International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.4, pp 1660-1666, Oct-Dec 2012

Development And Validation Of A Stability Indicating HPLC Method For Estimation Of Ceftriaxone And Sulbactam In Sterile Powder For Injection

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Abstract: A simple high performance liquid chromatographic method without using any ion pairing reagent was developed for simultaneous estimation of ceftriaxone and sulbactam in sterile powder for injection. The separation was obtained on Acclaim 120 C_{18}(250 x4.6 i.d)mm, 5µm, stainless steel column with the mobile phase consisting of a mixture of methanol, potassium phosphate buffer(pH 7.0) and triethylamine in the ratio of 23:77:0.2, delivered at a flow rate of 1.15mL/min. The eluents were monitored at 230nm. The validation of the method was performed as per the ICH guidelines for accuracy, precision, linearity, specificity and robustness. The correlation coefficient of the ceftriaxone and sulbactam was 0.998 and 0.999 respectively and the % relative standard deviation for the intra-day and inter-day precision was not more than 1% for both the drugs. The method was found to be specific as none of the degraded products co-eluted with the drugs peak. The accuracy of the method was ascertained by % recovery of the pure drug added which was 99.42% and 101.18% respectively, for ceftriaxone and sulbactam.
Key words: Ceftriaxone, Sulbactam, HPLC, Ion-pairing reagent, Sterile powder for injection.

INTRODUCTION

Ceftriaxone (CTX) is a broad spectrum third generation cephalosporin which is parenterally indicated in several infectious diseases[1]. It has excellent penetration into extravascular spaces and an increased resistance to degradation by β-lactamases. It is used as a routine prophylactic antibiotic for the patients undergoing orthopedic surgery[2]. CTX sodium is chemically known as, (Z)-7-[2-(2-aminothiazol-4-yl)-2methoxyiminoacetyl amido]-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)thiamethyl]-3-cephem-4-carboxylic acid, disodium salt[3,4]. CTX contains a highly acidic, heterocyclic system on the 3-thiamethyl group. This unusual dioxotriazine ring system is believed to confer the unique pharmacokinetic properties of this agent. The chemical structure of CTX is shown in Fig. 1. CTX is listed in the United States Pharmacopoeia[5], British Pharmacopoeia[6] and Indian Pharmacopoeia[7]. Sulbactam sodium is chemically known as (2S,5R)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide, belongs to a class of penicillanic acid sulfones[5]. Sulbactam (SLB) is an irreversible inhibitor of many bacterial β-lactamases, i.e. it binds to β-lactamases more readily than CTX. SLB does not have antibacterial activity when used alone; it synergistically expands ceftriaxone's spectrum of activity against many strains of β-lactamase-producing bacteria. The chemical structure of SLB is shown in Fig. 2.

Figure 1 – Chemical Structure of Ceftriaxone Sodium

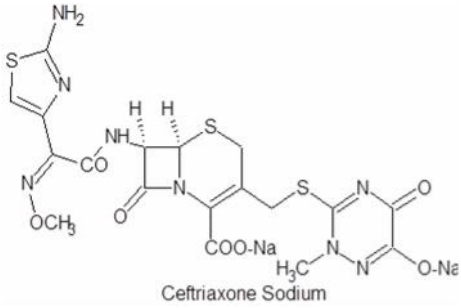
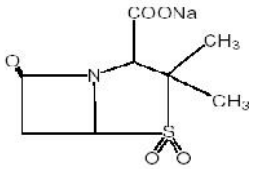


Figure 2 – Chemical Structure of Sulbactam Sodium



Several analytical methods including spectrophotometry[8,9,10,11], liquid chromatography [12,13,14], differential-pulse adsorptive stripping voltammetry[15] and TLC[16,17] have already been reported for the determination of CTX, either alone or in combination with other drugs. For SLB, there are several spectroscopic method along with ampicillin[18,19] ceftriaxone[20] and cefotaxime[21]. HPLC methods of SLB are available along with tazobactam[22], piperacillin[23] and amoxicillin[24]. The analysis of SLB has also been carried out along with CTX but there are certain disadvantages such as use of ion pairing reagent[25] and high percentage of organic phase[26] . In this paper, for the first time, development and validation of a new assay method is described for the analysis of CTX along with SLB in sterile powder for injection dosage form without using any ion pairing reagent.

EXPERIMENTAL

Chemicals and Reagents **-** CTX sodium and SLB was obtained as gift sample from Alkem Laboratories, Sikkim, India. Methanol and potassium phosphate used were of HPLC grade and purchased from S.D Fine Chem Ltd., Mumbai, India. Triethylamine used was of AR grade and purchased from S.D Fine Chem Ltd., Mumbai, India. Commercially available sterile dry powder for injection vials containing CTX and SLB in the ratio of 2:1 was purchased from the local market (I-Tax-S, Sanjeevani Biotech, India).

Instrumentation **-** Shimadzu LC-20 AT liquid chromatography equipped with a 20µL loop, in isocratic mode with Prominence SPD-20A UV-Visible detector was used for quantitative HPLC determination. The HPLC system was equipped with the Spinchrom (Shimadzu) software for data collection and processing. Sartorius(CP225D) electronic balance was used for weighing the materials.

Chromatographic Conditions- The chromato graphic separation was performed on Acclaim 120 C_{18}(250 x4.6 i.d)mm, 5µm, stainless steel column. The mobile phase consisting of a mixture of methanol, potassium phosphate buffer (pH 7.0) and triethylamine in the ratio of 23:77:0.2, was delivered at a flow rate of 1.15mL/min. The mobile phase was filtered through 0.22µm membrane filter and degassed by sonication prior to use. Separation was performed at ambient temperature and detection was made at 230nm.The injection volume was 20µL.

Preparation of standard solution – Weighed accurately and transferred 100mg of CTX and 50mg of SLB in a 100 ml volumetric flask, dissolved with 70 ml of mobile phase and sonicated for 10 minutes and made up the volume with the mobile phase. Pipetted out 5 ml from this solution and diluted to 50ml with the mobile phase.

Preparation of sample solution – An appropriate weight of the sample containing 100mg of ceftriaxone was transferred in a 100ml volumetric flask, dissolved with 70 ml of mobile phase and sonicated for 10 minutes and made up the volume with the mobile phase. The solution was filtered through 0.22µ filter and the filtrate was diluted with the mobile phase to give a final concentration of 100µg/mL of CTX and 50µg/mL of SLB.

Method Development – The wavelength of maximum absorption of SLB was determined to be 220nm so the choice of buffer was not acetate as it produces huge background noise in this wavelength region. The method development started with the acetonitrile and potassium phosphate buffer. In this mobile phase, the resolution of the two drugs was not acceptable, so to change the selectivity the organic phase was changed from acetonitrile to methanol. The resolution was found to be good with methanol, so it was selected as organic phase. The concentration of the buffer was optimised to be 50mm as it was found that at lower buffer concentration the

retention of SLB was poor and the peak asymmetry of CTX was also more. At 50mm buffer concentration, chromatographic properties of both the peaks were very good. The pH of the mobile phase was adjusted to 7.0 with the help of triethylamine as this pH was found to be most suitable for the stability of CTX. The cephalosporins are highly degradable drugs and for longer solution stability it is necessary to develop analytical methods at the pH at which the drug is most stable. The detection wavelength was fixed at 230nm as in shorter wavelengths the baseline disturbances are more due to presence of triethylamine.

System Suitability Parameters – The system suitability parameters such as peak tailing, number of theoretical plates, resolution, retention factor and percentage relative standard deviation of five replicate injections were established for the method and it is given in table 1. Representative chromatogram for the standard and sample of CTX and SLB is given in Figure 3 and 4, respectively.

Method Validation – The developed methods were validated for its accuracy, precision, linearity, specificity and robustness[27,28].

Accuracy - To a fixed and known amount of the drug in vial powder, pure CTX and SLB were added at three different concentration levels, and the total was found by the proposed method from which the percent recovery of pure drugs added were calculated. The results are shown in table 3.

Table 1 - System Suitability Parameters of Ceftriaxone and Sulbactam Assay Method

Name of the drug	Retention time (min)	Tailing factor	Retention factor	Theoretical plates	% RSD of five replicate injections	Resolution
Ceftriaxone	5.0	1.3	1.3	4500	0.59	2.2
Sulbactam	4.35	1.3	1.0	3800	0.66	

Figure 3 – Chromatogram of Ceftriaxone and Sulbactam Standard

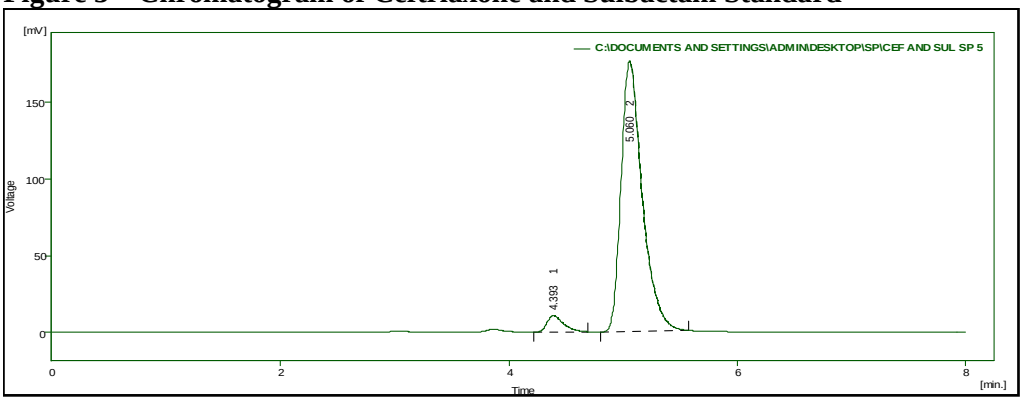
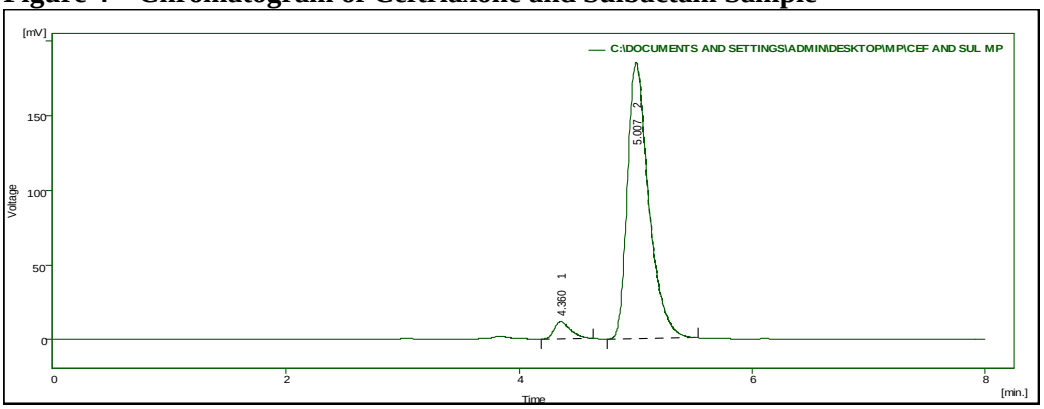


Figure 4 – Chromatogram of Ceftriaxone and Sulbactam Sample



Precision - Precision of the method was evaluated in terms of intra-day and inter-day precision. Intra-day and inter-day precision was reported as percentage relative standard deviation(RSD) on six separate weights of the sample at 100% test concentration. The precision data are summarized in table 4.

Linearity - The linearity of the method was checked by analyzing six solutions in the range of 10–75 µg/mL for SLB (10, 20, 25, 50, 60 and 75 µg/mL) and 20-150 µg/mL for CTX (20, 40, 50, 100, 120 and 150 µg/mL). Each solution was prepared in triplicate. The peak areas obtained from different concentrations of the drugs were used to calculate linear regression equations. The linearity data are summarized in table 2.

Specificity – Specificity of an analytical method may be defined as the ability to unequivocally determine the analyte in the presence of additional components such as impurities, degradation products, and matrix. The degradation products were formed by creating stress conditions such as acidic, basic, oxidative and thermal degradation and the data are summarized in table 5. The matrix was also prepared by mixing the commonly used excipients and injected to see any matrix effect. It was observed that there is no peak due to matrix at the retention time of CTX and SLB.

Robustness – To determine the robustness of the method the experimental conditions were deliberately altered and assay percentage, peak tailing and number of theoretical plates were evaluated in the changed conditions. The flow rate of the mobile phase was changed by 10% to 0.9mL/min and 1.1mL/min and the effect of pH was studied at 6.8 and 7.2 instead of 7.0. For all changes in conditions, the sample was analyzed in triplicate.

Table 2 – Linearity Result of the Method

Name of the drug	Concentration (µg/mL)	Regression equation	R^2
Ceftriaxone	20 - 150	Y=23.34x + 15.50	0.998
Sulbactam	10-75	y= 2.064x – 0.87	0.999

Table 3 - Recovery Result of the Method

Name of the drug	S.No.	Standard drug conc. (µg/mL) (a)	Sample drug conc. (µg/mL) (b)	Total drug conc. (µg/mL) (c)	Total amount found* (µg/mL) (d)	% Recovery of standard (d-b)/a X 100
Ceftriaxone	1	10	100	110	109.88	98.8
	2	15	100	115	114.79	98.65
	3	20	100	120	120.15	100.75
Sulbactam	1	5	50	55	55.05	101.0
	2	7.5	50	57.5	57.53	100.4
	3	10	50	60	60.2	102.0

Table 4 – Precision Study Result

	Ceftriaxone		Sulbactam	
Serial No.	Intra-day Precision (% assay)*	Inter-day Precision (% assay)*	Intra-day Precision (% assay)*	Inter-day Precision (% assay)*
1	100.12	100.48	100.21	99.67
2	100.23	101.38	99.34	100.09
3	101.74	101.91	100.07	98.64
4	99.97	101.92	98.37	99.30
5	99.37	101.89	98.18	98.84
6	99.07	99.76	98.47	100.58

***Average of three readings**

Table 5 - Results from Evaluation of the Forced Degradation Study of the Method

Stress Parameters	Sample treatment	Ceftriaxone		Sulbactam	
		Assay (%)	Degradation %	Assay (%)	Degradation %
Reference	Fresh solution	100.48	0	100.37	0
Acid hydrolysis	0.1M HCl for 30min	82.66	17.73	100.32	0
Base hydrolysis	0.1M NaOH for 15min	72.07	28.28	79.91	20.38
Oxidative	5.0% H_2O_2 for 30min	88.74	11.69	94.02	6.33
Light degradation	UV Light for 24 hrs	88.48	11.94	86.67	13.65

RESULTS AND DISCUSSION

Columns of different dimensions were investigated at the outset of this project to identify a suitable column and mobile phase to optimize the chromatography. It was found that the 250 x 4.6mm column was most suitable for the analysis in which number of theoretical plates, asymmetry factor, resolution and retention factor with the acceptable run time was obtained. The columns shorter than this is unable to separate the drugs properly and the longer columns were giving unnecessarily a longer run time. Different mobile phase compositions were evaluated for the optimum separation of these drugs. The use of methanol instead of acetonitrile was for the selectivity as it was found that the resolution between the two drugs was very poor with the acetonitrile as the organic phase. The pH of the buffer was adjusted to 7.0 with the help of triethylamine as the maximum stability of CTX is at this pH. The use of triethylamine serves the dual purpose, one it adjusts the pH to 7.0 and the other was to mask the polar silanol groups on the stationary phase thus reducing the tailing of the peaks. The effect of the flow rate was investigated by varying the flow rate of the mobile phase from 0.7 to 1.4 mL/min. However, a flow rate of 1.15 mL/min gave an optimal signal to noise ratio with a reasonable separation time and resolution, hence, permitted good analytical conditions.

The validation parameters such as accuracy, precision, specificity, linearity and robustness were performed as per the ICH guidelines. The proposed method was found to be linear in the range of 20-150µg/ml for CTX and 10-75µg/ml for SLB with the correlation coefficeient value of 0.998 and 0.999 for CTX and SLB, respectively. The percentage recovery obtained was 99.42% for CTX and 101.18% for SLB which confirms the accuracy of the method. The precision of the chromatographic method, reported as percent relative standard deviation(RSD), was estimated by measuring intra-day and inter-day assay precision on six separate weights of the sample at 100% test concentration. The %RSD values obtained were less than 1% for both CTX and SLB for both the intra-day and inter-day precision parameters. Stress studies were performed to evaluate the specificity of the method under four different stress conditions. Acid hydrolysis(0.1M HCl) for 30 minutes, base hydrolysis(0.1M NaOH) for 15 minutes, UV light degradation for 24 hrs and oxidative degradation(5.0% H_2O_2) for 30 minutes were carried out. It was observed that the degradation peaks eluted were not interfering with any of the drug peaks which confirm the specificity of the method. Robustness of the

method was checked by deliberately altering two critical parameters of the method. The flow rate was changed by 10% to 1.1mL/min and 0.9mL/min and the pH of the mobile phase buffer was changed from 7.0 to 6.8 and 7.2. The difference in the retention time and peak area (for a given CTX and SLB concentration) caused by the aforementioned minor alterations were found to be insignificant. The rigorous analysis of the validation results indicate that the presence of excipients in the injection formulation did not interfere with the final determination of the active components.

CONCLUSION

The proposed reversed phase HPLC method was found to be simple, precise, linear, accurate, specific and robust. The proposed method is suitable for the determinations of the drug, either in bulk or in injection, without interference from commonly used additives, and it could be used in a regular quality control laboratory.

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