditions don't affect the results and hence the method was found to be robust.

3.2.5 LOD and LOQ

Very sensitive detections limits of 0.15µg/ml and 0.03µg/ml was observed for CFZ and SBT respectively in the developed method confirms that the method was applicable for the analysis of drugs up to very lowest concentrations.

3.2.6 Forced degradations:

As shown in Table 4, Peroxide and UV light stress led to the faster effect on CFZ degradation with about 9% and SBT degrade 13% during acidic stress study. In high temperature and direct sunlight conditions, both the drugs SBT and CFZ were found more stable with degradation under 5% (Table 4). Any peak of product degradation was observed in all stress conditions. Figure IV shows the acidic and peroxide stress conditions showing the clear separation of degradation compounds.

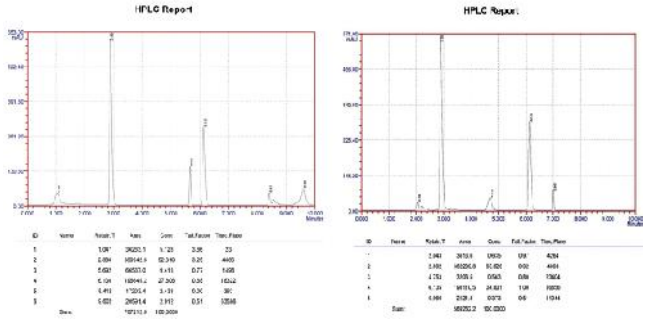

Figure 5: Acidic and Peroxide Chromatograms in forced degradation study for CFZ and SBT

3.2.7: Formulation analysis:

The method was applied for the routine analysis of CFZ and SBT in pharmaceutical formulations. The solution prepared from Vitazid-SB© vials containing 500 mg of SBT and 1000 mg of CFZ were analyzed in the optimized method and % assay was calculated using calibration curve. The % assay was found to be 98.454 and 98.885 for CFZ and SBT respectively. In the sample chromatogram (Figure V), no additional detections were observed conformed that the method was found to be specific and formulation excepients doesn't interfere the elution of CFZ and SBT.

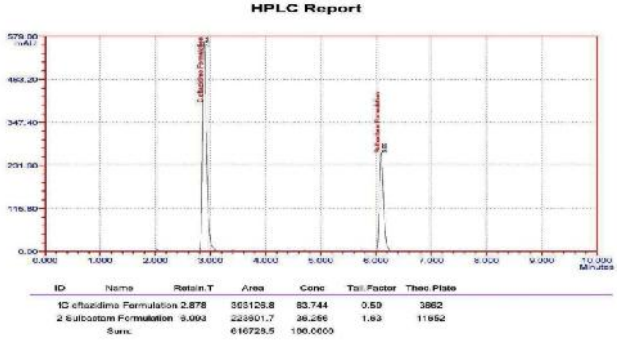

Figure 6: Formulation chromatogram for CFZ and SBT

Table 1: Optimized Chromatographic conditions for CFZ and SBT

S NO	Parameter	Results
1	MP	Water: Methanol: Acetate Buffer 50:30:10 (v/v)
2	Wavelength	236nm
3	Stationary Phase	Waters C-18 (250mm x 4.6mm, 5µm) column
4	pH of MP	5.8
5	Pump Mode	Isocratic
6	Flow Rate	1.0ml/min
7	Run Time	10min
8	Pump Pressure	10.2±4MPa

Table 2: Linearity results for CFZ and SBT

Ceftazidime		Sulbactam	
Concentration (µg/ml)	**Peak area**	**Concentration (µg/ml)**	**Peak area**
10	120020	5	104207
20	218392	10	140015
30	323030	15	182503
40	399298	20	226122
50	493468	25	267741
60	602520	30	316805

Table 3: Summary results for CFZ and SBT

S NO	Parameter	CFZ	SBT
1	Range	10-60µg/ml	5-30µg/ml
2	% RSD in Intra-day Precision	1.099	1.333
3	% RSD in Iner-day Precision	1.213	1.534
4	% Recovery in 50% spiked level	99.873	100.186
5	% Recovery in 100% spiked level	100.643	99.592
6	% Recovery in 150% spiked level	100.967	100.062
7	% Change for MP +ve change	0.497	0.024
8	% Change for MP -ve change	0.580	1.780
9	% Change for pH +ve change	0.771	0.773
10	% Change for pH -ve change	0.818	0.492
11	% Change for WL +ve change	1.483	0.223
12	% Change for WL -ve change	0.035	0.377
13	% RSD in Ruggedness study	0.996	1.142
14	LOD	0.15µg/ml	0.03µg/ml
15	LOQ	0.50µg/ml	0.10µg/ml
16	Formulation Assay (%)	98.454	98.885

Table 4: Forced Degradation study results for CFZ and SBT

S. No	Condition	No of additional detections	Ceftazidime			Sulbactam		
			Area	**% Obtained**	**% Stability**	**Area**	**% Obtained**	**% Stability**
1	Acidic	4	369942	92.6481	7.3519	196645	86.9641	13.0359
2	Aqueous	0	395007	98.9254	1.07464	224465	99.2672	0.73279
3	Base	2	376315	94.2441	5.75585	212988	94.1916	5.80837
4	Light	1	391961	98.1625	1.83747	217386	96.1366	3.8634
5	Peroxide	3	362209	90.7114	9.28855	198110	87.612	12.388
6	Thermal	1	381525	95.5489	4.45106	218526	96.6408	3.35925
7	UV	3	363139	90.9444	9.05564	202909	89.7343	10.2657

4. Conclusion:

It is a well known that validation procedure is an integral part of the analytical method development. Therefore, the developed method was validated according to the guidelines. A simple and rapid stability-indicating high-performance liquid chromatographic method is developed and validated for the simultaneous determination of SBT and CFZ in pharmaceutical dosage forms. This analytical method is specific, linear, precise, accurate, and robust for a rapid determination of this drug and can be used for studying the stability and degradation kinetics of SBT and CFZ. Based on the results, it can be concluded that there is no other co-eluting peak with the main peaks and that the method is specific for estimation of SBT and CFZ.

5. References

[1] NJ White, DA Dance, W Chaowagul, Y Wattanagoon, V Wuthiekanun, N Pitakwatchara. Halving of mortality of severe melioidosis by ceftazidime. *Lancet*, 1989, 2 (8665): 697–701.

[2] "Ceftazidime. The American Society of Health System Pharmacists. Retrieved 8 December 2016.

[3] Hamilton, Richart, Tarascon Pocket Pharmacopoeia 2015 Deluxe Lab Coat Edition. Jones & Bartlett Learning. 2015, p. 87.

[4] Fischer, Janos; Ganellin, C. Robin, Analogue based Drug Discovery. John Wiley & Sons., 2006, p. 495.

[5] "WHO Model List of Essential Medicines" (PDF). *World Health Organization*. October 2013. Retrieved 22 April 2014.

[6] MA Totir, MS Helfand, MP Carey, A Sheri, JD Buynak, RA Bonomo, PR Carey. Sulbactam forms only minimal amounts of irreversible acrylate-enzyme with SHV-1 beta-lactamase. *Biochemistry*, 2007, 46(31): 8980-7.

[7] MS Helfand, MA Totir, MP Carey, AM Hujer, RA Bonomo, PR Carey. Following the reactions of mechanism-based inhibitors with beta-lactamase by Raman crystallography. *Biochemistry*, 2003, 42(46): 13386-92.

[8] H Moreno Ade, HR Salgado. Development of new high – performance liquid chromatographic method for the determination of Ceftazidime. *J AOAC Int*., 2008, 91(4); 739-43

[9] Rajendra Bandal, Harole Mangesh, Pise Kalyan, A validated stability indicating RP-HPLC method for simultanious determination of avibactam and ceftazidime in bulk and pharmaceutical dosage from. *World Journal of Pharmacy and Pharmaceutical Sciences*, 2016, 5(7): 1611-1621.

[10] T Desai Hemant, B Patel Nirav, B Panchal Kalpesh. Development and validation of stability indicating method for simultaneous estimation of ceftazidime and tazobactam injection using RP-UPLC method. *World Journal of Pharmacy and Pharmaceutical Sciences*, 2014, 4(2): 610-622.

[11] K Rabindra Nanda, V Ashwini, Shelke. Development and Validation of RP-HPLC Method For The Simultaneous Estimation Of Ceftazidime Sodium And Tazobactam Sodium In Marketed Formulation. *International Journal of PharmTech Research*, 2013, 5(3); 983-990.

[12] Pinak Patel, Vidisha Patel, Gautam Chauhan, Development and validation of high performance thin layer chromatographic method for simultaneous estimation of ceftriaxone sodium and tazobactam sodium in their combined pharmaceutical formulation. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2014, 6(5); 623-629.

[13] NVV Jagan Mohan Reddy, S Ganapaty. A validated stability indicating RP-HPLC method for simultaneous determination of tobramycin and ceftazidime in pharmaceutical formulations. *International Journal of Pharmacy*, 2015, 5(3); 976-984.

[14] D Chaitanya, K Uma Maheswar, V Phani Kumar, CH Phani.RS. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Avibactam and Ceftazidime in Bulk drug and injection dosage Form. *Actapharmica*, 2016, 3(1); 127-131.

[15] K Nanda Rabindra, V Shelke Aswini, S Panchaware Madhav. UV Spectrophotometric for Simultaneous Estimation of Ceftazidime Sodium and Tazobactum sodium in Dry Powder Injection. *Asian journal of research in Chemistry*, 2012, 5(5); 586-590.

[16] Masoom Raza Siddiqui, Abu Tariq, Manu Chaudhary, K Dinesh Reddy, Prithvi Singh Negi. Development and Validation of High Performance Liquid Chromatographic Method for the Simultaneous Determination of Ceftazidime and Sulbactam in Spiked Plasma and Combined Dosage form-Zydotam. *American Journal of Applied Sciences*, 2009, 6(10); 1781-1787.

[17] R Patel Sannil. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Meropenem and Sulbactam Sodium in Combined Dosage Form. *International Journal of Pharmamedix India*, 2013, 1(2): 336-346.

[18] RK Nanda, VV Bhagwa, SE Potawale, SC Hamane. Development and validation of a HPTLC method for simultaneous densitometric analysis of Cefotaxime Sodium and sulbactam sodium as the bulk drugs and in the pharmaceutical dosage form. *Journal of Pharmacy Research* 2010, 3(7); 1667-1669

[19] AB Devkhile, KA Shaikh, Method development and validation for third generation cephalosporine by UV-Visible spectrophotometer. *International Research Journal of Pharmac*y, 2011, 2(1): 222-229

[20] L Mohan Krishna, P Jayachandra Reddy, V Jaya Sankar Reddy, KVS Prasada Rao. Assay of Ceftazidime in bulk and its pharmaceutical formulations by visible spectrophotometry. *Rasayan J. Chem.*, 2011, 4 (3); 561-566

[21] Hardik B Shah, Ashim Kumar Sen, Aarti Zanwar, AK Seth, Method development and validation for Ceftazidime injection by UV-Visible Specrophotometer. *An International Journal of Pharmaceutical Sciences*, 2013, 4(3); 333-342.

[22] FM Patel, J Dave, N Patel. Spectrophotometric methods for simultaneous estimation of Cefuroxime sodium and Sulbactam sodium in Injecton. *IJPSR*, 2012, 3(9); 3513-3517.

[23] Manoj D Raut, SP Ghode, Rahul Kale, Makarand V Puri, Hemant S Patil. Spectrophotometric method for the simultaneous estimation of Cefotaxime Sodium and Sulbactum in Parentral dosage forms. *International Journal of ChemTech Research*, 2011, 3(3); 1506-1510

[24] B Palani Kumar, A Thenmozhi, D Sridharan. A RP- HPLC method for simultaneous estimation of ceftriaxone sodium and sulbactam sodium in injection dosage form. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2010, 2(3); 34-36

[25] Anjali Patel, Laxman Prajapati , Amit Joshi, Mohammadali Kharodiya, Sandip Patel. Simultaneous estimation of Cefepime hydrochloride and sulbactam sodium in combined dosage form. *Journal of Chemical and Pharmaceutical Research*, 2015, 7(4); 860-865