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SIMULTANEOUS DETERMINATION AND METHOD VALIDATION OF CEFTRIAXONE SODIUM AND SULBACTAM SODIUM BY REVERSE PHASE ION - PAIR HPLC

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ABSTRACT

A simple, precise, accurate, rapid and reproducible reverse phase ion-pair high performance liquid chromatographic procedure was developed for simultaneous determination of ceftriaxone sodium and sulbactam sodium in sterile powder for injection. The mobile phase used was a combination of acetonitrile: 0.03M phosphate buffer (55:45,v/v) and the pH was adjusted to 5.0 by addition of triethylamine, 3.2 g/L tetraheptyl ammonium bromide was added as ion-pair reagent. The detection of the combined dosage form was carried out at 230 nm and a flow rate was set to 1 mL/min. Linearity was obtained in the concentration range of 50.0 to 400.0 μg/mL of ceftriaxone sodium and 25.0 to 200.0 μg/mL of sulbactam sodium with co-relation coefficient of 0.9999 and 0.9998, respectively. The results of the analysis were validated statistically and recovery studies confirmed the accuracy of the proposed method.

Key words: Ceftriazone sodium, Sulbactam sodium, Ion-pair HPLC.

INTRODUCTION

Ceftriaxone sodium (CFT) is a third generation, semisynthetic, broad spectrum cephalosporin antibiotic for the treatment of susceptible infections. The antibacterial action of ceftriaxone is due to the inhibition of cell wall synthesis. Ceftriaxone has a high degree of stability in the presence of beta-lactamases, both penicillinases and cephalosporinases of gram-negative and positive bacteria. Chemically ceftriaxone sodium is (6*R*, 7R)-7-[[(*Z*)-(2-aminothiazolyl) (methoxyimino) acetyl] amino]-3-[[(2-methyl-6-oxido-5-oxo-2,5-dihydro-1,

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2, 4-triazin-3-yl)sulphanyl]methyl]-8-oxo-5-thia1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate. (Fig. 1). It is official in USP NF[1] and BP[2]. Literature survey revealed few analytical methods, RP-HPLC method[3-5], spectrophotometric method[6], flow injection analysis[7], and HPTLC method[8], for determination of ceftriaxone sodium.

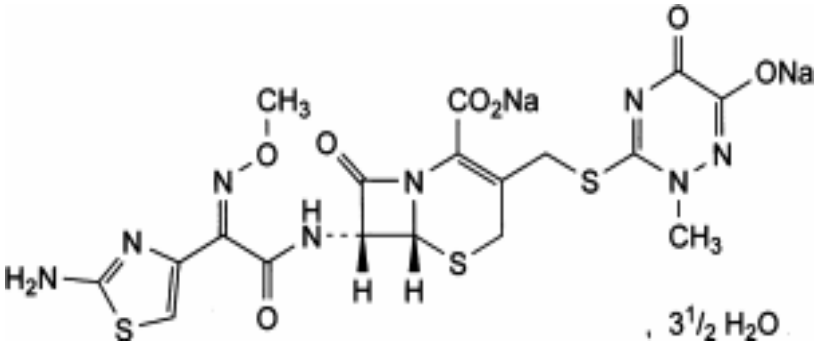

Fig. 1: Chemical structure of ceftriaxone sodium

Sulbactam sodium (SLBT) is a competitive, irreversible beta-lactamase inhibitor and has good inhibitory activity against the clinically important plasmid mediated beta-lactamases most frequently responsible for transferred drug resistance and is chemically 4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-, 4,4-dioxide, sodium salt. (Fig. 2) Few analytical methods have been reported for the estimation of sulbactam sodium[9-16]. Sulbactam sodium is official in USP NF[17].

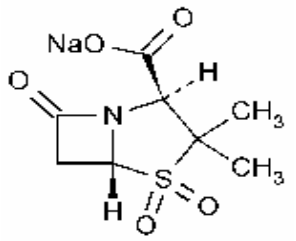

Fig. 2: Chemical structure of sulbactam sodium

EXPERIMENTAL

Materials

Pharmaceutical grade ceftriaxone sodium was obtained as a gift sample from Plethico Pharmaceuticals Limited, Indore, (M.P.) and sulbactam sodium (Working Standard No. QC/S-07-II) was obtained as a gift sample from Cadila Pharmaceuticals Limited,

Ahmedabad, (Gujrat). Sterile powder for injection (XTUM* 375, Batch No, SV0611106 was procured from the local market. (Label claim: Ceftriaxone sodium USP equivalent to 250 mg ceftriaxone and sulbactam sodium USP equivalent to 125 mg sulbactam) marketed by Shreya Life Sciences Pvt. Ltd. Andheri, Mumbai-400 099. All chemicals used were of HPLC grade and were purchased from Spectrochem, Mumbai, India.

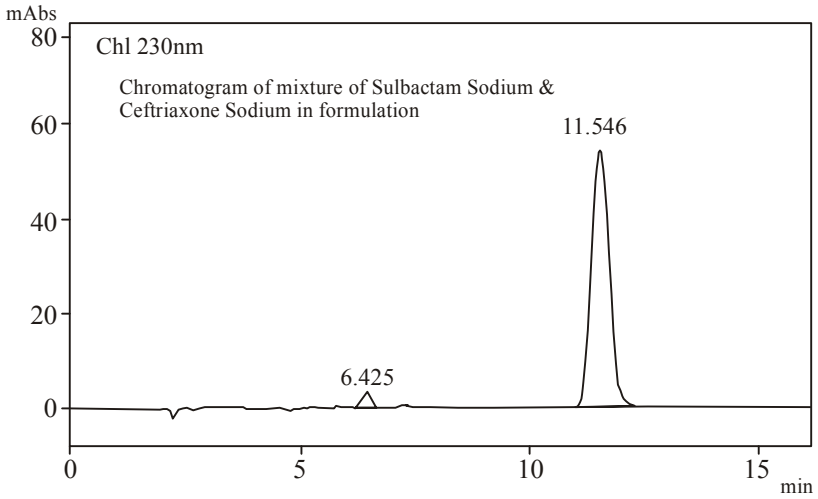

Fig. 3: Typical chromatogram of ceftriaxone and sulbactam in sterile powder for injection. (Retention time is 11.54 min for CFT and 6.42 min for SLBT)

Equipment used

Shimadzu LC 10AT VP HPLC system LC system used consist of pump (Model Shimadzu; LC-10 AT VP) with universal loop injector (Rheodyne 7725) of injection capacity 20 μL. Detector consists of photodiode array detector SPD-10 AVP, Shimadzu; the reversed phase column used was Luna C_{18} (5 μm, 25 cm x 4.6 mm i.d.) phenomenex, USA, at ambient temperature.

RP-HPLC method

Preparation and selection of mobile phase

Among the several mobile phases used for the simultaneous estimation of ceftriaxone and sulbactam, acetonitrile: 0.03M phosphate buffer (55:45,v/v) ratio was found to be most suitable and the pH was adjusted to 5.0 by adding triethylamine, 3.2 g/L tetraheptyl ammonium bromide was added as ion pair reagent and was filtered through 0.2 micron membrane filter.

Preparation of calibration curves

Mixed stock solutions of ceftriaxone sodium and sulbactam sodium with concentrations 1000 µg/mL and 500 µg/mL, respectively were prepared in the mobile phase. From the above stock solutions, dilutions were made in the concentration range of 50.0 to 400.0 µg mL^{-1} of ceftriaxone sodium and 25.0 to 200.0 µg mL^{-1} of sulbactam sodium. All solutions were stored at room temperature. These solutions were shown to be stable during the period of study. A volume of 20 µL of each standard after filtration by 0.2 micron membrane filter was injected into column. All measurements were repeated five times for each concentration and respective calibration curves were constructed by plotting the peak area versus the corresponding drug concentration. The slope and correlation coefficients were determined, which were found to be 0.9999 for ceftriaxone sodium and 0.9998 for sulbactam sodium.

Analysis of sterile powder for injection dosage form

To determine the content of ceftriaxone sodium and sulbactam sodium in sterile powder for injection (Label claim: 250 mg of ceftriaxone and 125 mg of sulbactam); five vials were weighed, the average weight of powder was determined. Then 82.5 mg of powdered sample was taken, which is equivalent to 50 mg of ceftriaxone and was dissolved in small amount of mobile phase by stirring for 2 minutes and the volume was made up to 50 mL by mobile phase. The stock solution was filtered by 0.2-micron membrane filter by

Table 1: Results of analysis of sterile powder for injection

Drug	Conc. taken (µg)	Conc. found (µg)	S.D.	% RSD
Ceftriaxone sodium	50.0	49.95	0.2247	0.45
	100.0	99.80	0.5788	0.58
	150.0	150.10	0.4352	0.29
	200.0	200.39	0.4608	0.23
Sulbactam sodium	25.0	24.96	0.1447	0.58
	50.0	49.97	0.1748	0.35
	75.0	75.08	0.4654	0.62
	100.0	99.52	0.2687	0.27

(Results are mean of five replicates)

using the instrument called injection filter. Then with the help of pipette, 0.5, 1.0, 1.5 and 2.0 mL of the filtered solution was taken in volumetric flasks and diluted up to 10 mL of each respectively, which contain 50:25, 100:50, 150:75 and 200:100 µg/mL, respectively. A 20 µL of the above dilutions were injected one by one to the HPLC with the help of Hammilton syringe and the amounts of CFT and SLBT were determined. The results are reported in Table 1.

Recovery studies

To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method. From the total amount of drug found, the percentage recovery was calculated. The results of the analysis are reported in Table 2.

Table 2: Results of recovery studies

Qnty. taken CFT (µg)	Qnty. added CFT (µg)	% recovery CFT	Qnty. taken SLBT (µg)	Qnty. added SLBT (µg)	% recovery SLBT
50.0	40.0	99.60	25.0	20.0	98.82
50.0	50.0	100.32	25.0	25.0	100.18
50.0	60.0	100.65	25.0	30.0	99.76
100.0	80.0	99.00	50.0	40.0	98.73
100.0	100.0	98.01	50.0	50.0	101.10
100.0	120.0	101.85	50.0	60.0	100.66

(Results are mean of five replicates)

RESULTS AND DISCUSSION

The HPLC method was found to be simple, accurate, economic and rapid for routine simultaneous estimation of ceftriaxone sodium and sulbactam sodium, in sterile powder for injection at single wavelength. The regression: 0.9999 and 0.9998 intercept: – 21698 and – 1110.71 and slope: 27204 and 1585.2 were found to be for ceftriaxone sodium and sulbactam sodium, respectively. Recovery was in the range of 98 – 102%; the value of standard deviation and percentage relative standard deviation were found to be less than 2%; which shows the high precision of the developed method (Tables 1 and 2). Evaluation,

repeatability, precision and ruggedness studies of ceftriaxone sodium and sulbactam sodium from marketed formulation are shown in Tables 3 and 4.

Table 3: Evaluation study of ceftriaxone sodium and sulbactam sodium in marketed formulation

Parameters	CFT	SLBT
Label Claim (mg)	250	125
Amount found (mg)	250.49	124.39
% of Label claim	100.19	99.51
± S.D.	1.12	0.72
% RSD	0.44	0.58
SEM	0.50	0.32
(Results are mean of five replicates)		

Table 4: Repeatability, accuracy, precision and ruggedness studies of ceftriaxone sodium and sulbactam sodium

Drug	Std. Conc.	Mean (peak area)	S.D.	% RSD
Ceftriaxone sodium	50.0	1551379	3619	0.23
	100.0	3106021	5676	0.81
	150.0	4641665	2526	0.05
	200.0	6261818	33706	0.53
Sulbactam sodium	25.0	40911	262	0.64
	50.0	82759	495	0.59
	75.0	122632	347	0.28
	100.0	162708	511	0.31
(Results are mean of five replicates)				

In proposed method, HPLC conditions were optimized to obtain an adequate separation of eluted compounds. Amongst the various mobile phases used, acetonitrile:

0.03M phosphate buffer in 55:45 (v/v) ratios, the pH was adjusted to 5.0 by addition of triethylamine and 3.2 g/L tetraheptylammonium bromide was added as ion-pair reagent and it was found robust with 1 mL/min flow rate. Mobile phase and flow rate selection was based on peak parameters such as height, tailing, theoretical plates, capacity factor, run time and resolutions. A typical chromatogram of ceftriaxone sodium and sulbactam sodium is shown in Fig. 1. The optimum wavelength for detection was 230 nm at which detector response was best obtained. The average retention time for ceftriaxone sodium and sulbactam sodium was found to be 11.54 ± 0.05 min. and 6.45 ± 0.05 min., respectively. According to USP XXIV (621)[18], system suitability tests are an integral part of chromatographic method. They are used to verify reproducibility of the chromatographic system. To ascertain the effectiveness of the method, system suitability study and method validation were carried out and its results are reported in Table 5.

Table 5: Results of validation studies

SST and other parameters	Ceftriaxone sodium	Sulbactam sodium
*Theoretical plates (N)	4210	4257
*Resolution (R_s)	9.20	--
Linearity range (μg mL^{-1})	50 - 400	25 - 200
Percentage recovery (%)	98 - 102	98 - 102
Drug recovered (250:125 mg)	250.006	124.916
LOD (μg mL^{-1})	2.632	2.312
LOQ (μg mL^{-1})	7.976	7.003
*Tailing factor	1.06	1.10
*Capacity factor	3.63	1.59
*Retention time (Minutes)	11.5	6.4
Slope (m)	27204	1585
Intercept (b)	-21698	-1110.7
Correlation coefficient	0.9999	0.9998
Accuracy/trueness (%)	98 - 102	98 - 102
Specificity/selectivity	No interference	No interference
Stability of sample solution	> 24 Hrs.	> 24 Hrs

*Calculated at 5% peak height

CONCLUSION

The proposed method is accurate, simple, rapid, reproducible and selective for the simultaneous estimation of ceftriaxone sodium and sulbactam sodium in sterile powder for injection.

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