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Research Article

AN RPHPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFTRIAXONE SODIUM AND SULBACTAM SODIUM IN PARENTERAL DOSAGE FORM

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ABSTRACT

Simple rapid, accurate, and precise isocratic liquid chromatographic method has been develop with UV detection at 230 nm is described for simultaneous determination of Ceftriaxone sodium and sulbactam sodium in parenteral by using Agilent 1200 Series HPLC. Chromatographic separations of two drugs was achieved on a C-18 column using a mobile phase consisting of Ammonium acetate Buffer (50mm) and Methanol (90:10 % v/v) adjusted to pH 7.0 with triethylamine, 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 20-100µg/mL for Ceftriaxone sodium and 10-50 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 Oct-2-ene-2-carboxylic acid[1]. Sulbactam is 4-thia-1-azabi cycloheptanes-2-carboxylic acid, 3, 3-dimethyl-7-oxo-4, 4- dioxide, sodium salt[2]. Both Ceftriaxone and Sulbactam are listed in the Indian Pharmacopoeia[3] and the British Pharmacopoeia, respectively[4]. Ceftriaxone is a third generation cephalosporin beta-lactam antibiotics used in the treatment of bacterial infections caused by susceptible, usually gram positive organism[5]. Sulbactam is an irreversible inhibitor of beta lactamase; it does not allow the enzyme to interact with the β lactum antibiotics and protect it[6]. Ceftriaxone shows lower MICs than Cefotaxime for several gram-negative organisms. Therefore, combination of broad spectrum Ceftriaxone sodium and broad spectrum Sulbactam sodium is more effective against beta lactamase producing bacteria than the single drug alone. The dose of individual drug is also less in the combined dosage form that may cause less adverse effects. Literature survey reveals that there are only few HPLC[7,8] and Spectroctrophotometric[9] methods available for the determination of both drugs, simultaneously. Reported UV method has used a specific mode that is only available in the sophisticated instruments.

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[10,11,12,13], HPLC[14-17], HPTLC[18], capillary electrophoresis[19] and differential pulse adsorptive stripping voltammetry[20], Polarographic[21].The aim of the present study was to develop a simple, sensitive, accurate, versatile, and fast HPLC method for the simultaneous estimation of Ceftriaxone sodium and Sulbactam sodium in pharmaceutical injection dosage form.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals used were of analytical grade, and HPLC grade Methanol was used. Double distilled water filtered through 0.2µm filter (MILLI PORE) was used to prepare solutions; pharmaceutical grade Ceftriaxone sodium and sulbactam sodium were procured from Hindustan Antibiotics Ltd. Pimpri Pune, which was certified to be 98.5% and 99.7% respectively. Parenteral formulation, 1.5gm injection containing ceftriaxone sodium 1gm and sulbactam sodium 0.5 gm were obtained from local market.

Apparatus

Chromatographic separation was performed on Agilent 1200 series liquid chromatographic system equipped with quaternary pump, UV/Vis detector and manually Injector. LC solution software was employed for data collecting and processing. Weighing was done on Metler balance.

Chromatographic conditions

Chromatographic Separation was achieved on ODS Hypersil C-18 (250mm × 4.6 mm,µ5 ,) column. The mobile phase consisting of Ammonium phosphate buffer (50Mm) and methanol (90:10% v/v) adjusted to pH7.0 with Triethylamine, was delivered at rate of 1.0mL/ minute. The mobile phase was filtered through 0.20 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 20 µL with a run time of 15 min.

Preparation of standard solution

Accurately weighed quantity of Ceftriaxone sodium (RS) 10 mg and sulbactam sodium (RS) 5 mg was dissolved in water, and further diluted to get 20µg/mL of Ceftriaxone sodium and 10µg/mL of sulbactam.

Preparation of sample solution

A quantity of powder equivalent to 10mg of Ceftriaxone sodium and 5mg of Sulbactam sodium were accurately weighed and transferred into volumetric flask and sonicated for 10minutes and filtered through Whatmann filter No.41 paper. The further dilution was made to get desired concentrations. All dilution made with mobile phase.

Asaay

From the above sample solution 20µL 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 Ammonium phosphate buffer with pH 7.0 was finally selected. Binary mixture of Ammonium phosphate buffer and methanol (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 10.062 ± 0.006 and 3.249 ± 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 Formulation

Drug Name	Label Claim (mg/injection)	Mean Peak Area		Amount found ±SD (mg/injection)	%Label
		Standerd	sample		
Ceftriaxone sodium	1.0 gm	1876.835	1860.568	1.0023 ± 0.05	100.21± 0.5
Sulbactam	0.5 gm	94.976	93.235	0.510 **±**0.02	102.00**±**0.41

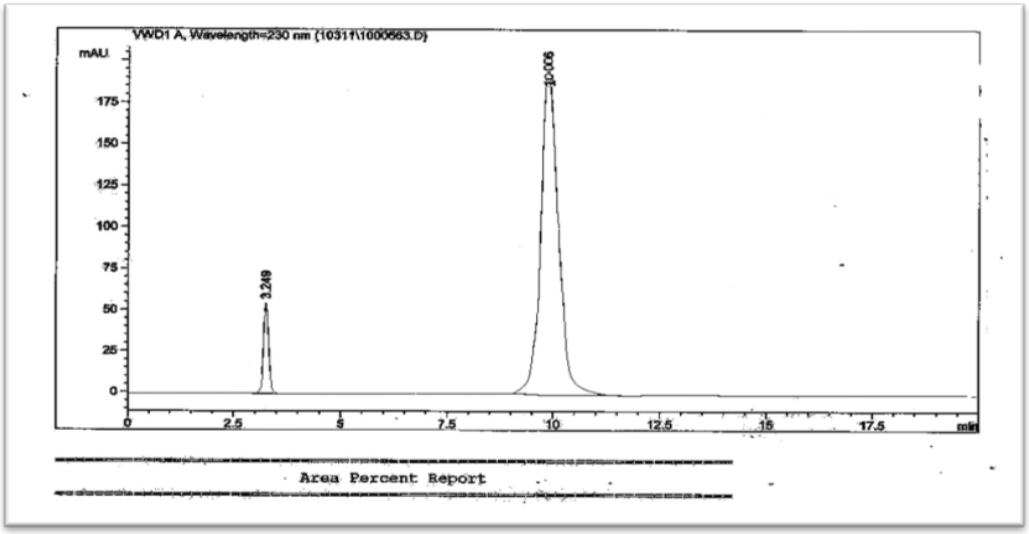

Fig. 1: Chromatogram of Sulbctam Sodium and Ceftriaxone Sodium

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.

Linearity

Under the experimental conditions described above, linear calibration curves for both Ceftriaxone sodium and Sulbactam sodium were obtained with five concentration level each. The r^2 for Ceftriaxone sodium was 0.991 and for Sulbactam sodium r^2= 0.9994. The linearity range of Ceftriaxone sodium was 20-100 µg/mL and 10-50 µg/mL for Sulbactam sodium.

Table 2: System suitability parameters

Sr. no	Parameters	Obtained values Ceftriaxone sodium	Sulbactum sodium
1	Theoretical plates	3256	3850
2	Tailing factor	1.20	1.22
3	Asymmetry	1.16	1.13
4	%RSD of peak retention time	0.895	1.569

Table 3: Linearity study of Ceftriaxone sodium:

Sr No.	Concentration (µg/mg)	Area mAU	RSD	Correlation coefficient
1	10	333.6		
2	20	667.2		
3	30	1000.8		
4	40	1334.4		
5	50	1668.36	0.9645	0.9991
6	60	1998.35		
7	70	2335.2		
8	80	2568.32		
9	90	3002.4		
10	100	3336		

*Average of six determinations.

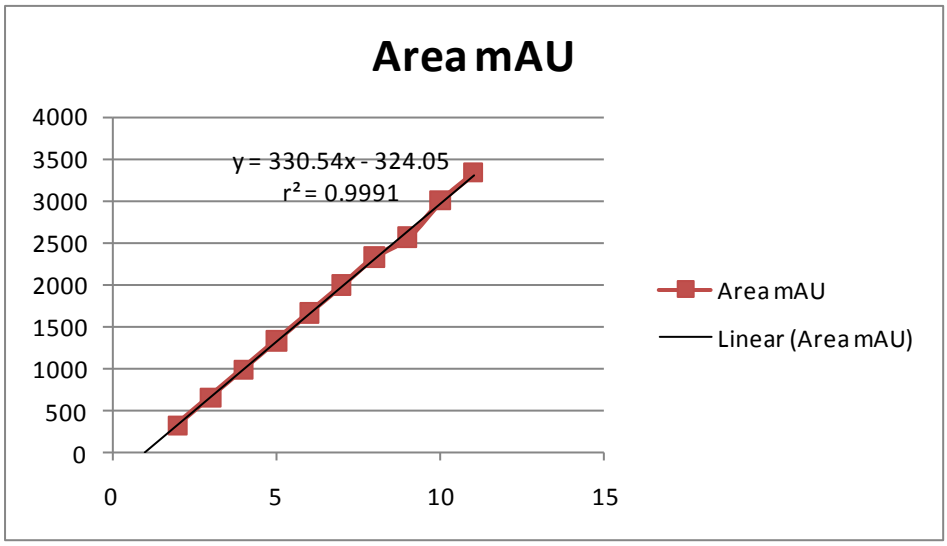

Fig. 3: Calibration curve for Ceftriaxone

Table 4: Linearity study of Sulbactum sodium:

Sr No.	Concentration (µg/mg)	Area mAU	RSD	Correlation coefficient
1	10	76.94		
2	20	152.76		
3	30	221.78	0.3276	0.9994
4	40	304.4		
5	50	380.22		

*Average of six determinations.

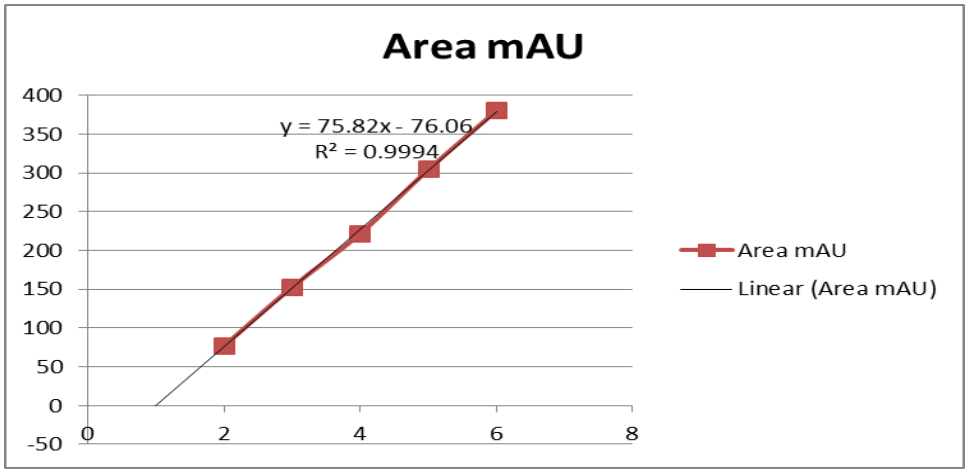

Fig. 4: Calibration curve for Ceftriaxone.

Limit of detection and limit of quantitation

The LOD was calculated to be 1µg/mL for Ceftriaxone sodium and 0.05 µg/mL for Sulbactam sodium. And the LOQ of Ceftriaxone sodium and Sulbactam sodium were found to be 4 µg/mL and 2 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	
	Sulbactum sodium	**Ceftriaxone sodium**
Linearity	10-50 µg/mL	20-100 µg/mL
Correlation coefficient	0.9994	0.9991
Limit of detection	0.05 µg/mL	1µg/mL
Limit of Quantitation	2 µg/mL	4 µg/mL
Accuracy	1.569	0.895
% Recovery		
50%	99.44%	99.85%
80%	97.78%	97.89%
100%	99.65%	98.90%
Precision	0.648	0.457
Robustness	99.69	100.26
Ruggedness	98.562	100.420

CONCLUSION

The method offers simplicity, selectivity, precision, time saving and accuracy by using economical mobile phase. The method is easy to use for laboratory use. 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. The United States Pharmacopoeia, 30th Revision, U.S. Pharmacopoeial Convention, Inc., Rockville, MD. 2007.
3. Indian Pharmacopoeia, Vol. 1, Govt. of India ,Ministry of Health and Family Welfare. New Delhi; Published by The Controller of Publications; 1996,p.148.
4. United States Pharmacopoeia, United States Pharmacopoeial Convention. Inc, Rockville, MD, 2004, p.343
5. www.Rxlist.com
6. Goodman Gilman's, The Pharmacological basis of therapeutics, 10th ed. McGraw-Hill: London, (2001); p.569-620.
7. 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.
8. B.Palanikumar, A.Thenmozi, And D.Sridharan, An RPHPLC method for simultaneous estimation of Ceftriaxone sodium and Sulbactum sodium in injection dosage form., International journal of pharmacy and pharmaceutical sciences. 2010, volume 2, No. 3
9. Sridharan Durairaj*, Simultaneous Estimation of Ceftriaxone Sodium and Sulbactam Sodium using Multi-Component Mode of Analysis., International Journal of Chem Tech Sciences., Vol.2, No.4, pp 2177-2181, Oct-Dec 2010
10. W. Zhao, Y. Zhang, Q. Li. "Indirect spectrophotometric determination of ceftriaxone sodium with n-propyl alcohol-ammonium sulfate-water system by extraction floatation of copper (II), "*Clin. Chim. Acta.* 2008; 391:80-848.
11. 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.
12. 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.
13. 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.
14. G. G. Granich, D. J. Krogstad. "Ion pair high-performance liquid chromatographic assay for ceftriaxone sodium," *Antimicrob. Agents Chemother. 987;* 31: 385-388.
15. V. Ascalone, L. Dal Bol. "Determination of ceftriaxone sodium, a novel cephalosporin, in plasma, urine and saliva by highperformance liquid chromatography on NH2 bonded-phase column" *J.* Chromatogr. 1983; 273:357-366.
16. S. Joshi. "HPLC separation of antibiotics present in formulated and unformulated samples" *J. Pharm. Biomed. Anal.* 2002; 28:795-809.
17. 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.
18. 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.
19. 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.
20. S. Altinoz, A. Temizer, S. Beksac. "Determination of ceftriaxone sodium in biological material by differential-pulse adsorptive stripping voltammetry" *Analyst.* 1990; 115:873-874.
21. Subba Reddy gv, Vijaya Narasimha, Reddy sj, "Polarographic reduction behaviour of some potential Antibiotic Cephalosporins" International Journal of Pharmacy and Pharmaceutical sciences Vol 2 Suppl 4 2010