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Simultaneous Spectrophotometric Estimation of Cefotaxime Sodium and Sulbactam Sodium in Pharmaceutical Dosage Form

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Abstract: Three simple, accurate and reproducible spectrophotometric methods have been developed for the simultaneous estimation of Cefotaxime Sodium and Sulbactam Sodium in pharmaceutical dosage forms. The first method involves determination using the simultaneous equation method, the sampling wavelengths selected are 233.5 nm and 264 nm over the concentration ranges of 5-35 mcg ml^{-1} and 2.5-17.5 mcg ml^{-1} for Cefotaxime Sodium and Sulbactam Sodium respectively. The second method is the Area Under Curve method (AUC), the sampling wavelength ranges selected are 238.5-228.5nm and 269-259nm with linearity in the concentration ranges of 5-35 mcg ml^{-1} and 2.5-17.5 mcg ml^{-1} for Cefotaxime Sodium and Sulbactam Sodium respectively. The third method involves determination using the Multicomponent Mode Method, the sampling wavelengths selected are 233.5 nm and 264 nm over the concentration ranges of 5-35 mcg ml^{-1} and 2.5-17.5 mcg ml^{-1} for Cefotaxime Sodium and Sulbactam Sodium respectively. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guidelines.

Key Words: Cefotaxime Sodium, Sulbactam Sodium, Simultaneous equation method, Area Under Curve method (AUC) and Multicomponent Mode Method.

Introduction

Cefotaxime Sodium (CEFO) is Sodium (6R,7R)-3-[(acetyloxy)methyl]-7-[[(2Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate is officials in IP[1]. Literature survey reveals several spectroscopic [3-4], HPLC [5-7] and HPTLC [8] methods for the estimation of Cefotaxime Sodium individually as well as in combination with other drugs.

Sulbactam Sodium (SBT) is chemically 4-Thia-1-aza-bicyclo [3, 2, 0] heptane 2-carboxylic acid, 3, 3 dimethyl-7-oxo-4, 4 dioxo, sodium salt is officials in USP [2]. Literature survey reveals UV spectroscopic [9-10] and HPLC [11-12] methods for the estimation of Sulbactam Sodium individually as well as in combination with other drugs Cefotaxime Sodium and Sulbactam Sodium are available in combined pharmaceutical dosage form for the treatment of lower respiratory tract infections and urinary tract infections. Not a single UV or HPLC method is reported so far for the simultaneous analysis of Cefotaxime Sodium and Sulbactam Sodium in their combined dosage form. So a need was felt to develop new methods to analyze the drugs simultaneously. A successful attempt has been made to estimate the two drugs simultaneously by UV spectrophotometric analysis. This paper describes three simple, rapid, accurate, reproducible and economical methods for the simultaneous determination of Cefotaxime Sodium and Sulbactam Sodium in parenteral formulations using simultaneous equation method, Area under Curve method (AUC) and Multicomponent Mode Method.

Experimental

Instrumentation:

A Shimadzu UV/Visible spectrophotometer, model 1700 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction was employed. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample. An ultrasonic cleaner (Art No.400014CL) was used for sonicating the injection sample solution.

Reagents and Chemicals:

Analytical pure samples of Cefotaxime Sodium and Sulbactam Sodium (Hindusthan Antibiotic Limited, Pimpri, Pune, India) were used in the study. The pharmaceutical dosage form used in this study was Taximax (Alkem laboratories Limited, India.) labeled to contain 1000 mg Cefotaxime Sodium and 500 mg of Sulbactam Sodium.

Preparation of Standard Stock Solution:

Standard stock solutions (100 mcg ml^{-1}) of Cefotaxime Sodium and Sulbactam Sodium were prepared by dissolving separately 10 mg of drug each in 100 ml 0.1M NaOH. The working standard solutions of these drugs were obtained by dilution of the respective stock solution with 0.1M NaOH.

Preparation of Sample Stock Solutions:

An accurately weighed powder sample equivalent to 10 mg of Cefotaxime Sodium was transferred to a 100 ml volumetric flask and dissolved in 0.1M NaOH and sonicated for 15 minutes and volume made to 100ml with 0.1M NaOH. It was then filtered through Whatmann filter paper No.41. The solution was suitably diluted with 0.1M NaOH to obtain sample solutions containing Cefotaxime Sodium and Sulbactam Sodium in the concentrations ratio of 2:1 mcg ml^{-1} respectively as in the formulation.

Method I:

Simultaneous Equation Method

Construction of calibration curve and formation of simultaneous equation method

For the simultaneous equation method, 233.5nm, and 264 nm were selected as the two sampling wavelengths. Fig.1 represents the overlain UV spectra of Cefotaxime Sodium and Sulbactam Sodium. Cefotaxime Sodium and Sulbactam Sodium exhibited linearity with absorbances in the range of 5-35 mcg ml^{-1} and 2.5-17.5 mcg ml^{-1} at their respective selected wavelengths. Co-efficient of correlation were found to be 0.9993 and 0.999 for Cefotaxime Sodium and Sulbactam Sodium respectively. The optical characteristics and regression values for the calibration curves are presented in Table 1. For simultaneous estimation of Cefotaxime Sodium and Sulbactam Sodium, mixed standards containing Cefotaxime Sodium and Sulbactam Sodium in a concentration ratio of 2:1 mcg ml^{-1} each were prepared by appropriate dilution of the standard stock solutions with 0.1M NaOH. The absorbances of the mixed standard solutions were measured at the selected wavelengths. A set of two simultaneous equations were established using the mean absorptivity coefficients of Cefotaxime Sodium and Sulbactam Sodium at the selected λ's.

$A1 = 54.14\ C_{CEFO} + 32.25\ C_{SBT}$……(I) at 233.5nm ($\lambda_1$)

$A2 = 41.29\ C_{CEFO} + 88.50\ C_{SBT}$ …… (II) at 264nm (λ_2)

Where- 54.14 and 41.29 are absorbtivity values of Cefotaxime Sodium at λ_1 and λ_2 respectively.

32.25 and 88.50 are absorbtivity values of Sulbactam Sodium at λ1, and λ2 respectively.

A1, A2 are the absorbance of mixed standards at λ1, and λ2 respectively.

C_{CEFO} and C_{SBT} are concentrations in g L^{-1}.

The concentration of C_{CEFO} and $C_{SBT\ in}$ mixed standard and injection formulation can be obtained by solving equation (I) and (II).

Method II:

Area Under Curve Method

For the Area under curve method (AUC), 238.5-228.5nm and 269-259nm were selected as the two sampling wavelength intervals. Fig.2 represents the overlain UV spectra of Cefotaxime Sodium and Sulbactam Sodium with AUC ranges. Cefotaxime Sodium and Sulbactam Sodium exhibited linearity in the concentration range of 5-35 mcg ml^{-1} and 2.5-17.5 mcg ml^{-1} at their respective selected wavelength intervals. Coefficients of correlation were found to be 0.9992 and 0.9992 for Cefotaxime Sodium and Sulbactam Sodium respectively. The optical characteristics and regression values for the calibration curves are presented in Table 1. For the simultaneous estimation, mixed standards containing Cefotaxime Sodium and Sulbactam Sodium in the ratio of 2:1 mcg ml^{-1} were prepared by appropriate dilution of the standard stock solutions. The AUC of the mixed standard solutions were measured at the selected wavelength intervals. A set of two simultaneous equations were established using the mean absorptivity coefficients of Cefotaxime Sodium and Sulbactam Sodium at the selected wavelength intervals.

$A1 = 547.7\ C_{CEFO} + 412.5\ C_{SBT}$……(III) at 238.5-228.5nm (λ1- λ2)

$A2 = 314.9\ C_{CEFO} + 866.9\ C_{SBT}$ …… (IV) at 269-259 nm (λ3- λ4)

Where 547.7 and 314.9 are mean absorbtivity values of CEFO at (λ1- λ2) and (λ3- λ4) respectively.

412.5 and 866.9 are mean absorbtivity values of SBT at (λ1- λ2) and (λ3- λ4) respectively.

A1 and A2 are the absorbance of mixed standards at (λ1- λ2) and (λ3- λ4) respectively.

C_{CEFO} and C_{SBT} are concentrations in g L^{-1}.
The concentration of C_{CEFO} and C_{SBT} in mixed standard and injection formulation can be obtained by solving equation (III) and (IV).

Method III:
Multicomponent Mode Method

For the analysis of Cefotaxime Sodium and Sulbactam Sodium by multicomponent method of analysis, the multicomponent mode of the UV visible spectrophotometer was used. For multicomponent method of analysis, 233.5nm, and 264 nm were selected as the two sampling wavelengths for Cefotaxime Sodium and Sulbactam Sodium respectively. The drugs showed linearity in the concentration ranges of 5-30 mcg ml^{-1}, 2.5-17.5 mcg ml^{-1} with regression coefficient (r^2) values of 0.9988, 0.9986 for Cefotaxime Sodium and Sulbactam Sodium respectively. Six mixed standards in ratio of 2:1 mcg ml^{-1} showing linearity within the Beer's concentration range of Cefotaxime Sodium and Sulbactam Sodium were prepared by appropriate dilution of standard stock solutions (100 mcg ml^{-1}).In multicomponent mode of the instrument, the mixed standards were scanned over the range of 190-400 nm at the selected sampling wavelengths. The overlain spectra of the six mixed standards were then employed to determine the concentration of the drugs in sample solutions by analysis of the spectral data of sample solution with reference to that of mixed standards.

Assay of Injection Formulation:

Powder equivalent to 10 mg of Cefotaxime Sodium and 5 mg of Sulbactam Sodium was weighed and dissolved in 100 mL 0.1M NaOH with the aid of ultrasonication for 30 min. The solution was then filtered through Whatmann filter paper No.41 and diluted further to obtain final concentration of 10 mcg ml^{-1} of Cefotaxime Sodium and 5 mcg ml^{-1} of Sulbactam Sodium. The sample solutions were analyzed as per the procedure for mixed standards. The concentrations of each drug in sample solutions were calculated using equations (I) and (II) for the simultaneous equation method, equations (III) and (IV) for the Area under curve method and using the multicomponent mode of the instrument for the Multicomponent method of analysis. The proposed methods were validated as per ICH guidelines [13].The accuracy of the proposed methods were determined by performing recovery studies at 80%, 100% and 120% of the test concentration. The results of the analysis and statistical validation data of the injection formulation are given in table 1. The statistical validation data of recovery study are given in table 2.

Table 1: Optical Characteristics and Validation Data of Cefotaxime Sodium and Sulbactam Sodium.

Parameters	**Cefotaxime Sodium**			**Sulbactam Sodium**		
	Method-A	**Method-B**	**Method-C**	**Method-A**	**Method-B**	**Method-C**
Working wavelengths	233.5nm	238.5nm-288.5nm	233.5nm	264nm	269nm-259 nm	264nm
Beer-Lamberts Law range (mcg mL^{-1})	5-30	5-30	5-30	2.5-17.5	2.5-17.5	2.5-17.5
Precision*						
Interday (%RSD)	0.15013	0.0422	0.24562	0.1760	0.1114	0.20046
Intraday (%RSD)	0.05645	0.1069	0.1623	0.2322	0.1558	0.21193
LOD (mcg mL^{-1})*	0.03054	0.01567	0.20058	0.2308	0.01894	0.07453
LOQ (mcg mL^{-1})*	0.09255	0.04750	0.60782	0.06994	0.05740	0.22587
Regression Values:						
I. Slope*	0.0541	0.53661	0.05416	0.08833	0.8687	0.08766
II. Intercept*	0.01186	0.08175	0.01366	0.00751	0.06001	0.00561
III. Regression Coefficient (r^2)*	0.9993	0.9992	0.9988	0.999	0.9992	0.9986

*Denotes average of six estimations

Table 2: Statistical Validation Data of Injection Formulation

Component	Amount present(mg)	Method	% Amount Found	S.D.*	% R.S.D.*
Cefotaxime Sodium	1	A	100.05	0.01751	0.04766
	1	B	100.2	0.02429	0.16187
	1	C	99.98	0.01593	0.210993
Sulbactam Sodium	8	A	100.47	0.01897	0.251926
	8	B	99.97	0.01095	0.146195
	8	C	100.6	0.01831	0.12208

* Denotes average of six estimations

Injection Formulation, Taximax manufactured by Alkem laboratories Limited, India.

Where,

Method A – Simultaneous equation method

Method B – Area Under Curve method (AUC)

Method C – Multicomponent Mode Method

Table 3: Statistical Validation of Recovery Studies

Level of % recovery	Methods	% Recovery*		% R.S.D.*	
		Cefotaxime Sodium	Sulbactam Sodium	Cefotaxime Sodium	Sulbactam Sodium
80	A	100.1	100.5	0.02136	0.08555
	B	100.1	99.91	0.2889	0.2996
	C	99.92	100.4	0.2074	0.1890
100	A	100.15	100.6	0.0333	0.06626
	B	99.79	100.2	0.1453	0.1388
	C	99.75	100.1	0.1712	0.3902
120	A	100.2	100.5	0.05901	0.07
	B	100.1	99.91	0.1048	0.1050
	C	100.3	100.2	0.3022	0.1851

*Denotes average of three estimations at each level of recovery.

Table 4: Ruggedness Study

Method	Parameter	% Mean		S.D.*		% R.S.D.*	
		Cefotaxime Sodium	Sulbactam Sodium	Cefotaxime Sodium	Sulbactam Sodium	Cefotaxime Sodium	Sulbactam Sodium
Simultaneous Equation method	**Instrument**	99.95	100.8	0.02041	0.01549	0.13624	0.20489
	Analyst	99.99	100.7	0.01549	0.02066	0.10333	0.2736
Area Under Curve (AUC) Method	**Instrument**	99.90	100.1	0.006325	0.005477	0.04222	0.07302
	Analyst	99.84	100.2	0.008165	0.01169	0.05454	0.15565
Multicomponet Method	**Instrument**	100.1	100.5	0.03613	0.02078	0.240706	0.27556
	Analyst	100.1	100.4	0.03754	0.01194	0.24993	0.158566

*Denotes average of three estimations at each level of recovery.

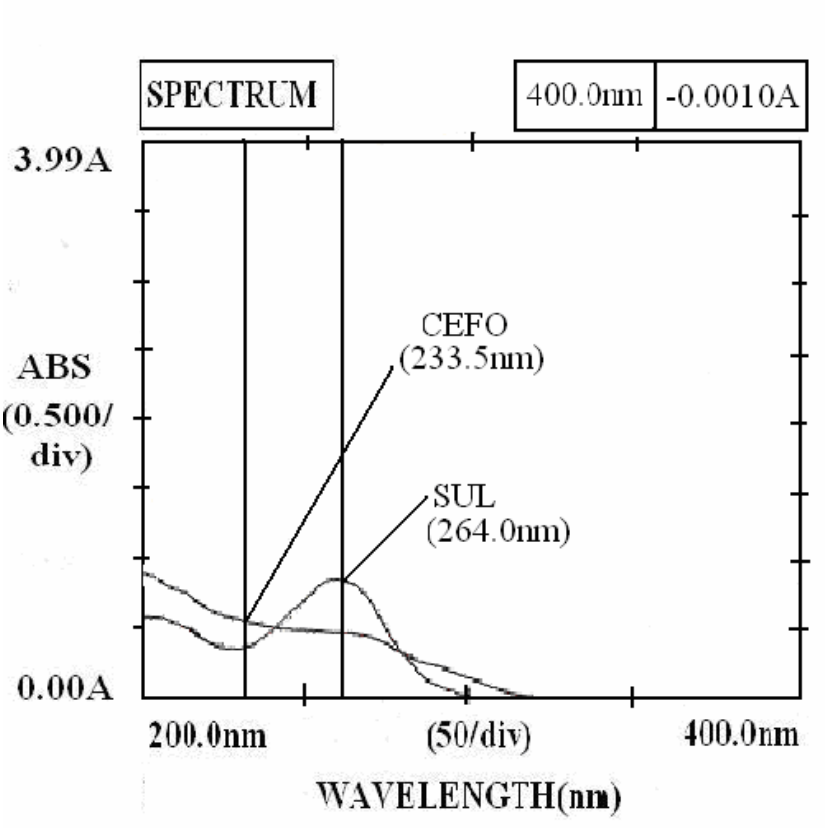

Fig.1: Overlain Spectra of Cefotaxime Sodium and Sulbactam Sodium in Simultaneous Equation Method.

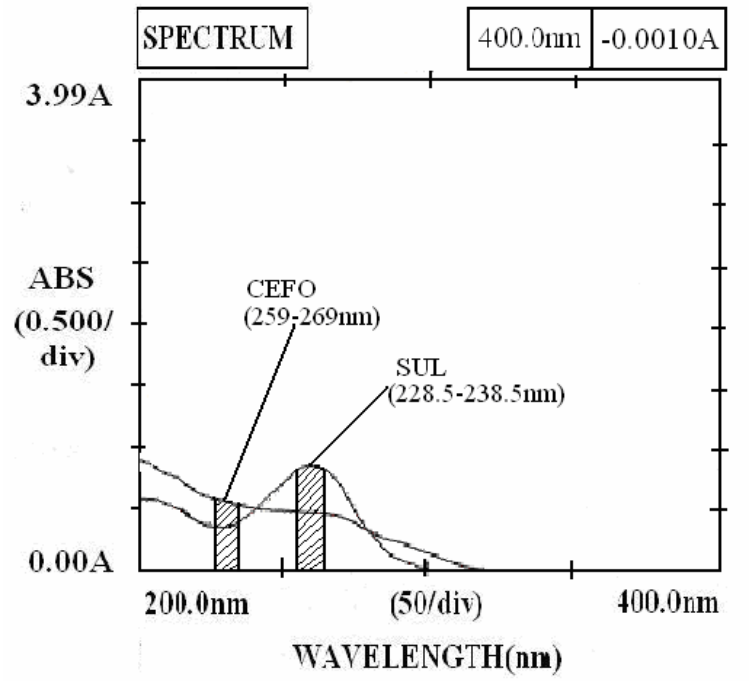

Fig. 2: Overlain spectra of Cefotaxime Sodium and Sulbactam Sodium in Area Under Curve (AUC) method

Results and Discussion:

Under the experimental conditions described, calibration curve, assay of injection and recovery studies were performed. The developed methods were validated as per ICH guidelines for linearity, repeatability, intermediate precision (inter-day and intra-day precision studies), LOD, LOQ as shown in Table 1. The mean % content of 100.04% and 100.25 % formulation by the developed methods were 100.07% and 100.34% respectively (Table 2). The mean % recoveries of Cefotaxime Sodium and Sulbactam Sodium were found to be 100.04% and 100.25 % respectively (Table 3). The ruggedness of the developed methods was determined by evaluating the effect of change in instruments and analysts on the % mean content of drugs. The statistical validation data of ruggedness study is given in table 4.

Conclusion:

Cefotaxime Sodium and Sulbactam Sodium are available in combined pharmaceutical dosage form for the treatment of of Lower respiratory tract infection. Here, three simple UV spectrophotometric methods (Simultaneous equation method, Area Under Curve method (AUC), Multicomponent Mode Method) were developed for their simultaneous analysis. The standard deviation, RSD and standard error calculated for the methods are low, indicating high degree of precision of the methods. The RSD is also less than 2% as required by ICH guidelines. The % recovery was between 98-102% indicating high degree of accuracy of the proposed methods. The developed methods are simple, rapid, precise, accurate and can be employed for the routine estimation of Cefotaxime Sodium and Sulbactam Sodium in both bulk and injection dosage form.

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