

Spectrophotometric method for the simultaneous estimation of Cefotaxime Sodium and Sulbactam in Parentral dosage forms.

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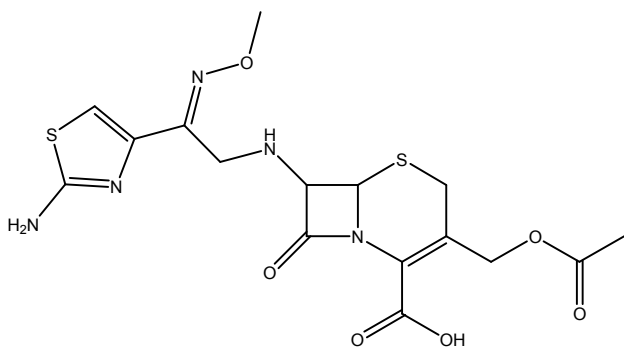
Abstract: Two simple, accurate and reproducible spectrophotometric methods have been developed for the simultaneous estimation of Cefotaxime Sodium and Sulbactam Sodium in parentral dosage forms. The first method involved determination using the simultaneous equation method. The sampling wavelengths selected are 231.0 nm and 260.2 nm over the concentration ranges of 5-30 mcg ml⁻¹ and 2.5-15 mcg ml⁻¹ for Cefotaxime Sodium and Sulbactam Sodium respectively^[3,4,5]. The second method is the Q-absorption ratio method and the sampling wavelength selected are 234.2 and 260.2 nm with linearity in the concentration ranges of 5-30 mcg ml⁻¹ and 2.5-15 mcg ml⁻¹ for Cefotaxime Sodium and Sulbactam Sodium respectively carried out^[6,7,8,9]. Both the methods were found to be accurate, precise and reproducible. These methods were applied to the assay of the drugs in marketed formulation, which were found in the range of 98.0% to 102.0% of the labeled value for both cefotaxime and sulbactam. Hence, the methods herein described can be successfully applied in quality control of combined pharmaceutical dosage form.

Key Words: Cefotaxime Sodium, Sulbactam Sodium, Simultaneous equation method, Q-absorption ratio method.

Introduction

Cefotaxime Sodium (CEF) is Sodium (6R, 7R)-3-[(acetyloxy) methyl]-7-[[[(2Z) 2(2aminothiaz4-yl)-2-(methoxy imino) acetyl] amino]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate is official in IP^[1]. It belongs to a third generation of cephalosporin. It is used for infections of the respiratory tract, skin, bones, joints, urogenital system, meningitis, and septicemia gastroenteritis.

Sulbactam Sodium (SBT) is chemically 4-Thia-1-azabicyclo [3, 2, 0] heptanes 2-carboxylic acid, 3, 3dimethyl-7-oxo-4, 4 di-oxo, sodium salt is official in USP^[2]. Literature survey reveals UV spectroscopic and HPLC 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.

**Structure: sulbactam sodium**

A detailed survey literature was carried out and revealed that several method based on varied techniques, viz. High Performance Liquid Chromatography, High Performance Thin Layer Chromatography and Spectrophotometry were available individually for both cefotaxime and sulbactam. No method is available for the simultaneous estimation of these drugs by UV spectrophotometric method. So, a successful attempt has been made to estimate the two drugs simultaneously by UV spectrophotometric analysis. Paper describes two simple, rapid, accurate, reproducible and economical methods for the simultaneous determination of Cefotaxime Sodium and Sulbactam Sodium in parenteral formulations using simultaneous equation method and Q-absorption ratio method [10,11,12].

EXPERIMENTAL:**Materials and Methods**

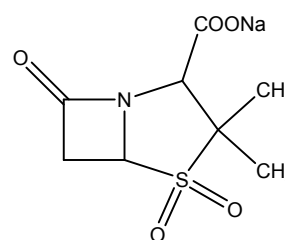
A Shimadzu UV/Visible spectrophotometer, model 1601(Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction was employed. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample. An ultrasonic cleaner (ArtNo.400014CL) was used for sonicating the injection sample solution.

Reagents and Chemicals:

Analytical pure samples of Cefotaxime Sodium and Sulbactam Sodium (Hindustan Antibiotic Limited, Pimpri, Pune, India) were used in the study. The pharmaceutical dosage form used in this study was Haltax-s (Hindustan antibiotic 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

**Structure: cefotaxime sodium**

prepared by dissolving separately 10 mg of drug each in 100 ml 0.1M KOH. The working standard solutions of these drugs were obtained by dilution of the respective stock solution with 0.1M KOH.

Preparation of Sample Stock Solutions:

An accurately weighed powder sample equivalent to 10mg of Cefotaxime Sodium was transferred to a 100 ml volumetric flask and dissolved in 0.1M KOH and sonicated for 10 minutes and volume made to 100ml with 0.1M KOH. It was then filtered through Whatmann filter paper No.41. The solution was suitably diluted with 0.1M KOH 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, 231.0nm, and 260.2 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 absorbance 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 KOH. 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 Concentrations in the sample were obtained by using following equations-

$$C_x = \frac{A_1 a_{y2} - A_2 a_{x1}}{A_{x1} a_{y2} - a_{x2} a_{y1}} \dots \dots \dots \text{Eq. (i)}$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{y1} a_{x2} - a_{y2} a_{x1}} \dots \dots \dots \text{Eq. (ii)}$$

Where, A1 and A2 are absorbances of mixture at 231.0 nm and 260.2 nm respectively, ax1 and ax2 are absorptivities Of cefotaxime at λ1 and λ2 respectively and ay1 and ay2 are absorptivities of sulbactam at λ1 and λ2 respectively. Cx and Cy are concentrations of cefotaxime and sulbactam respectively.

Method II

(Absorbance ratio or Q-analysis method):

From the overlain spectrum of cefotaxime and sulbactam, two wavelengths were selected one at 234.2 nm which is the isoabsorptive point for both the drugs and the other at 260.2 nm which is λ_{max} of sulbactam. The absorbances of the sample solutions prepared in a similar manner as in the previous method were measured and the absorptivity values for both drugs at the selected wavelengths were also calculated. The method employs Q values and the concentrations of drugs in sample solution were determined by using the following formula:

For cefotaxime-

$$C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{a_{x1}} \dots \dots \dots \text{Eq (iii)}$$

For sulbactam

$$C_y = \frac{A_1}{a_{x1}} - C_x \dots \dots \dots \text{Eq (iv)}$$

Where,

$$Q_m = \frac{\text{Absorbance of sample at 234.2 nm}}{\text{Absorbance of sample at 260.2 nm}}$$

$$Q_x = \frac{\text{Absorptivity of cefotaxime at 234.2 nm}}{\text{Absorptivity of sulbactam at 260.2nm}}$$

$$Q_y = \frac{\text{Absorptivity of cefotaxime at 234.2 nm}}{\text{Absorptivity of sulbactam at 260.2nm}}$$

Sodium and Sulbactam Sodium at the selected l's.

A = Absorbance of sample at isoabsorptive point, a1 and a2 = Absorptivities of cefotaxime and sulbactam respectively at isoabsorptive point.

Assay of Injection Formulation:

Marketed powdered injection formulation (Haltax-S) containing 1g of Ceftriaxone Sodium and 0.5g of Sulbactam Sodium were analyzed by this method. Weighed accurately 15mg from the sample container. Diluted with 0.1M Potassium hydroxide, finally to get the concentration 10 µg/mL and 5µg/mL of Ceftriaxone sodium and Sulbactam sodium. The sample was than analyzed by using the two method simultaneous equation method and Q- absorption ratio method in which the 0.1M Potassium hydroxide was used as blank. The concentration of each component was obtained by the spectral data of the sample solution with reference to that of the mixed standard. Results of analysis are shown in table. The proposed methods were validated as per ICH guidelines [13]. The accuracy of the proposed methods were determined by performing recovery studies at 50%, 80% and 100% 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.

VALIDATION

The method was validated according to ICH Q2 B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte.

Accuracy:

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (50%, 80% and 100%). Percent recovery for cefotaxime and sulbactam, by both the methods, was found in the range of 97.44% to 100.17%.

Linearity:

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of cefotaxime and sulbactam. For simultaneous equation method and Q analysis, the Beer- Lambert's concentration range was found to be 2.5-30 µg/ml for cefotaxime and sulbactam.

Precision:

Precision was studied to find out intra and inter-day variations in the test method of cefotaxime and sulbactam. Calibration curves prepared in medium were run in triplicate in same day and for three days. %RSD (relative standard deviation) were calculated which should be less than 2 %. The results are tabulated in Table 2.

Table No. 1. Results of Commercial Sample Analysis

The % drug obtained and % recovery value are mean of five determinations. S.D. indicates Standard deviation of five determinations.

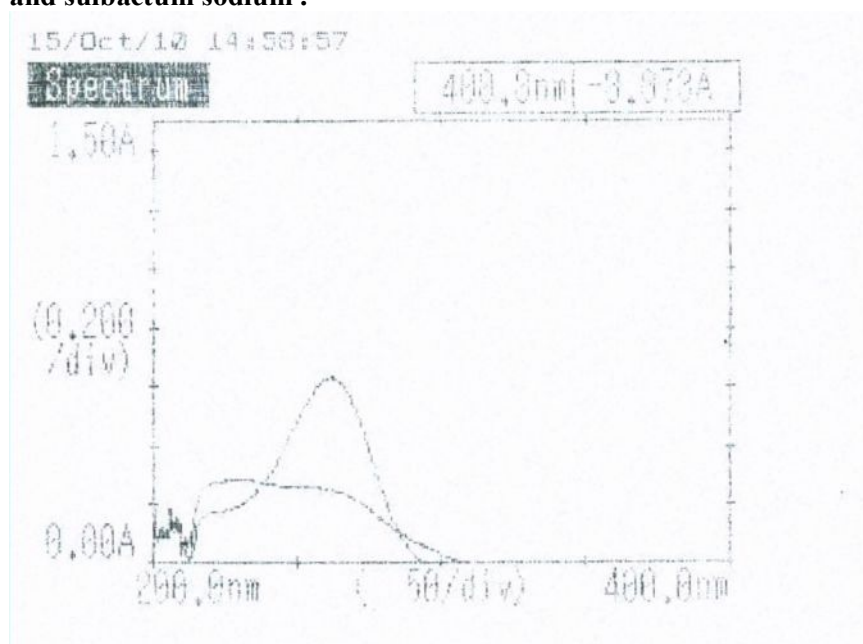
Method	Drug	Labeled Drug (Mg/vial)	Amount obtained (mg)	% Drug obtained	± RS.D.*
A	Cefotaxime	1000	1004.45	100.89	0.05286
	Sulbactam	500	498.00	99.60	0.04262
B	Cefotaxime	1000	1000.80	100.0	0.05296
	Sulbactam	500	497.99	99.59	0.04286

S.D.* = Standard deviation, n= 5, RSD= Relative standard deviation.

Table 2: Optical characteristics & validation of the proposed methods

Parameters	Method A		Method B	
	Cefotaxime	sublactum	Cefotaxime	sublactum
Linearity range (µg/ml)	10 -25	5- 12.5	10-25	5 – 12.5
Beer-Lambert Law (µg/ml)	5- 30	2.5- 15	5- 30	2.5- 15
Accuracy (% Recovery)	99.25	97.78	99.60	97.81
*Ruggedness (±S.D.)				
Interday	1.0525	0.7723	0.9015	0.8945
Intraday	1.8785	1.9980	1.2350	1.0978
Different analyst	1.4411	1.8540	0.3565	0.4712

* Indicates the ±Standard deviation (S.D.) value of the % estimation at different Conditions where n=3.

Figure I: The overlain UV-absorption spectra of 10ug/ml solution of cefotaxime sodium and sublactum sodium .

Results and Discussion

The methods were developed under the experimental conditions described. The assay of injections 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, ruggedness as shown in Table 2. The mean % content of 100.04% and 100.25 % formulation by the developed methods were 100.07% and 100.34% respectively (Table 1). The mean % recoveries of Cefotaxime Sodium and Sulbactam Sodium were found to be 100.04% and 100.25 % respectively (Table 1). The ruggedness of the developed methods was determined by evaluating the effect of change in instruments and analysts on the %mean content of drugs.

Conclusion:

Cefotaxime Sodium and Sulbactam Sodium are available in combined pharmaceutical dosage form for the treatment of of Lower respiratory tract infection.

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Here, two simple UV spectrophotometric methods (Simultaneous equation method, Q-absorption ratio 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 97-102% 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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