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Research Article

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Simultaneous estimation of cefepime hydrochloride and sulbactam sodium in combined dosage form

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ABSTRACT

A simple accurate, and precise effective simultaneous equation spectrophotometric method has been developed for estimation of cefepime hydrochloride and sulbactam sodium in injection dosage form. The Beer lambert law followed at concentration range 24-52 µg/ml and 16-26 µg/ml of cefepime hydrochloride and sulbactam sodium. The proposed method was validated and applied for estimation of cefepime hydrochloride and sulbactam sodium in combined dosage form.

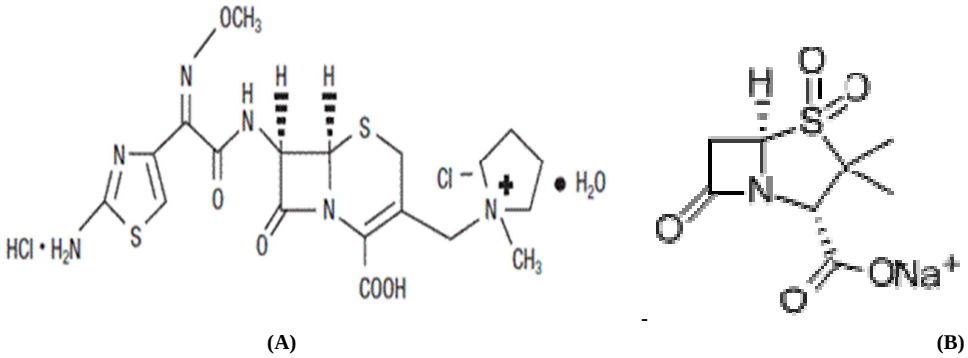
Keywords: combined dosage, spectrophotometric, cefepime hydrochloride, sulbactam sodium, simultaneous equation.

INTRODUCTION

Cefepime hydrochloride is chemically 7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino) acetamido)-3-((1-methylpyrrolidinium-1-yl)methyl)-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-carboxylate. Cefepime hydrochloride belongs to cephalosporins class [1]. It is a semi synthetic analogue of kanamycin, which is active against most of gram-negative bacteria including gentamycin- and tobramycin-resistant strains. The drug is official in Indian Pharmacopoeia, British Pharmacopoeia, and United State Pharmacopoeia [2-4]. Several analytical methods have been developed for cefepime hydrochloride alone and in combination with several other drugs [5-8].

Sulbactam sodium is a β-lactamase inhibitor. This drug is given in combination with β-lactam antibiotics to inhibit β-lactamase, an enzyme produced by bacteria that destroys the antibiotics.[9]

Chemically sulbactam sodium is Sodium (2S,5R)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo (3.2.0)heptane-2-carboxylate 4,4-dioxide [10]. The drug is official in Indian Pharmacopoeia and British Pharmacopoeia [11-12]. Several analytical methods including UV, HPTLC, RP-HPLC have been developed for sulbactam sodium [16].



(A) **(B)**

Figure 1: Chemical structure of (A) Cefepime hydrochloride (b) Sulbactam sodium

Detailed survey of analytical method literature revealed HPLC method has been reported for combination of cefepime hydrochloride and sulbactam sodium [18]. The present work describes the simultaneous equation method for estimation of simultaneous equation method. Further method was validated as per ICH guidelines [18].

EXPERIMENTAL SECTION

Instrumentation

Shimadzu UV/Visible double beam spectrophotometer (UV 1800) with 1cm matched quartz cells were used for the spectral measurement. The spectrophotometer was equipped with UV probe software.

Chemicals and Reagents

Pure sample of Cefepime hydrochloride and sulbactam sodium were kindly gifted from Montage Laboratories Pvt. Ltd. Himatnagar, Gujarat.

Commercial injection formulation-Supime was (Venus Remedies Limited) was purchased from local market. All other reagents used were of AR grade.

Solvent system: 0.1 N NaOH (pH 8 with audjusted with 0.1 N HCl).

Preparation of stock solution

Accurately weighed 100 mg cefepime hydochloride and 100 mg sulbactam sodium reference standard was transferred to 100 ml volumetric flask individually, and was dissolved in minimum quantity of 0.1N NaOH. The volume was dilute up to the mark with 0.1N NaOH. The aliquots from the standard stock solution were pipette out for further dilution whenever needed to prepare working standard solution.

Preparation of working standard solution:

Accurately measured 1 ml of std stock solution was pipette out into 10 ml volumetric flask and diluted using 0.1N NaOH up to the mark to prepare the conc. of 100 µg/ml of cefepime hydochloride and 100 µg/ml of sulbactam sodium.

Selection of wavelength for analysis of cefepime hydrochloride and sulbactam sodium

The aliquots of cefepime hydochloride and sulbactam sodium stock solution were taken and diluted with the 0.1N NaOH individually, such that the final concentration of cefepime hydochloride and sulbactam sodium was 24 µg/ml and 12µg/ml respectively. The solution was scanned over the range of 200-400 nm using UV-visible Spectrophotometer and spectrum was recorded. The wavelength ((λ1, λ2) at which maximum absorbance was obtained was considered as λ_{max} of the drug. These two wavelengths were used to measure absorbance of cefepime hydochloride and sulbactam sodium. The overlain spectra are shown in figure 2.

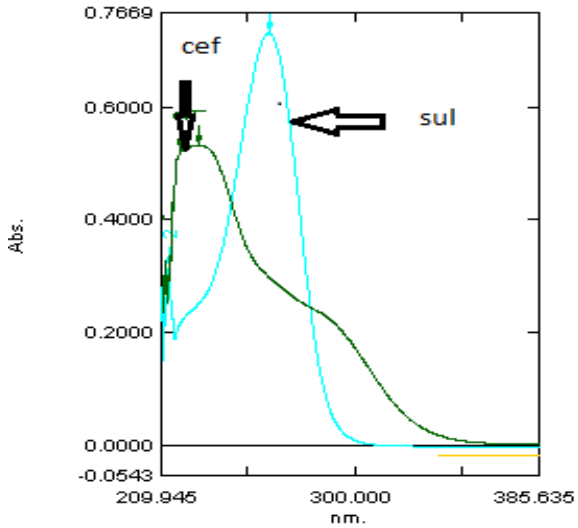


Figure 2: Overlay spectra of cefepime hydrochloride (24 µg/ml) and sulbactam sodium (12 µg/ml)

Preparation of calibration curve

Accurately measured working standard solution of cefepime hydochloride and sulbactam sodium were transferred to a set 10 ml volumetric flask individually and diluted with 0.1N NaOH to get different range of concentration of cefepime hydochloride and sulbactam. The absorbance of each solution was measured at selected wavelengths. Calibration curves were constructed by plotting absorbance versus concentration value for cefepime hydochloride and sulbactam sodium.

Development of method

Simultaneous equation method

The formula for cefepime hydochloride and sulbactam sodium was developed by adopting following proposed equation for the purpose. Absorptivity of cefepime hydochloride and sulbactam sodium was determined and the values are used to constitute the equation**.**

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the absorbance maximum of the other, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method).

$$\mathrm{Cx} = \frac{\mathrm{A2ay1} - \mathrm{A2ay2}}{\mathrm{ax2ay1} - \mathrm{ax1ay2}}$$

$$\mathrm{Cy} = \frac{\mathrm{A1ax2} - \mathrm{A2ax1}}{\mathrm{ax2ay1} - \mathrm{ax1ay2}}$$

a_{x1} and a_{x2} =The absorptivity of cefepime hydochloride at 227 nm and 260 nm respectively.
a_{y1} and a_{y2} = The absorptivity of sulbactam at 227 nm and 260 nm respectively.
A_1 and A_2 =The absorbance of the diluted sample at 227 nm and 260 nm respectively

Method validation

Linearity range

Calibration curve of cefepime hydochloride and sulbactam sodium was developed individually by preparing different concentration of cefepime hydochloride and sulbactam sodium measuring the absorbance at two selected wavelengths 227 nm and 260 nm. The higher value of the regression coefficient confirmed the adherence to beer's law.

Precision:
Variation of results within same day is called intraday precision and variation of results amongst days is called interday precision. Intra-day precision of the proposed method was evaluated by assaying freshly prepared solutions of cefepime hydochloride and sulbactam sodium in triplicate at three different concentrations. Interday precision was evaluated by using freshly prepared solutions of cefepime hydochloride and sulbactam sodium in triplicates at three different days. The amount of drugs determined and % RSD found.

Accuracy
Accuracy was determined by calculating the recovery of cefepime hydochloride and sulbactam sodium by standard addition method. To a fixed amount of sample injection solution of sulbactam sodium (12µg) and cefepime hydrochloride (24µg) and different amount of standard sock solution cefepime hydochloride (19.2,24,28.8) and Sulbactam Sodium 9.6,12,14.4 was added.

Specificity
Specificity is the ability of the method to measure the analyte in the presence of other relevant components. The evaluation of specificity of the method was determined against placebo.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):
The limit of detection (LOD) and the limit of quantitation (LOQ) of all selected combination of drugs were derived by calculating the signal to-noise ratio using the following equations as per the ICH guidelines.

$$LOD = \frac{SD}{Slope} X\ 3.3 LOQ = \frac{SD}{Slope} X\ 10$$

Where, S.D -standard deviation of the response

Analysis of marketed formulation
A powder quantity equivalent to 1000 mg cefepime hydrochloride and 500 mg sulbactam sodium was accurately weighed and transferred to volumetric flask dissolved in small quantity of 0.1N NaOH. The content was diluted up to 100 ml using 0.1N NaOH. Accurately measured 1ml of sample stock solution was pipette into 100 ml volumetric flask and diluted using 0.1N NaOH up to the mark to prepare the concentration of 100 µg/ml of cefepime hydochloride and 50 µg/ml of sulbactam Sodium. 2.4 ml solution from sample was taken into 10 ml volumetric flask and diluted up to mark by 01 N NaOH to get 24 µg/ml of cefepime hydrochloride and 12 µg/ml of sulbactam sodium.

RESULTS AND DISCUSSION

Cefepime hydrochloride and sulbactam sodium show appropriate absorbance at 227 nm and 260 nm respectively, so these two wavelengths were selected for simultaneous estimation. The linearity range for cefepime hydrochloride and sulbactam sodium was found to be 24-52µg /ml and 12-26 µg /ml respectively. Calibration spectra and curves are shown in Figure 3 and 4.

The precision **(**% RSD) values for cefepime hydochloride and sulbactam Sodium were found to be 0.104% and 0.048% (Table 1). Relative standard deviation was less than 2 %, which indicates that the proposed method is repeatable

The recovery experiments were performed by the standard addition method. The mean recoveries were found 100.98 % and 99.94 % for cefepime hydochloride and sulbactam sodium respectively. The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 1.

LOD values for cefepime hydrochloride and sulbactam sodium were found to be 0.041 and 0.0132. LOQ value cefepime hydrochloride and sulbactam sodium were found to be 0.12 and 0.032 (Table 1). These data show that method is sensitive for the determination of cefepime hydrochloride and sulbactam sodium.

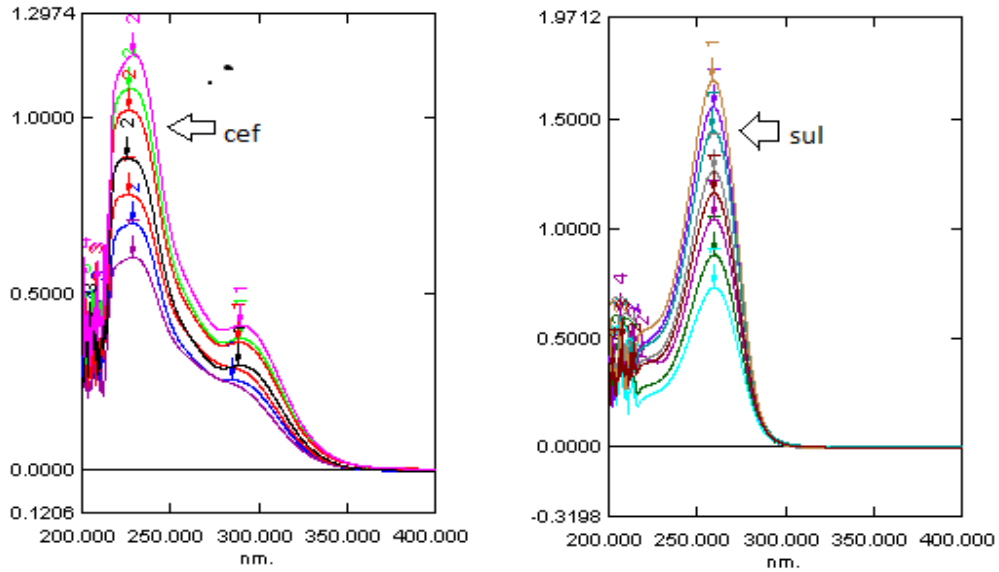


Figure 3: Calibration spectra of cefepime hydrochloride (24-52µg/ml) at 227nm and sulbactam(12-26µg/ml) at 260nm

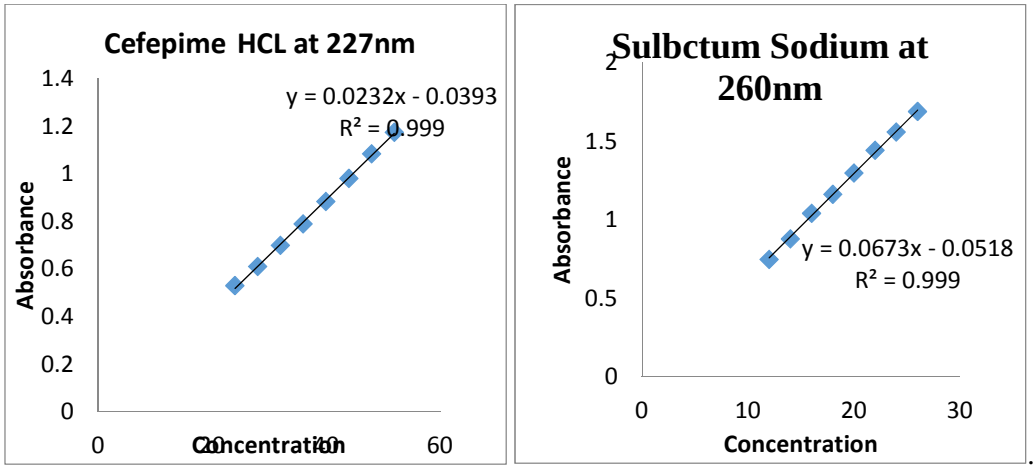


Figure 4: Calibration curves of cefepime hydrochloride (24-52µg/ml) at 227nm and sulbactam sodium (12-26µg/ml) at 260nm

Analysis of marketed injection formulation (1000 mg cefepime hydrochloride and 500 mg of sulbactam sodium) was carried using developed method. The % contents were found 100.9 and 102.8 for cefepime hydrochloride and sulbactam sodium respectively, which was in good agreement with the label claims (Table 2)

Table 1: Optical characteristics and validation of proposed method

Parameters		Cefepime hydrochloride 227nm	Sulbactam sodium 260 nm
Linearity Range(µg/ml)		24-52µg/ml	12-26µg/ml
Regression equation		y = 0.0232x -.0393	y = 0.0671x -0.0495
Correlation co-efficient (r^2)		0.999	0.999
Accuracy (% Recovery)	80%	102.29	101.9
	100%	102.63	98.98
	120%	98.03	98.95
Precision (%RSD)	Intra day	0.104	0.048
Repeatibility		0.055	0.055
LOD(µg/ml)		0.041	0.0132
LOQ(µg/ml)		0.12	0.032

Table 2: Assay of Cefepime hydrochloride (CEF) and Sulbactam Sodium (SUL) in injection dosage form.

Injection Formulation	Labeled Claim (mg/Injection)		Amount found (mg/Injection)		Potency (%)	
	CEF	SUL	CEF	SUL	CEF	SUL
Supime (Venus remedies)	1000	500	1009	514.57	100.9	102.8

CONCLUSION

The results and the statistical parameters show that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. Therefore, the method can be used for the determination of cefepime hydochloride and sulbactam sodium either in bulk or in the dosage formulations without interference with commonly used excipients and related substances.

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