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RESEARCH ARTICLE

SIMULTANEOUS QUANTIFICATION OF SULBACTUM SODIUM AND CEFTRIAXONE SODIUM IN PARENTERAL DOSAGE FORM BY NOVEL LC METHOD

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ABSTRACT

A simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of Sulbactum sodium (SBS) and Ceftriaxone sodium (CTS) in pharmaceutical dosage forms. The RP-HPLC, separation was carried out by using Agilent XDB, C_{18} (150 x 4.6 mm, 5µ) analytical column and detection was carried out at 218nm by using variable wavelength detector. The mobile phase consists of buffer (0.02 M Sodium Dihydrogen phosphate, pH-3 was adjusted with ortho phosphoric acid): Acetonitrile in the ratio of 82:18 % v/v delivered at a flow rate of 1.0 ml / min and wavelength of detection at 218nm. The retention times of SBS and CTS were found to be 2.375 and 2.989 min respectively. The developed method was validated according to ICH guidelines. The results indicates that the method was found to be simple, rapid, precise and accurate and can be adopted to routine methods of analysis of SBS and CTS in Pharmaceutical dosage forms.

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INTRODUCTION

Subactum Sodium (SBS) (Fig.1) is chemically (2S, 5R)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo (3.2.0) heptane-2-carboxylic acid 4, 4-dioxide. It is a beta-lactamase inhibitor, given in combination with beta-lactam antibiotics to inhibit beta-lactamase. Ceftriaxone Sodium (CTS) (Fig.2) is chemically (6R, 7R)-7 {((2Z)-2-(2-amino-1, 3-thiazol-4-yl)->2-(methoxyimino) acetyl) amino}-3-{((2-methyl- 5, 6-dioxo1, 2, 5, 6-tetrahydro-1, 2, 4-triazin-3-yl) thio) methyl}-8-oxo-5-thia-1-aza bicycle (4.2.0) oct-2-ene-2-carboxylic acid. It is a third-generation cephalosporin antibiotic which having broad spectrum activity against Gram-positive and Gram-negative bacteria. Literature survey revealed that SBS with Cefatoxime sodium can be estimated by Spectrophotometric method (Nanda *et al*., 2010; Manoj *et al*., 2011). CTS can be estimated singly and also combination with Cefotoxime Sodium by Spectrophotometric method (Revathi Ethiraj *et al*., 2014; Patel *et al*., 2006). But CTS and SBS can be estimated by Spectrophotometric and chromatographic methods such as UPLC (Patel Nirav *et al*., 2012), HPLC (Palanikumar *et al*., 2010; Rahul S Kale *et al*., 2011; Sanjay Mohan Shrivastava *et al*., 2009), LC-MS/MS (Payasi *et al*., 2010) methods have been reported. HPLC trials were conducted by using different mobile phases and columns. Finally a Novel HPLC method was developed and validated. The present study describes a simple, sensitive, accurate and precise HPLC method for the estimation of SBS and CTS in pure form and its pharmaceutical dosage forms.

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MATERIALS AND METHODS

Reagents and Materials

SBS and CTS API's gift samples were obtained from Senthan labs, Banglore. The marketed formulation (Cefiray-S 15) was procured from Amray Healthcare; Himachal Pradesh, India in the local market. The label consists of 1gm of Ceftriaxone sodium and 500 mg of Sulbactam sodium. The chemicals which we used i.e Acetonitrile, Sodium dihydrogen phosphate, Water and Ortho phosphoric acid used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.

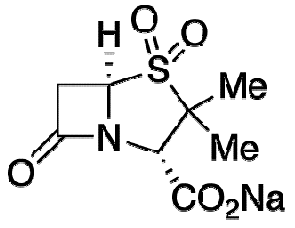


Fig.1. Structure of Sulbactum sodium

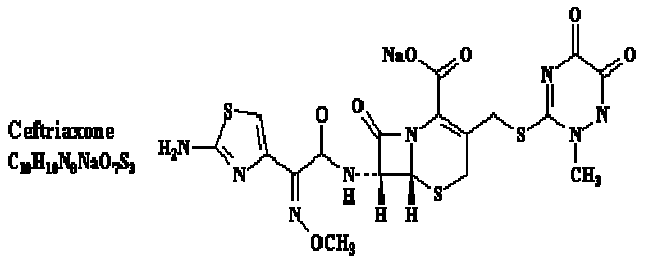


Fig.2. Structure of Ceftriaxone sodium

Instrumentation

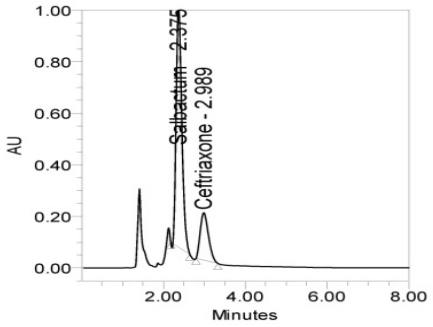
Table 1. Instruments and specifications

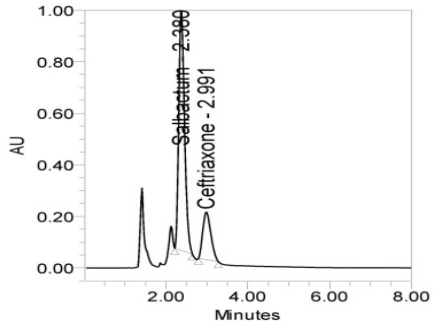
Instrument	HPLC
Make and model	Agilent 1200 Series HPLC system
Specification	Quaternary Gradient
Sampling mode	Auto sampler
Detection	Variable wavelength detector
Software	EZChrom

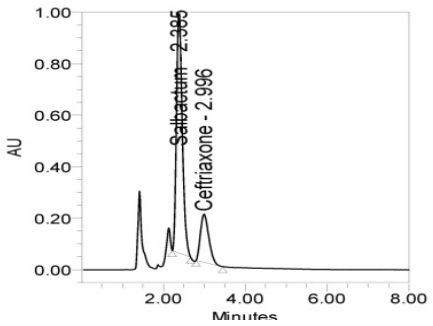
Experimental

Chromatographic conditions

Agilent XDB, C_{18} (150 x 4.6mm, 5µ) was the column used for separation. Mobile phase consisting of a mixture of Buffer (0.02M sodium dihydrogen phosphate, pH-3 was adjusted with ortho phosphoric acid) and Acetonitrile in the ratio 82:18 v/v was delivered at a flow rate of 1.0 ml/min with detection at 218nm. The mobile phase was filtered through a 0.45 nylon filter and sonicated for 15 min. Analysis was performed at ambient temperature.







Peak Name: Salbactum

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Salbactum	2.375	10580351	2366	1.35
2	Salbactum	2.380	10512550	2347	1.30
3	Salbactum	2.385	10502305	2363	1.31

Peak Name: Ceftriaxone

	Peak Name	RT	Area	USP Plate Count	USP Tailing	Resolution
1	Ceftriaxone	2.989	2142207	2971	1.32	1.9
2	Ceftriaxone	2.991	2208362	2986	1.24	1.9
3	Ceftriaxone	2.996	2138150	2846	1.38	1.9

Fig.3. A Representative chromatogram of CTS and SBS

Method development: Procedure for Buffer Preparation

Buffer (0.02M sodium dihydrogen phosphate, pH-3was adjusted with ortho phosphoric acid) and Methanol in different proportions were tried and finally Buffer (0.02M sodium dihydrogen phosphate, pH-3 was adjusted with ortho phosphoric acid), Acetonitrile (82:18 v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The chromatogram of working standard solution is shown in Fig 3.

Preparation of standard solution

Accurately Weighed and transferred 50mg of CTS and 25mg of SBS working standards into a 100 ml clean dry volumetric flask, add 70ml of mobile phase, sonicated for 5 minutes and make up to the final volume with mobile phase. The solution was filtered through 0.45µ nylon membrane filter. The concentration containing 500µg/ml of CTS and 250µg/ml of SBS were prepared.

Procedure for analysis of tablets

Marketed powdered Parenteral formulation (Cefiray-S 15) consists 1g of CTS and 500mg of SBS were analyzed by this method. Accurately weighed the quantity of dry powder of the Injection which is equivalent to 50mg of CTS and 25mg of SBS, transfer into a 100 ml of clean dry volumetric flask, add 70ml of mobile phase, sonicate it for 5 minutes and final volume was made up with mobile phase. The solution was filtered through 0.45µ nylon membrane filter. 1ml of the above solution was diluted to 10ml with mobile phase to get 50µg/ml of CTS and 25µg/ml of SBS. The Assay results were noted in Table.10.

Calibration curve

Aliquots of (5, 7.5, 10, 12.5 & 15ml) mixed working standard solution of CTS and SBS were transferred into a series of 10ml volumetric flasks and appropriately diluted with mobile phase.

The method was found to be linear over a concentration range of 50 to 150% linear level. Calibration curves were obtained by plotting the response (area of drug peak) versus concentration of drug (ppm). Regression equations were calculated and results are tabulated (Table 2 and Table 3) and (Fig.4 and Fig.5).

Table 2. Linearity of CTS

Linearity Level (%)	Concentration (ppm)	Peak Area	Statistical Analysis	
0	0.00	0.00	Slope	3162
50	250	791017		
75	375	1178928	y- Intercept	-3730
100	500	1564583		
125	625	1971296	R^2	0.999
150	750	2379126		

Table 3. Linearity of SBS

Linearity Level (%)	Concentration (ppm)	Peak Area	Statistical Analysis	
0	0.00	0.00	Slope	26597
50	125	3241824		
75	187.5	5107252	y- Intercept	-19296
100	250	6490093		
125	312.5	8292152	R^2	0.999
150	375	9999654		

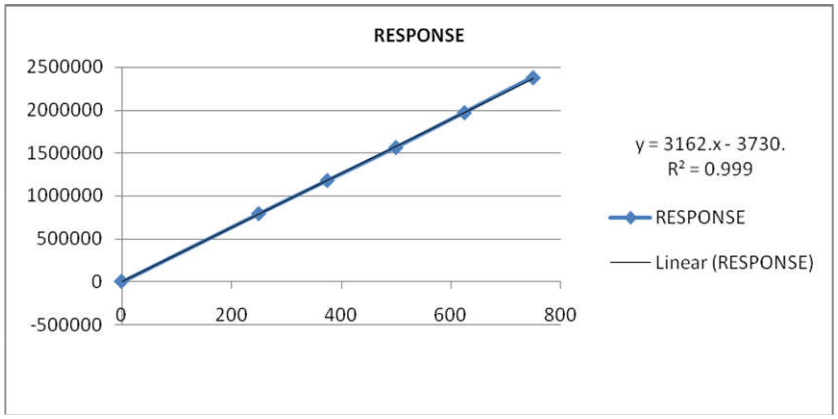


Fig.4. CTS Linearity Graph (Amount ppm Vs Peak Area)

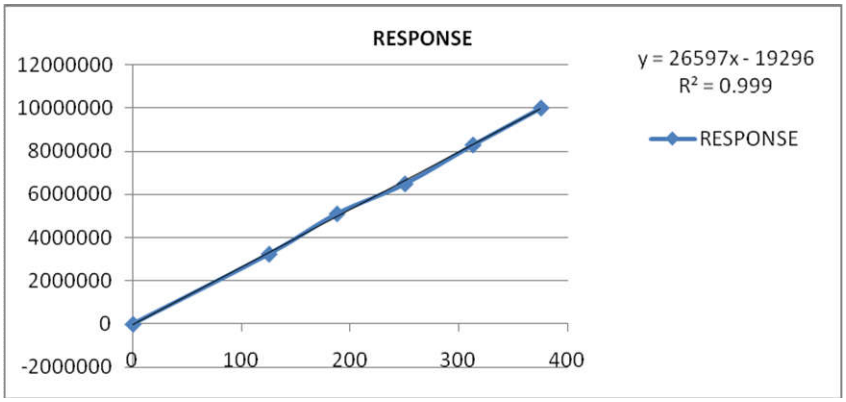


Fig.5. SBS Linearity Graph (Amount ppm Vs Peak Area)

Table 4. System Suitability test Parameters of CTS

Injection	RT	Peak Area	% Area	USP Plate count	USP Tailing	Resolution
1	2.967	1450815	100.00	2342	1.34	2.2
2	2.979	1453075	100.00	2323	1.29	2.2
3	2.996	1452219	100.00	2371	1.34	2.2
4	3.020	1453647	100.00	2329	1.35	2.2
5	3.026	1455377	100.00	2295	1.35	2.1
6	3.027	1456756	100.00	2313	1.38	2.2
Mean		1453649				
SD		2146.3				
% RSD		0.1				

Table 5. System Suitability test Parameters of SBS

Injection	RT	Peak Area	% Area	USP Plate count	USP Tailing	Resolution
1	2.318	7027622	100.00	2290	1.42	2.2
2	2.332	7016689	100.00	2295	1.44	2.2
3	2.352	7023689	100.00	2281	1.42	2.2
4	2.375	7045241	100.00	2277	1.43	2.2
5	2.386	7035090	100.00	2281	1.40	2.1
6	2.388	7044563	100.00	2285	1.41	2.2
Mean		7032149				
SD		11534.4				
% RSD		0.2				

Table 6. System Precision of CTS

Injection	RT	Peak Area	USP Plate Count	USP Tailing	Resolution
1	3.009	1496636	2140	1.35	2.0
2	3.012	1445595	2168	1.36	2.0
3	3.016	1458847	2167	1.37	2.0
4	3.023	1440356	2197	1.35	2.0
5	3.026	1437792	2225	1.27	2.1
6	3.028	1453655	2197	1.36	2.1
Mean		1455480			
SD		21669.3			
% RSD		1.5			

Table 7. System Precision of SBS

Injection	RT	Peak Area	USP Plate Count	USP Tailing	Resolution
1	2.389	6973303	2301	1.32	2.0
2	2.393	6952942	2304	1.34	2.0
3	2.397	6963759	2273	1.34	2.0
4	2.398	6941014	2289	1.37	2.0
5	2.402	6901944	2303	1.36	2.1
6	2.404	6922657	2247	1.38	2.1
Mean		6942603			
SD		26647.6			
% RSD		0.3			

Table 8. Accuracy of CTS

Concentration % of spiked level	RT	Area	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	2.992	732565	101.78	Mean	101.87
50% Sample 2	2.993	730494	100.50	SD	0.42
50% Sample 3	2.993	736542	101.337	%RSD	0.41
100 % Sample 1	2.990	1492174	102.65	Mean	101.02
100% Sample 2	2.991	1456045	100.16	SD	1.4
100% Sample 3	2.993	1457318	100.25	%RSD	1.3
150% Sample 1	2.989	2142207	98.24	Mean	99.19
150% Sample 2	2.991	2208362	101.27	SD	1.80
150% Sample 3	2.996	2138150	98.05	%RSD	1.82
Acceptance criteria	%RSD should not be more than 2.0				

Table 9. Accuracy of SBS

Concentration % of spiked level	RT	Area	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	2.383	3577674	101.75	Mean	99.92
50% Sample 2	2.384	3467244	98.611	SD	0.60
50% Sample 3	2.384	3495609	99.417	%RSD	0.60
100 % Sample 1	2.381	6908489	98.24	Mean	98.37
100% Sample 2	2.383	6917077	98.36	SD	0.13
100% Sample 3	2.385	6927202	98.50	%RSD	0.13
150% Sample 1	2.375	10580351	100.30	Mean	99.84
150% Sample 2	2.380	10512550	99.66	SD	0.40
150% Sample 3	2.385	10502305	99.56	%RSD	0.40
Acceptance criteria	%RSD should not be more than 2.0				

Table 10. Analysis of Marketed Parenteral formulation

Formulation	Analyte	Label claim (mg)	Amt. found (mg)	%label claim estimated
	Ceftrioxone sodium	1000mg	996.9	99.69
Injection	Sulbactum sodium	500mg	501.1	100.22

Table 11. Summary of validation parameters

Parameter	RP-HPLC Method	
	Ceftrioxone sodium	Sulbactum sodium
Mean % recovery	99.19-101.87	98.37-99.92
Precision		
a)Intraday precision	0.04	0.12
b) Inter-day precision	1.04	0.99
System Suitability		
Retention time (min)	2.989	2.375
Theoretical plates	Not less than 1000	Not less than 1000
Tailing factor	Less than 2.0	Less than 2.0
Resolution	Not more than 3.0	Not more than 3.0
Regression line		
Concentration at linear level (%)	50-150	50-150
Concentration range (ppm)	250-750	125-375
Slope	3162	26597
y-Intercept	-3730	-19296
Correlation coefficient (R^2)	0.999	0.999

Method Validation

System Suitability & Method Precision (Repeatability)

The system suitability parameters like asymmetry of the chromatographic peak, peak resolution and theoretical plates, %RSD of peak area for 6 replicate injections were evaluated & results are tabulated (Table.4 and Table.5). The precision of the instrument was checked by repeatedly injecting (n=6) solutions of CTS and SBS (50µg/ml & 25µg/ml) without changing the parameters (Table.6 and Table.7).

Intermediate Precision (Reproducibility)

The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, solutions of standard and sample were repeated thrice in a day and %RSD for response factor was calculated. In the inter-day variation studies, injections of standard and sample solutions were made on three consecutive days and %RSD was calculated. From the obtained data the developed RP-HPLC method was found to be precise.

Accuracy (Recovery studies)

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed injection solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated. It indicates that the method was accurate. The Accuracy data was shown in Table 8 and 9.

RESULTS AND DISCUSSION

The proposed method was found to be linear in the concentration range of 50 to 150% linear level for CTS and SBS. The method was specific, since excipients in the formulation did not interfere in the estimation of CTS and SBS. Accuracy of the method was indicated by recovery values. Precision is reflected by %RSD values less than 2.0. These low values suggest the sensitivity of the developed method. Validation parameters were summarized in Table 11.

Conclusion

The developed RP-HPLC method was simple, sensitive, precise and accurate and hence can be used in routine for the determination of Sulbactam Sodium and Ceftrioxone Sodium in bulk as well as in pharmaceutical preparations.

REFERENCES

Manoj D.Raut, S.P.Ghode, Rahul.S.Kale, Makarand.V.Puri, Hemant.S.Patil, 2011. "Spectrophotometric method for the simultaneous estimation of Cefotaxime Sodium and Sulbactum in Parentral dosage forms, *International Journal of ChemTech Research*", 3(3), 1506-1510.

Nanda R.K, Bhagwat V.V, Potawale S.E, Vidyasagar N.C, Mishra. R 2010. "Simultaneous Spectrophotometric Estimation of Cefotaxime Sodium and Sulbactam Sodium in Pharmaceutical Dosage Form", *International Journal of ChemTech Research*, 2(3), 1612-1617.

Palanikumar.B, Thenmozhi.A, and Sridharan.D. 2010. "An RP-HPLC Method for Simultaneous Estimation of Ceftriaxone Sodium and Sulbactum Sodium in Injection dosage form", *International Journal of Pharmacy and Pharmaceutical Sciences*, 2(3):34-36

Patel Nirav B∗, Arvadiya Alpesh C, Jansari Sneha K, Mistry Vipul D, Desai Hemant T. 2012. "Development and Validation of Stability Indicating Method for Simultaneous Estimation of Ceftriaxone and Sulbactam Injection using RP-UPLC Method", *IOSR Journal of Pharmacy*, 2(6), 29-37.

Patel. S.A, Patel. N.M and Patel. M.M. 2006. "Spectrophotometric Estimation of Cefotaxime and Ceftriaxone in Pharmaceutical Dosage Forms", *Indian J. Pharm. Sci*., 68 (1), 101-103.

Payasi A, Chaudhary M, Gupta A, Dwivedi VK, Bhatnagar A. 2010. "Pharmacokinetic study of sulbactomax", *J Toxicol Sci*., 35(4), 459-464.

Rahul S Kale, Hemant K Jain, Prashant D Ghode, Ganesh S Mhaske, Makarand V Puri, Manoj D Raut, Hemant S Patil, 2011. "An RP-HPLC method for Simultaneous estimation of Ceftriaxone sodium and Sulbactum sodium in Parenteral Dosage form", *International Journal of Pharmacy and Pharmaceutical Sciences*, 3(5), 406-409.

Revathi Ethiraj, Ethiraj Thiruvengadam, Venkattapuram Saravanan Sampath, Abdul Vahid, and Jithin Raj, 2014. "Development and Validation of Stability Indicating Spectroscopic Method for Content Analysis of Ceftriaxone

Sodium in Pharmaceuticals", *International Scholarly Research Notices*, 1-5.

Sanjay Mohan Shrivastava, Rajkumar Singh, Abu Tariq, Masoom Raza Siddiqui, Jitendar Yadav, P. S. Negi, Manu Chaudhary, 2009. "A Novel High Performance Liquid Chromatographic Method for Simultaneous Determination of Ceftriaxone and Sulbactam in Sulbactomax", *International Journal of Biomedical Science*, 5(1), 37-43.

Sridharan Durairaj, Thenmozhi Annadurai, B.Palani kumar and S.Arunkumar, 2010. "Simultaneous Estimation of Ceftriaxone Sodium and Sulbactam Sodium using Multi-Component Mode of Analysis", *International Journal of ChemTech Research*, 2(4), 2177-2181.

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