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Simultaneous estimation of Sulbactam and Ceftazidime in combined pharmaceutical dosage form by Visible, Ultraviolet and First order derivative Spectrophotometric methods

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ABSTRACT

Sulbactam and Ceftazidime in combined dosage form is indicated for the treatment of bacterial infections, intra abdominal infections, gynecological infections, skin or soft tissue infections, surgical infections etc. The aim of the present work is to develop simple, precise, accurate and reproducible spectrophotometric methods for estimation of Sulbactam and Ceftazidime. Method A is based on UV spectrophotometry and method B is based first order UV derivative spectrophotometry. Visible spectroscopic method is also developed by AMP and INH derivative methods. UV simultaneous estimation was achieved by using distilled water as solvent with absorption (λ max) at 241 nm for Ceftazidime and 223 nm for Sulbactam. In AMP method 428 nm is taken, as this wave length give maxima for Ceftazidime and for Sulbactam NIH method 601 nm is taken (λ max). The linearity was established over the concentration range of 5-30 µg/ml for Sulbactam and 10-60 µg/ml for Ceftazidime with correlation coefficient (r^2) of 0.999 for both the drugs. The methods were validated as per the International Conference on Harmonization (ICH) guidelines. Both methods were applied to the assay of the drugs in marketed formulation, which were found in the range of 98.0% to 100.0% of the labeled value for both Sulbactam and Ceftazidime. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method. Hence, the methods herein described can be successfully applied in quality control of combined pharmaceutical dosage forms.

Keywords: Ceftazidime (CFZ), Sulbactam (SBT), Spectrophotometry, First Order Derivative Method, ICH guidelines.

1. INTRODUCTION

Sulbactam and Ceftazidime combination is available as injection dosage form indicated for the treatment of different infections like bacterial, Intra-abdominal, gynecological, skin or soft tissue, surgical and other conditions. Sulbactam is a β-lactamase inhibitor used to inhibit β-lactamase enzyme that produced by bacteria that destroys antibiotic activity. Sulbactam (Figure 1) is an irreversible inhibitor of β-lactamase and it binds to the enzyme and does not allow it to degrade the antibiotic. Sulbactam administered in combination with other β-lactam antibiotics, as its antibacterial activity is too weak to have any clinical importance. The IUPAC name of Sulbactam is (2S,5R)-3,3-dimethyl -7-oxo-4-thia-1-azabicyclo [3.2.0]heptanes-2-carboxylic acid 4,4-dioxide with molecular formula $C_8H_{11}NO_5S$ and molecular mass 233.243.

Ceftazidime (Figure 2) is a third generation cephalosporin having broad-spectrum β-lactam antibiotic used to treat lower respiratory tract, skin, urinary tract, blood stream, joint and abdominal infections, and meningitis [1]. It is a semi synthetic drug belongs to cephalosporins class [2,3]. Ceftazidime was commercial available from 1984 [4] and is listed as Essential Medicines by World Health Organization [5] and is available as a generic medication. It has activity against both gram positive and negative bacteria. Specifically this drug is used for joint infections, meningitis, pneumonia, sepsis, urinary tract infections, malignant otitis externa, pseudomonas aeruginosa infection, and vibrio infection. It is

given as injection into a vein or muscle [6, 7]. The drug is available as injection and works by inhibition of cell wall synthesis via affinity for penicillin-binding proteins (PBPs). The IUPAC name of Ceftazidime is (6R, 7R,Z)-7-(2-(2-aminothiazol-4-yl)-2-(2-carboxypropan-2-yloxyim-ino)acetamido)-8-oxo-3-(pyridinium-1-ylmethyl) -5-thia-1-aza-bicyclo [4.2.0] octa-2-ene-2-carboxylate with molecular formula $C_{22}H_{22}N_6O_7S_2$ and molecular mass 546.58.

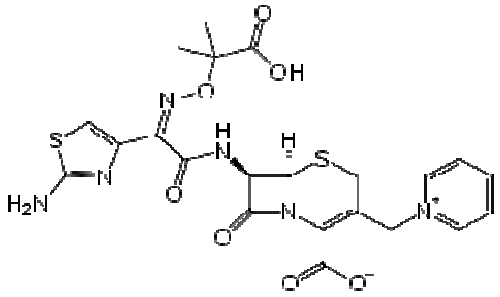

Figure - 1: Chemical structures of Ceftazidime (CFZ).

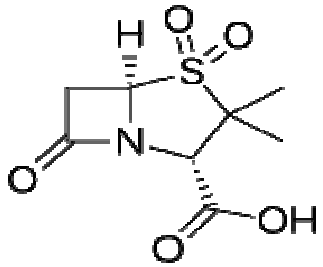

Figure - 2: Chemical structures of Ceftazidime Sulbactam (SBT)

Study of literature has provided information that very few analytical methods have been reported with Ceftazidime (CFZ) and Sulbactam (SBT) drugs. Spectrophotometry methods have been reported with Ceftazidime [8-10], Ceftazidime in combination with other drugs[12] and Sulbactam in combination with other drugs [13-16]. There are few liquid chromatography (RP-HPLC) methods [17-26] reported for the analysis of Ceftazidime and Sulbactam separately and in combination with each other. In the present study, it is undertaken to validate these two drugs with UV and Visible spectrophotometric methods.

2. MATERIALS AND METHODS

2.1. Instrumentation

Tec comp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Ultrasonicator (1.5L) was used to sonicate the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

2.2. MATERIALS

Analytical pure samples of Ceftazidime (Biochem Pharmaceutical Limited, Mumbai, India), and Sulbactam (Solitaire Pharmacia Private Limited, Chandigarh, India) were used in this study. The pharmaceutical dosage form used in this study was Vitazid-SB procured from the local pharmacy and labeled to contain Sulbactam-500mg and Ceftazidime - 1000mg per vial.

2.3. Standard Solutions

Stock solutions of Ceftazidime (CFZ) and Sulbactam (SBT) were prepared in distilled water as solvent. Working standard solutions were freshly obtained by diluting the stock standard solutions with distilled water during the day of analysis.

2.4. Preparation 4-Amino Phenazone solution [AMP]

500 mg of 4-Amino Phenazone was accurately weighed and dissolved in 100 ml of methanol containing 1 ml of conc. HCl.

2.5. Iso Nicotanic hydrazide [INH] solution

800 mg of Iso Nicotanic hydrazide was accurately weighed and dissolved in 100 ml of methanol containing 1 ml of conc. HCl.

2.6. Sample Preparation

Ten vials of Ceftazidime and Sulbactam (Vitazid-SB; Ceftazidime – 1000 mg and Sulbactam – 500 mg) were mixed and a uniform formulation sample was prepared. It was soaked in 5 ml diluents and was keep it for solubility for 1 hr. Then it was filtered and makes up to 10 ml with same diluents to make 100 µg/ml stock solutions. From this, with proper dilution, a concentration of 40 µg/ml of Ceftazidime was prepared. As per the label claim of the two drugs a Sulbactam concentration 40 µg/ml was obtained. The resultant solution was used for the simultaneous estimation of Ceftazidime and Sulbactam in combined dosage forms.

2.7. Methodology

2.7.1. Simultaneous equation method

From the stock solution 100 µg/ml, working standard solutions of drugs were prepared by appropriate dilution and were scanned for the entire UV range. λ maximum of Sulbactam has been found at 223 nm and Ceftazidime at 241 nm (Figure 3). And the calibration curves were determined in the

concentration range of 5-30 µg/ml for Sulbactam and 10-60 µg/ml for Ceftazidime drugs.

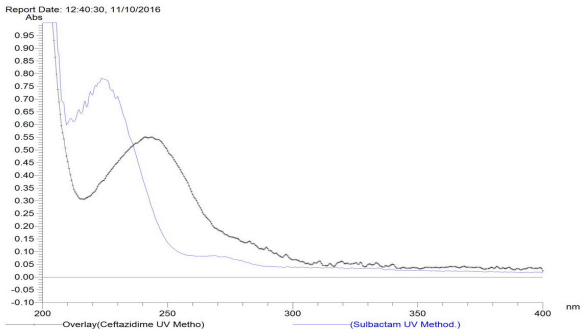

Figure - 3: Overlain Ultraviolet spectra of Sulbactam (SBT) and Ceftazidime (CFZ) in the developed method.

At the absorbance of these standard solutions calibration curves were plotted at these wavelengths (Figure 4 and 5). The proposed method was validated according to the Unites States Pharmacopeia (USP) and International Conference on Harmonization (ICH) guidelines in terms of linearity and range, precision, accuracy. The simultaneous analysis of the drugs were carried using the following equation

$$C_x = A_2a_{y1}-A_1a_{y2}/a_{x2}a_{y1}-a_{x1}a_{y2}$$

$$C_y = A_1a_{x2}-A_2a_{x1}/a_{x2}a_{y1}-a_{x1}a_{y2}$$

Where:

a_{x1}= Absorptivity of Sulbactam at 223 nm

a_{x2}= Absorptivity of Sulbactam at 241 nm

a_{y1}= Absorptivity of Ceftazidime at 241 nm

a_{y2}= Absorptivity of Ceftazidime at 223 nm

A1 and A2 are the absorbance of the diluted sample at 223 nm and 241 nm respectively.

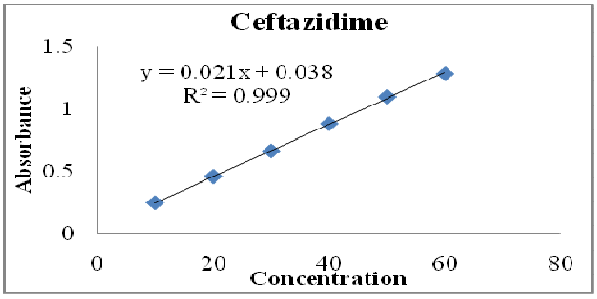

Figure - 4: Calibration graph for Ceftazidime.

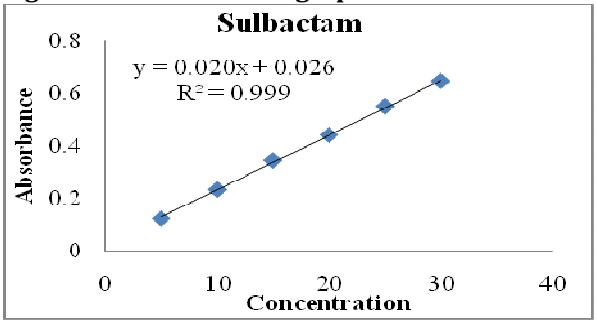

Figure - 5: Calibration graph for Sulbactam.

2.7.2. First derivative spectroscopy method:

First derivative spectroscopy on the basis of zero-crossing measurements involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelength of the derivative spectrum of another component. The working standard solutions of Sulbactam and Ceftazidime were scanned in the wavelength range of 400 to 200 nm to obtain overlain spectra (Figure 6). In this method, 223 nm was selected for the determination of Sulbactam and 241 nm for Ceftazidime. First-derivative technique (D1) traced with Δλ= 2 nm was used to resolve the spectral overlapping. The calibration curves were checked for linearity and linear behavior was observed in the concentration range of 5-30 µg/ml for Sulbactam and 10-60 µg/ml for Ceftazidime (Figure 7 and 8).

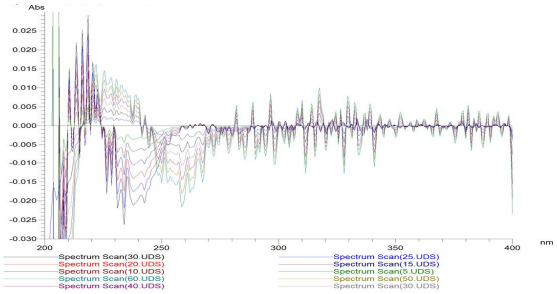

Figure - 6: Overlay spectra of Sulbactam and Ceftazidime in I order derivative method

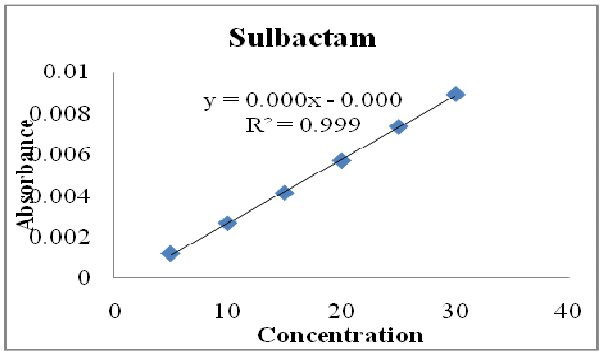

Figure - 7: Calibration graphs for I order derivative method of Sulbactam.

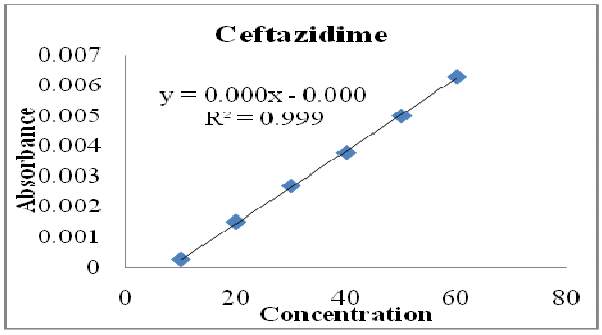

Figure - 8: Calibration graphs for I order derivative method of Ceftazidime.

2.7.3. Visible spectrophotometry method

2.7.3.1. AMP method for Ceftazidime

Standard calibration solutions (0.5-3.0 ml; 100 µg/ml) were prepared from standard stock solution of Ceftazidime by transferred aliquots of solution into a series of 10 ml calibrated tubes. Then 3.0 ml of 4-Amino Phenazone solution was added to each tube and kept aside for 15 min. Later the solution in each tube was made up to 10 ml with methanol. The absorbance was measured at 428 nm against the reagent blank.

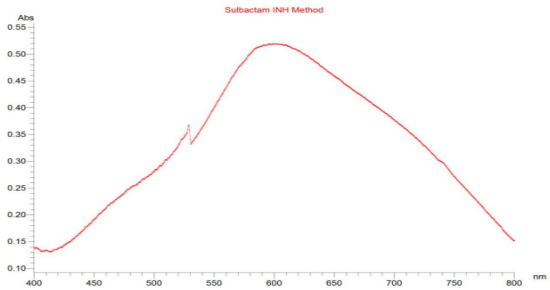

Figure - 9: Visible spectrum of Sulbactam (INH method).

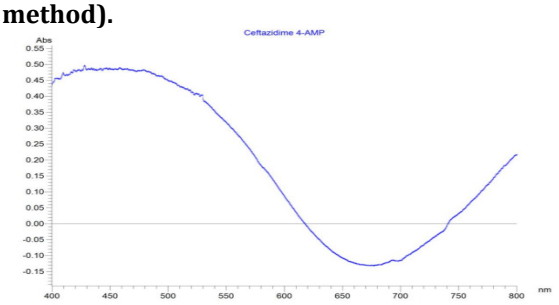

Figure - 10: Visible spectrum of Ceftazidime (AMP method).

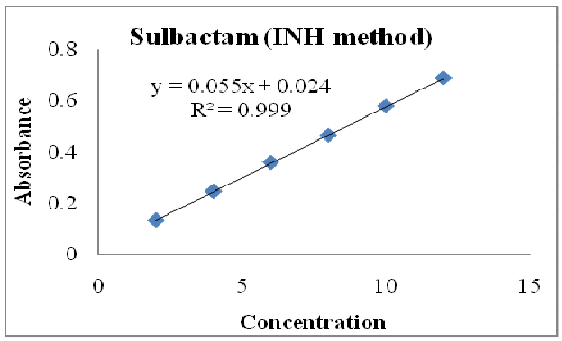

Figure - 11: Calibration graphs for visible methods of Sulbactam.

2.7.3.2. INH method for Sulbactam

Standard calibration solutions (0.5-3.0ml; 40µg/ml) were prepared from standard stock solution of Sulbactam by transferring aliquots of drug solution into a series of 10 ml calibrated tubes. Then 2.0 ml of INH solution was added to each tube and heated for 10 min at 60^{0}C. Later the solution in each tube was cooled and made up to 10 ml with methanol. The absorbance was measured at 601 nm against the reagent blank. Spectra of Sulbactam and Ceftazidime derivatives are presented in Figure 9 and 10 and the calibration curves for these two drugs are given in Figure 11 and 12.

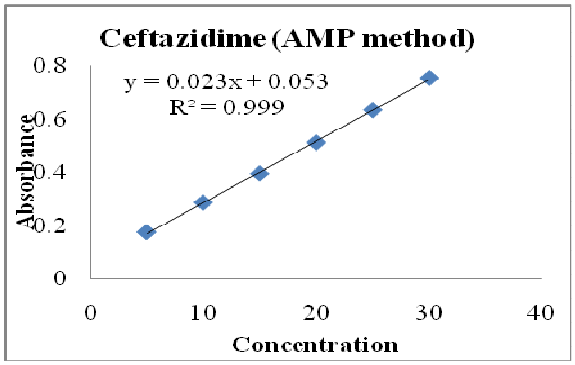

Figure - 12: Calibration graphs for visible methods of Ceftazidime.

3. RESULT AND DISCUSSION

3.1. Simultaneous equation method

The Beer- Lambert's concentration range of simultaneous equation method was found to be 5-30µg/ml for Sulbactam at 223 nm and 10-60 µg/ml for Ceftazidime at 241 nm. The correlation coefficient was found to be 0.999 for both Sulbactam and Ceftazidime respectively (Table 1). Precision was determined by calculating relative standard deviation (% RSD) for repeatability in intraday and inter-day estimation. % RSD of Sulbactam was found to be 0.422, 0.417 and 0.597 for intraday, inter-day and ruggedness tests respectively and the % RSD of Ceftazidime was 0.261, 0.372 and 0.397 for intraday, inter-day and ruggedness tests respectively. The values of LOD and LOQ are 0.15 µg/ml and 0.30 µg/ml for Sulbactam and 0.50 µg/ml and 1.0 µg/ml for Ceftazidime respectively. The accuracy of method was determined by calculating mean percentage recovery at 50,100 and 150 % level. The % recovery ranges from 99.85 to 101.4 for Sulbactam and 99.72 - 100.36 for Ceftazidime respectively. Marketed vials are analyzed and the amount of drug determined by these proposed methods are 98.897 and 99.741 for Sulbactam and Ceftazidime respectively (Table 2). The method can be successfully used for simultaneous estimation of Sulbactam and Ceftazidime in combined dosage form.

3.2. First order derivative Spectrophotometric method

Beer's law is obeyed in the concentration range of 5-30 µg/ml for Sulbactam at 223 nm and 10-60 µg/ml for Ceftazidime at 241 nm.

Correlation coefficient was greater than 0.999 for both the drugs (Table 3). The proposed methods were also evaluated by the assay of commercially available vials containing Sulbactam and Ceftazidime. The results of formulation analysis found 98.897 and 99.741 for Sulbactam and Ceftazidime respectively (Table 4). Recovery was found in the range of 99.12 to 99.92% for Sulbactam and 99.23 to 100.26 %, for Ceftazidime. The precision results were found to be within the limit where % RSD values for Sulbactam found to be 0.257, 0.402 and 0.721 for intraday, inter day and ruggedness studies. And also %RSD values for Ceftazidime found to be 0.497, 0.968 and 0.695 for intraday, inter day and ruggedness studies. The values of LOD and LOQ were 0.15 µg/ml and 0.30µg/ml for Sulbactam and 0.50 µg/ml and 1.0 µg/ml for Ceftazidime respectively.

Table - 1: Linearity test results of Simultaneous equation method

Sulbactam		Ceftazidime	
Conc	**Absorbance**	**Conc**	**Absorbance**
5	0.126	10	0.246
10	0.235	20	0.456
15	0.345	30	0.664
20	0.441	40	0.881
25	0.549	50	1.098
30	0.645	60	1.284

Table - 2: Formulation assay results of Simultaneous equation method

Drug	Brand Name	Label Claim	Amount Prepared	Amount Found	% Assay
Sulbactam	Vitazid-SB	500 mg	20 µg/ml	19.779 µg/ml	98.897
Ceftazidime		1000 mg	40 µg/ml	39.896 µg/ml	99.741

Table - 3: Linearity results of first derivative method

Ceftazidime		Sulbactam	
Concentration	**Absorbance**	**Concentration**	**Absorbance**
10	0.00025	5	0.00121
20	0.00149	10	0.00268
30	0.00268	15	0.00415
40	0.00378	20	0.00571
50	0.00499	25	0.00735
60	0.00628	30	0.00892

Table - 4: Formulation assay results of first derivative method

Drug	Brand Name	Label Claim	Amount Prepared	Amount Found	% Assay
Sulbactam	Vitazid-SB	500 mg	20 µg/ml	19.779 µg/ml	98.897
Ceftazidime		1000 mg	40 µg/ml	39.896 µg/ml	99.741

Table - 5: Linearity test results of colorimetric methods

Sulbactam (INH method)		Ceftazidime (AMP method)	
Concentration in µg/ml	**Absorbance**	**Concentration in µg/ml**	**Absorbance**
2	0.134	5	0.175
4	0.246	10	0.286
6	0.358	15	0.396
8	0.464	20	0.513
10	0.578	25	0.635
12	0.687	30	0.756

Table - 6: Validation results of colorimetric methods

Validation parameter	Sulbactam (INH method)	Ceftazidime (AMP method)
Intraday precision	0.402	0.362
Inter-day precision	0.519	0.504
Ruggedness	0.502	0.366
Recovery	100.17-100.83%	100.15-100.75%
LOD	0.05µg/ml	0.10µg/ml
LOQ	0.20µg/ml	0.40µg/ml

Table - 7: Formulation assay results of colorimetric methods

Drug	Brand Name	Label Claim	Amount Prepared	Amount Found	% Assay
Sulbactam	Bulk drug	----	20 µg/ml	19.906 µg/ml	99.53
Ceftazidime	ORZID	250 mg	40 µg/ml	39.74 µg/ml	99.35

4.3. Visible Spectrophotometric methods

Sulbactam and Ceftazidime colorimetric methods were developed with INH (Iso Nicotanic hydrazide) and AMP (4-Amino Phenazone) methods respectively. Both reagents react with the drugs resulting in the formation of bluish green colour for Sulbactam and orange yellow for Ceftazidime, which showed λ max at 601nm and 428nm against blank respectively. Amino group in 4-AMP and INH condenses with –OH group present in Sulbactam and Ceftazidime respectively to form a colour complex. This method obeyed Beer-Lambert's law in the concentration range of 2-12 µg/ml for Sulbactam and 5-30 µg/ml Ceftazidime respectively (Table 5). All the results with Validation study are within the limit and are presented in (Table 6). The quantitative estimation of Ceftazidime injection formulation (ORZID – 250 mg) in pharmaceutical dosage with proposed method was found to be 99.35%. Thus the method is useful for the determination of Ceftazidime in pharmaceutical formulations. Due to the unavailability of single marketed formulations of Sulbactam, bulk drug samples were analyzed and 99.53% assay has been achieved (Table 7).

5. CONCLUSION

The proposed methods are simple, accurate, precise, sensitive and can be successfully applied for routine quantitative estimation of Sulbactam and Ceftazidime formulation dosage forms. The scan results were very clear and obey Beer's law to a certain extent, which enables rapid quantization of many samples in routine quality control. They also show good linearity and sensitivity. A minimal interference was observed from excipient. These results show the method could find practical application as a quality control tool for analysis of Sulbactam and Ceftazidime from their different pharmaceutical dosage forms in a quality control laboratory.

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