International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 2, Issue 3, 2010

Research Article

AN RP- HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFTRIAXONE SODIUM AND SULBACTAM SODIUM IN INJECTION DOSAGE FORM

B.PALANIKUMAR*, A.THENMOZHI, AND D.SRIDHARAN

Department of Pharmaceutical Analysis, Arulmigu Kalasalingam College of pharmacy, Anand Nagar, Krishnankoil- 626 190
Email: palanipapers@rediffmail.com

Received: 25 Jan 2010, Revised and Accepted: 23 Feb 2010

ABSTRACT

An isocratic liquid chromatographic method with UV detection at 230 nm is described for simultaneous determination of Ceftriaxone sodium and sulbactam sodium in Cetriax-s 1.5gm injection. Chromatographic separations of two drugs was achieved on a Hypersil ODS C-18 column (250mm X 4.6mm, i.d., 5µm,) using a mobile phase consisting of 10mM Potassium Dihydrogen Orthophosphate and acetonitrile (90:10 % v/v) adjusted to pH 5.0 with Potassium hydroxide, in the flow rate of 1.0mL/min. The optimum separation was achieved in less than 15minutes. The developed Liquid Chromatographic method offers symmetric peak shape, good resolution and reasonable retention time for both drugs. The method was validated as per ICH guidelines. The developed method obeys beer's law over the concentration range of 140-250µg/mL for Ceftriaxone and 75 to 160 µg/mL for sulbactam sodium.

Keywords: *Ceftriaxone sodium, Sulbactam sodium, Simultaneous, HPLC.*

INTRODUCTION

Ceftriaxone sodium is chemically known as di-sodium (6R, 7R) - 3[(acetyl-oxy) methyl]-7-[[2Z)-(2-amino-4-thiazolyl) (methoxyamino)-acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4. 2. 0.]Oct-2-ene-2-carboxylic acid[1]. Ceftriaxone is a cephalosporin beta-lactam antibiotics used in the treatment of bacterial infections caused by susceptible, usually gram positive organism[2]. Sulbactam is 4-thia-1-azabi cyclo [3.2.0] heptanes -2-carboxylic acid, 3,3-dimethyl-7-oxo-4,4- dioxide, sodium salt[3]. It is an irreversible inhibitor of beta lactamase; it binds the enzyme and does not allow it to interact with the antibiotic[4]. Both Ceftriaxone and Sulbactam are listed in the United States Pharmacopoeia and the British Pharmacopoeia. Literature survey reveals that there are only one HPLC[5] method is available for the determination of both drugs simultaneously. It was found that there are few analytical methods reported for Ceftriaxone sodium and Sulbactam sodium either in individually or in combination with other drugs by spectrophotometry[6-9], HPLC[10-13], HPTLC[14], capillary electrophoresis[15] and differential pulse adsorptive stripping voltammetry[16]. The aim of the present study was to develop a simple, sensitive, accurate, versatile, speedy and time-saving HPLC method for the simultaneous estimation of Ceftriaxone sodium and Sulbactam sodium in pharmaceutical injection dosage form.

EXPERIMENTAL

Chemicals and reagents

All chemicals used were of analytical grade, and HPLC grade acetonitrile (Merck, Ltd, Mumbai) were used. Double distilled water filtered through 0.45µm filter (MILLI PORE) was used to prepare solutions; pharmaceutical grade Ceftriaxone sodium and sulbactam sodium were procured from Aurobindo chemicals and drugs Ltd. Pondicherry, which was certified to be 98.5% and 99.7% respectively. Commercial formulation, Cetriax-S injection containing ceftriaxone sodium 1gm and sulbactam sodium 0.5 gm were obtained from local market.

Apparatus

Chromatographic separation was performed on SHIMADZU liquid chromatographic system LC 2010 AT equipped with quaternary pump, Shimadzu variable UV/Vis detector SPD-20A and auto Injector. LC solution software was employed for data collecting and processing. Weighing was done on shimadzu balance (AY-120).

Chromatographic conditions

Chromatographic Separation was achieved on ODS Hypersil C-18 (250mm × 4.6 mm, 5µ.,) column. The mobile phase consisting of Potassium Dihydrogen Orthophosphate and acetonitrile (90:10% v/v) adjusted to pH5.0 with Potassium hydroxide, was delivered at rate of 1.0mL/ minute. 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. 25ºC and detection was made at 230 nm**.** The injection volume was 25 µL with a run time of 15 min.

Preparation of standard solution

Accurately weighed quantity of Ceftriaxone sodium (RS) 100 mg and sulbactam sodium (RS) 50 mg was dissolved in water, and volume made up to 50mL, from this 5mL of above is transferred to a 50mL volumetric flask and made up to 50mL with water to get 200µg/mL of Ceftriaxone sodium and 100µg/mL of sulbactam.

Preparation of sample solution

A quantity of powder equivalent to 100mg of Ceftriaxone sodium and 50mg of Sulbactam sodium were accurately weighed and transferred into a 50mL volumetric flask; 20mL of water was added and sonicated for 10minutes and filtered through Whatmann filter No.41 paper. The volume was made up to 50mL with water. From this 5mL of solution is transferred to 50mL volumetric flask and made up to volume with water.

Asaay

From the above sample solution 25µL solution was injected into the chromatographic system along with same concentration of standard solution and chromatogram was recorded. The peak area values of Ceftriaxone sodium and Sulbactam sodium were calculated. The amount of Ceftriaxone sodium and Sulbactam sodium in the solution were estimated using calibration curve method. Results of analysis are tabulated in table 1.

RESULTS AND DISCUSSION

Method development and validation

Taking in consideration the instability of Ceftriaxone sodium and sulbactam sodium in strong alkaline and strong acidic condition, the pH value of the mobile phase should be 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 potassium dihydrogen orthophosphate with pH 5.0 was finally selected. Binary mixture of acetonitrile and potassium dihydrogen Ortho phosphate buffer (90:10 % v/v) was optimized as mobile phase which produced symmetric peak shape, good resolution and reasonable retention time for both the drugs. The retention times of Ceftriaxone sodium and Sulbactam sodium for six repetitions were found to be 9.870 ±

0.006 and 5.750 ± 0.02min respectively. A typical chromatogram of a standard and sample solution is shown in Fig. 1. Since both Ceftriaxone sodium and Sulbactam sodium in the mobile phase have no significant UV maximum but end absorption, to ensure the sensitivity of the method, the wavelength of 230 nm was employed for the detection.

Table 1: Assay of tablets

Drug Name	Label Claim mg/injection	Mean Peak Area		Amount found* ±SD (mg/tab)	%Label claim ±SD
		Standard	Sample		
Ceftriaxone	1.0gm	12343483	12084893	1.002 ± 0.05	100.21± .50
Sulbactam	0.5mg	513505	512440	0.510 ±0.02	102.00±0.41

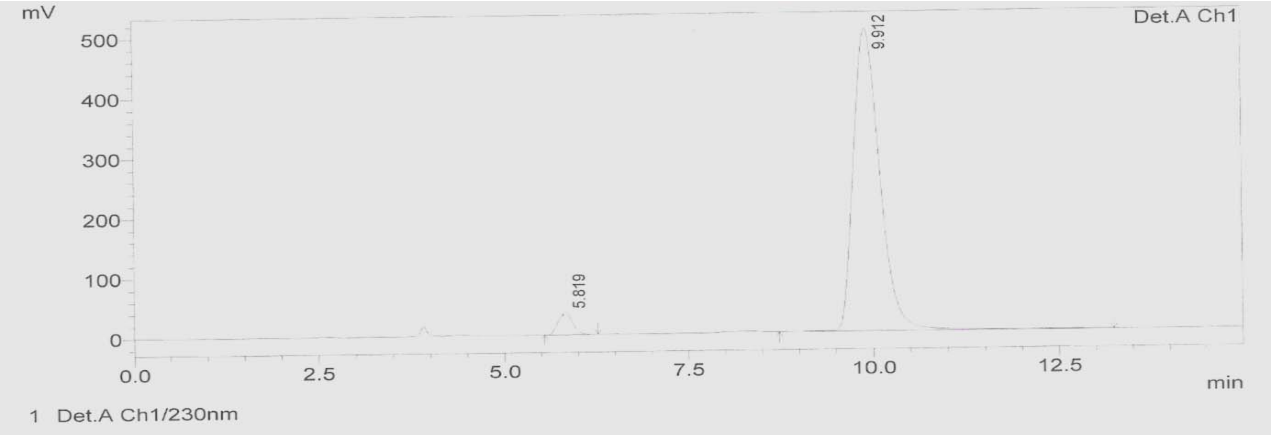

Fig. 1: Chromatogram of Sulbctam Sodium and Ceftriaxone Sodium

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 2), indicating the good performance of the system.

Table 2: System suitability parameters

S. no	Parameters	Obtained values	
		Ceftriaxone	Sulbactam
1.	Theoretical plates (n)	3367	3931
2.	Tailing factor (t)	1.2	1.1
3.	Asymmetry	1.15	1.14
4.	% RSD of peak retention time	1.674	0.887

Linearity

Under the experimental conditions described above, linear calibration curves for both Ceftriaxone sodium 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 =102337.93 C – 6763. 23 (r=0.99995, n=5) for Ceftriaxone sodium and A=8612.21 C–1152.33 (r=0.99996, n=5) for Sulbactam sodium. The linearity range of Ceftriaxone sodium was 140-250µg/mL and 75-150 µg/mL for Sulbactam sodium.

Limit of detection and limit of quantitation

The LOD was calculated to be 3µg/mL for Ceftriaxone sodium and 2 µg/mL for Sulbactam sodium. And the LOQ of Ceftriaxone sodium and Sulbactam sodium were found to be 12 µg/mL and 8 µg/mL, respectively.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by method to the assay value. Accuracy should be established across the specified range of the analytical procedure. The accuracy was then calculated as the percentage of analytes recovered by the assay. Mean recoveries (mean ± S.D.) for ceftriaxone sodium and sulbactam sodium from the combination formulation are shown in (Table 2) indicating good accuracy of the method.

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 to75, 100 and 125% of label of 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 3, indicating that acceptable precision was achieved for ceftriaxone sodium and sulbactam sodium, as revealed by relative standard deviation data (RSD<2.0% in all of the levels of the two drugs).

Table 3: Summary of validation parameters

Parameters	Data	
	Ceftriaxone	**Sulbactam**
Linearity range	140-250µg/ml	75-150µg/ml
Correlation coefficient	09978	0.9999
Limitof quantitation	0.12µg/ml	8µg/ml
Limitof detection	3 µg/ml	2 µg/ml
Accuracy %rsd (repeatability) n=6	0.3127	0.1554
%Recovery		
50%	99.33%	99.44%
100%	97.65%	97.78%
150%	98.09%	99.65%
Precision (%rsd)	0.5079	0.5999
Robustness	99.28%	98.96%
Ruggedness	99.01%	97.79%

CONCLUSION

The developed RP-HPLC method with UV-Visible detection for the estimation of Ceftriaxone sodium and Sulbactam sodium, offers simplicity, selectivity, precision and accuracy. It produces symmetric peak shape, good resolution and reasonable retention time for both drugs. So this method can be applicable for the simultaneous estimation of Ceftriaxone sodium and Sulbactam sodium in quality control studies for routine analysis.

REFERENCES

1. www.wikewpedia.com
2. www.Rxlist.com
3. The United States Pharmacopoeia, 30th Revision, U.S. Pharmacopoeial Convention, Inc., Rockville, MD. 2007.
4. Goodman Gilman's, The Pharmacological basis of therapeutics, 10th ed. McGraw-Hill: London,(2001); p.569-620.
5. Sanjay Mohan Shrivastava, Rajkumar Singh, and Abu Tariq, A novel HPLC method for simultaneous determination of Ceftriaxone and sulbactam in sulbacomax., International journal of biomedical sciences. 2009, Volume 5, No: 1.
6. W. Zhao, Y. Zhang, Q. Li. "Indirect spectrophotometric determination of sodium ceftriaxone sodium with n-propyl alcohol-ammonium sulfate-water system by extraction floatation of copper(II), "*Clin. Chim. Acta.* 2008; 391:80-848.
7. A. F. M. El-Walily, A. A. Gazy, S. F. Belal, E. F. Khamis. "Quantitative determination of some thiazole cephalosporins through complexation with palladium (II) chloride, "*J. Pharm. Biomed. Anal.* 2000; 22:385- 392.
8. S. A. Patel, N. M. Patel, M. M. Patel. "Spectrophotometric estimation of cefotaxime and ceftriaxone sodium in pharmaceutical dosage forms, "*Indian J Pharm Sci.* 2006; 68:101-103.
9. J. Haginaka, J. Wakai, H. Yasuda, T. Uno, T. Nakagawa. "Spectrophotometric determination of sulbactam by reaction with 1, 3, 4-triazole" *Analyst.* 1984;109: 1057-1059.
10. G. G. Granich, D. J. Krogstad. "Ion pair high-performance liquid chromatographic assay for ceftriaxone sodium," *Antimicrob. Agents Chemother. 987;* 31: 385-388.
11. V. Ascalone, L. Dal Bol. "Determination of ceftriaxone sodium, a novel cephalosporin, in plasma, urine and saliva by high-performance liquid chromatography on NH2 bonded-phase column" *J.* Chromatogr. 1983; 273:357-366.
12. S. Joshi. "HPLC separation of antibiotics present in formulated and unformulated samples" *J. Pharm. Biomed. Anal.* 2002; 28:795-809.
13. S. Al-Rawithi, R. Hussein, D. A. Raines, I. AlShowaier, W. Kurdi. "A sensitive assay for the determination of cefazolin or ceftriaxone sodium in plasma utilizing LC" *J. Pharm. Biomed. Anal.* 2000; 22:281-286.
14. J. S. Eric, D. Agbaba, D. Zivanov-Stakic, S. Vladimirov. "HPTLC determination of ceftriaxone sodium, cefixime and cefotaxime in dosage forms" *J. Pharma. Biomed. Anal.* 1998; 18:893-898. 10. D. Marini, F. Balestrieri. "Determination of ceftriaxone sodium by HPLC". *Farmaco.* 1986; 41:172-176.
15. I. Jelinek, H. Krejcirova, J. Dohan, Z. Roubal, V. Hola, V. Rejholec. "Determination of sulbactam in human serum using capillary isotachophoresis" *Cesk. Farm.* 1990; 39:305-307.
16. S. Altinoz, A. Temizer, S. Beksac. "Determination of ceftriaxone sodium in biological material by differential-pulse adsorptive stripping voltammetry" *Analyst.* 1990; 115:873-874.