American Journal of Applied Sciences 6 (10): 1781-1787, 2009
ISSN 1546-9239

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

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Abstract: Problem statement: To develop a sensitive method to determine simultaneously ceftizidime and sulbactam in spiked plasma and combined formulation. **Approach:** In this study an isocratic High performance liquid chromatographic method with UV detection at 230 nm was described for simultaneous determination of Ceftazidime and sulbactam sodium in plasma and combined dosage form. Chromatographic separation of two drugs was achieved on a Hypersil ODS C-18 column using a mobile phase consisting of a binary mixture of acetonitrile and tetrabutyl ammonium hydroxide adjusted to pH 5.0 with orthophosphoric acid in ratio 25:75. **Results:** The developed performance liquid chromatographic method offers symmetric peak shape, good resolution and reasonable retention time for both drugs. Linearity, accuracy and precision were found to be acceptable over the concentration range of 125-625 ppm for Ceftazidime and 62.5-312.5 ppm for sulbactam sodium. **Conclusion:** The results showed that this method could be well used for the simultaneous estimation of Ceftazidime and Sulbactam in plasma and combined formulation.

Key words: Liquid chromatography, ceftazidime pentahydrate, sulbactam sodium, tetrabutyl ammonium hydroxide

INTRODUCTION

Ceftazidime,[(6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamidol]-3-(pyridinum-1-ylmethyl)ceph-3-em-4-carboxylate] is a third generation cephalosporin antibiotics characterized by a broad antimicrobial spectrum and resistant to β lactamase producing organism in addition to its enhanced in vitro activity against a wide veriaty of gram negative organisms, particularly pseudomanas aeruginosa and Pseudomonas cepacia[1,2].

Sulbactam, Sodium (2S,5R)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide, like other β lactamase inhibitors, can be combined with one of many β lactam antibiotics to prevent their destruction by β lactamase for the treatment of infection due to variety of organism staphylococuus aureus, Haemophilius influenza[3]. Both ceftazidime[4] and sulbactam[4] are listed individually in USP.

A literature survey revealed that several methods have been used for estimation of ceftazidime alone which includes Spectrophotometry[5-9], High Performance Liquid Chromatography (HPLC)[10-12] and in combination with pyridine[13] Vancomycin[14] and cefepime[15] by HPLC. Sulbactam was assayed successfully by Spectrophotometry[16], Capillary Isotachophoresis[17], HPLC[18-21] and Gas Chromatography-Mass Spectrometry (GC-MS)[22]. Sulbactam along with clavulonic Acid[23-24], Tazobactam[25], Rifampicin[26] was determined by HPLC. Sulbactam in combination with ampicillin sodium, amoxicillin and piperacillin sodium were determined by UV spectrophotometry and HPLC respectively[27-30].

Zydotam (Ceftazidime-Sulbactam) is a sterile combination of Sulbactam sodium and ceftazidime pentahydrate available as dry powder for injection. Zydotam is supplied in strengths equivalent to 750 mg, 1.5 and 3.0 g with Solvent for injection. It is administered as intramuscular and intravenous injection after reconstitution with solvent supplied with the pack. The product Zydotam is manufactured by Venus Remedies Limited, India. Zydotam is a synergistic antimicrobial combination with marked in vitro antibacterial activity against a broad spectrum of

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organisms. Sulbactam not only potentates the antibacterial activity of Ceftazidime but also exhibits a moderate antibacterial activity. By forming a protein complex with beta-lactamases, Sulbactam irreversibly blocks their destructive hydrolytic activity. Thus, Sulbactam addition extends the spectrum of activity of Ceftazidime. As Sulbactam also binds with some penicillin binding proteins, sensitive strains are often rendered more susceptible to the Zydotam than Ceftazidime alone. In bacterial strains that produce either low amounts of beta-lactamase, or none at all, a synergistic effect is observed when sulbactam is associated with Ceftazidime that has a complementary affinity for the target sites. Zydotam is active against all the organisms sensitive resistant to Ceftazidime. The combination of Ceftazidime and Sulbactam is used to improve cost effectiveness of antimicrobial therapy as it replaces regimens that use multiple drug leading to high incidence of adverse reaction.

Although both the drugs are available in market in combined dosage from yet it lacks a validated method for its estimation in combined formulation. Thus for the quality control and research laboratory purpose it was the need to develop and validate an analytical method for the simultaneous determination of both Ceftazidime and Sulbactam in combined formulation.

The present communication describes isocratic high performance liquid chromatographic method for simultaneous determination of Ceftazidime pentahydrate and Sulbactam sodium, which would be a better alternate for the quality control of the developed formulation. As to the best of our knowledge there is no method present for the simultaneous determination of ceftazidime and Sulbactam in combined dosage form. This study achieved satisfactory results in terms of selectivity, linearity, precision and accuracy under simple chromatographic condition. Adding to its advantage, the method is time saving and requires no pretreatment.

MATERIALS AND METHODS

Reagents and standard: Ceftazidime pentahydrate and sulbactam sodium Reference Standards (RS) of United States Pharmacopoeia (USP) were bought from Sigma, United States. Zydotam, a Fixed Dose Combination (FDC) was obtained from manufacturer, Venus Remedies Limited, India. Each vial contains 1 g Ceftazidime and 0.5 g sulbactam. Tetrabutyl Ammonium Hydroxide (TBAH), acetonitrile and phosphoric acid was of chromatographic grade and was obtained from Merck, Mumbai India. All other chemicals were of analytical reagent grade unless specified.

Apparatus: Agilent 1200 series liquid chromatographic system equipped with G1311A quaternary pump was used for the chromatographic separation. Agilent variable UV/vis detector and a G1329A Auto Injector. EZ Chrome Elite software was employed for data collecting and processing.

Chromatographic conditions: Chromatographic Separation was performed on ODS Hypersil C-18 Stainless Steel column with dimension 250×4.6 mm, 5 µ (Thermo Electron Corporation). The mobile phase consisting of a binary mixture of Acetonitrile and TBAH adjusted to pH 5.0 with orthophosphoric acid in ratio 25: 75, was delivered at rate of 1.6 mL min^{-1}. The mobile phase was filtered through 0.45 µm membrane filter (Millipore) and degassed prior to use. Separation was performed at ambient temperature i.e., 25°C and detection was made at 230 nm. The injection volume was 20 µL with a run time of 12 min.

Preparation of standard sample dissolve an accurately weighed quantity of Ceftazidime (RS) 50 mg and sulbactam sodium (RS) 25 mg in mobile phase and dilute quantitatively and stepwise, if necessary, with mobile phase to obtain a solution having a known concentration of about 500 ppm of Ceftazidime and 250 ppm sulbactam sodium.

Preparation of sample solution: Transfer about 75 mg of Zydotam, (Ceftazidime pentahydrate and sulbactam sodium for injection), accurately weighed, to 100 mL volumetric flask. Add mobile phase, swirl to dissolve, dilute with mobile phase to volume and mix.

Data analysis: For determination of Ceftazidime and sulbactam separately inject equal volumes of the standard preparation and the assay preparation into the chromatograph, record the chromatograms and measure the responses for the major peaks.

DISCUSSION

Method Development and validation: A variety of mobile phase were investigated in the development of the HPLC method suitable for the analysis of Ceftazidime and Sulbactam in bulk and commercial dosage form. This include methanol-phosphate buffer (pH 3-7), Acetonitrile-phosphate buffer (pH 3-7) TBAH-Acetonitrile (pH 3-7). Taking in consideration the instability of ceftazidime and sulbactam sodium in strong alkaline and strong acidic condition, the pH value of the mobile phase should was limited within the range of 3-7. Since mild acidic pH favours the retention and separation of two drugs on C-18 column. After

some trials TBAH with pH 5.0 was finally selected. Acetonitrile is the most commonly used solvent for LC analysis and often is the first choice for many researchers. Therefore, a binary mixture of acetonitrile and TBAH buffer became the mobile phase for the determination of the two drugs. Firstly, various concentrations of TBAH buffer were tried to find the proper one to achieve our purpose. As a result, 0.1N TBAH buffer was found to be ideal for our analysis. Then, the proportion of acetonitrile and TBAH buffer in mobile phase was determined by varying the proportion of acetonitrile and TBAH buffer from 10:90, 20:80, 30:70-25:75. Finally, the 25:75 ratio of acetonitrile and 0.1 N TBAH buffer was used for the simultaneous determination of the two drugs, this system produced symmetric peak shape, good resolution and reasonable retention time, high theoretical plate value and asymmetry less than 2.0 in the case of both drugs. The retention times of Ceftazidime pentahydrate and sulbactam sodium for six repetition were 4.11±0.007 and 6.21±0.015 min respectively. A typical chromatogram of a sample solution is shown in Fig. 1.

Specificity: Specificity is the ability of the method to accurately measure the analyte in the presence of all potential sample components. The response of analyte in test mixtures containing analyte and all potential sample components in compound with response of a solution containing only analyte. The analyte peak is evaluated for peak purity from the nearest eluting peak. For this purpose a solution containing 500 ppm of Ceftazidime and sulbactam sodium was injected and peak purity was done. The acceptance criteria for peak purity is that the purity angle should be less than purity threshold. Result of peak purity analysis was found to be satisfactory, purity angle and purity threshold for Ceftazidime and sulbactam is 1.00 and 0.99, respectively.

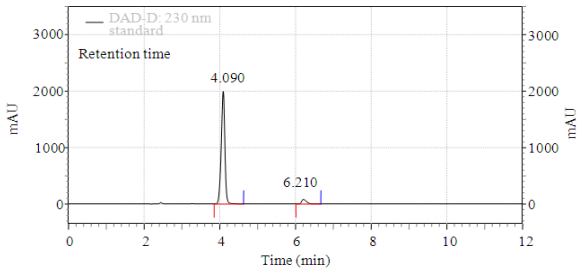

Fig. 1: A typical LC chromatogram of a mixture of Ceftazidime pentahydrate 500 ppm (Peak No. 1) and sulbactam sodium, 250 ppm (peak No. 2) achieved using the proposed method

RESULTS

Forced Degradation Study: This was demonstrated by carried out by treating the mixture of ceftazidime and Sulbactam with 0.5 N HCl, 0.5 N NaOH, 3% H_2O_2, heating in water bath at 80°C for 4 hour and keeping under UV light for 12 h. The samples were degraded to levels where the contents of ceftazidime and sulbactam in the samples were lowered to that of the original level.

Acid degradation: Accurately weighed quanlity of Ceftazidime and Sulbactam equivalent to 50 mg of ceftazidime and 25 mg of sulbactam was transferred to100 mL beaker. Then 10 mL 0.5 N HCl was added to it and heated in the water bath at 60°C for 4 h. Cooled and neutralized with 0.5 N NaOH, sample was diluted up to 100 mL with mobile phase. This solution was injected into the HPLC System. The chromatogram is shown in Fig. 2.

Thermal degradation: Accurately weighed quantity of Ceftazidime and Sulbactam equivalent to 50 mg of ceftazidime and 25 mg of sulbactam was transferred to100 mL beaker and was kept as such in oven at 100°C for 4 h. Cooled and sample was dissolved in 100 mL mobile phase. This solution was injected into the HPLC system. The chromatogram is shown in Fig. 3.

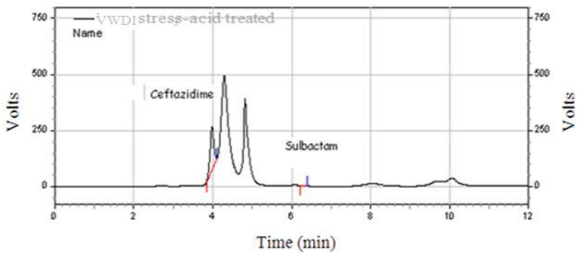

Fig. 2: Chromatogram of Ceftazidime pentahydrate and sulbactam sodium after subjected to acid degradation

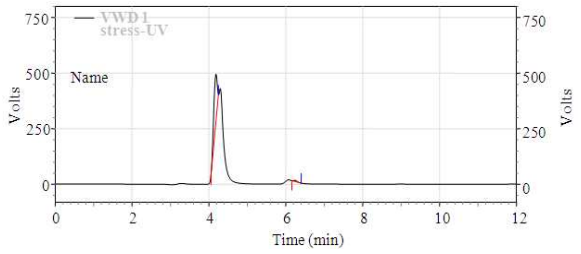

Fig. 3: Chromatogram of ceftazidime Pentahydrate and sulbactam sodium after subjected to Thermal degradation

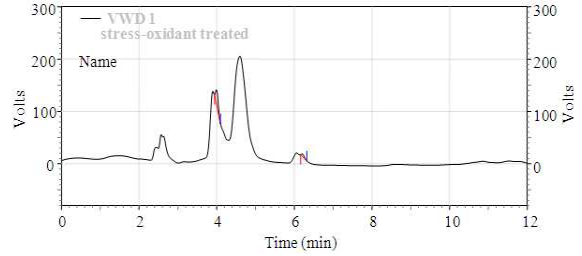

Fig. 4: Chromatogram of Ceftazidime Pentahydrate and sulbactam sodium after treating with oxidant

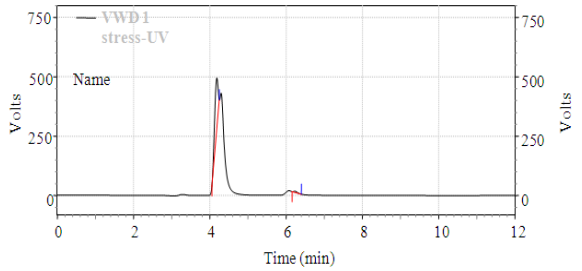

Fig. 5: Chromatogram of Ceftazidime Pentahydrate and sulbactam sodium after UV rays treatment

Peroxide degradation: Accurately weighed quanlity of Ceftazidime and Sulbactam equivalent to 50 mg of ceftazidime and 25 mg of sulbactam was transferred to100 mL beaker to it 10 mL of 3% H_2O_2 was added and kept in water bath at at 60°C for 4 h. Cooled and sample was dissolved in 100 mL mobile phase. This solution was injected into the HPLC system. The chromatogram is shown in Fig. 4.

UV degradation: Accurately weighed quantity of Ceftazidime and Sulbactam equivalent to 50 mg of ceftazidime and 25 mg of sulbactam was transferred to100 mL beaker and was kept as such under UV light for 12 h. Then sample was dissolved in 100 mL mobile phase. This solution was injected into the HPLC system. The chromatogram is shown in Fig. 5.

System suitability: System performance parameters of the developed HPLC method were determined by analyzing standard working solutions. Chromatographic parameters, such as number of theoretical plates (N), resolution (Rs), capacity factor (k) and selectivity factor (α) were determined. The results are shown in (Table 1), indicating the good performance of the system. System repeatability was determined by five replicate injections of a working standard solution and the Relative Standard Deviations (RSD) of peak areas of both drugs were calculated to evaluate the repeatability. It was found that RSD for both the drugs was less than 2.0%.

Table 1: System performance parameters for Ceftazidime and sulbactam sodium (n = 5)

Peak No.	Compounds	t_R (min)	N	K	R_S	α
1	Sulbactam	6.433	10509	246.948	--	--
2	Ceftazidime	4.107	10350	246.730	1.72	
	0.64					

Note: The chromatographic conditions used were: ODS Hypersil C-18 Column, mobile phase of acetonitrile and 0.1 N TBAH (25:75), flow rate of 1.6 mL min^{-1}, detection wavelength of 230 nm, room temperature 25°C, t_R: Retention time, N: Theoretical plates, K: Capacity factor, R_S: Resolution, α: Selectivity factor

Linearity: Under the experimental conditions described above, linear calibration curves for both Ceftazidime pentahydrate and sulbactam sodium were obtained with five concentration level each. Peak Area (A) and Concentration (C) of each drug substance was subjected to regression analysis to calculate the regression equation and the correlation coefficients. The regression equation obtained were A = -0.325+0.0000081 C(r = 0.9997, n = 5) for Ceftazidime and A = -0.3018+0.000154 C(r = 0.9995, n = 5) for sulbactam sodium. The individual linearity range was 125-750 ppm for ceftazidime and 62.5-375 ppm for sulbactam sodium. The results shows that within the tested concentration range there was excellent correlation between the peak area and the concentration of each drugs.

Limit of detection and limit of quantitation: Limit Of Detection (LOD) were established at a signal to noise ratio (S/N) of 3.3. Limit Of Quantification (LOQ) was established at a signal to noise ratio (S/N) of 10. LOD and LOQ were experimentally verified by six injection of Ceftazidime and sulbactam sodium at the LOD and LOQ concentration. The LOD was calculated to be 0.11 ppm and LOQ was found out to be 0.34 ppm.

Accuracy recovery: Accuracy was determined by applying the described method to synthetic mixtures of exipients to which known amount of each drug corresponding to 75, 100 and 125% of label of claim had been added. The accuracy was then calculated as the percentage of analyte recovered by the assay. Mean recoveries (mean ± SD) for Ceftazidime and sulbactam sodium from the combination formulation are shown in (Table 2) indicating good accuracy of the method for simultaneous determination of the two drugs.

Precision: System precision is the measure of the method variability that can be expected for a given analyst performing the analysis. Precision of the method was determined with the product. An amount of the product powder equivalent to 75, 100 and 125% of label claim was weighed accurately and assayed in five replicate determinations for each of the three weighing amounts. The results for precision are shown in Table 2,

Table 2: Accuracy and precision of the HPLC method for simultaneous determination of Ceftazidime and sulbactam sodium

	Accuracy		Precision (%)		
Drugs label claim	Mean recovery ± SD	RSD (%, n = 9)	75	100	125
Ceftazidime	98.69±0.12	0.12	0.19	0.11	0.39
Sulbactam	98.75±0.31	0.31	1.17	0.74	0.37

Note: The chromatographic conditions used were: ODS Hypersil C-18 Column, mobile phase of acetonitrile and 0.1 N TBAH (25:75), flow rate of 1.6 mL min^{-1}, detection wavelength of 230 nm, room temperature 25°C

Table 3: Assay results for Ceftazidime and sulbactam sodium sterile powder for injection (mean ± SD)

Batch No.	Sulbactam sodium	Ceftazidime
1	100.88±0.31	100.54±0.53
2	99.81±0.23	100.12±0.34
3	99.92±0.61	100.30±0.12

Note: The chromatographic conditions used were: ODS Hypersil C-18 Column, mobile phase of acetonitrile and 0.1 N TBAH (25:75), flow rate of 1.6 mL min^{-1}, detection wavelength of 230 nm, room temperature 25°C

indicating that acceptable precision was achieved for Ceftazidime and sulbactam sodium, as revealed by relative standard deviation data (RSD<2.0% in all of the levels of the two drugs.

Analytical solution stability: The stability of both the standard and the test was determined by monitoring the peak area of the standard solution and a sample solution of Ceftazidime and sulbactam sodium at 0, 6, 12 and 24 h at room temperature and refrigerated condition (2-8°C). The results shows that there is no significant difference in the area for 24 h.

Method application: The validated LC method was applied to the simultaneous determination of Zydotam for injection. The three batches of the sample were analyzed and the assay results, expressed as percentage of the label claim are shown in (Table 3). The result indicates that the amount of each drug in the injection corresponds to requirement.

Application of the proposed method in Spiked plasma: In a flcon tube Zydotam (containing Ceftazidime and Sulbactam inratio 2:1) was mixed with plasma to get a concentration of 1 mg mL^{-1}. The above plasma sample was then mixed with water and Acetonitrile (In ratio 1:2) to prepare a solution of 0.75 mg mL^{-1}, then the plasma sample was incubated for 30 min at 4°C and was centrifuged for 15 min at 5000 rpm and the superanent was collected and was analyzed as per the proposed method. The plasma sample was analyzed at 5 concentration level and recovery was found between 96.26-98.05% for Ceftazidime and 96.59-98.19% for Sulbactam. The chromatogram is shown in Fig. 6.

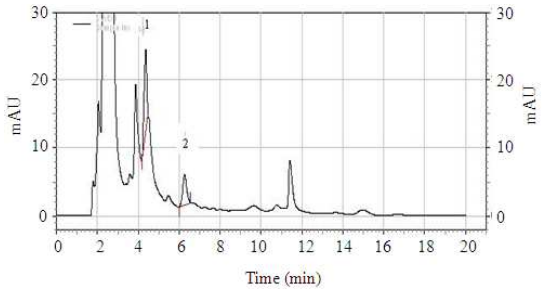

Fig. 6: A typical chromatogram of Ceftazidime (1) and Sulbactam (2) in spiked plasma sample

CONCLUSION

The developed Liquid chromatographic method with UV-Visible detection offers, sensitivity, precision and accuracy. It produces symmetric peak shape, good resolution and reasonable retention time for Ceftazidime and sulbactam sodium. Moreover there is no pretreatment of the sample which makes the method simple and easy to perform. It can be used for the simultaneous determination of Ceftazidime and sulbactam sodium in the pharmaceutical companies and research laboratories for routine analysis and in plasma samples.

ACKNOWLEDGMENT

The researchers are grateful to Chief General Manager, Domestic Operations, Venus Remedies Limited for providing samples of Zydotam.

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