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CALORIMETRIC DETERMINATION OF CEFTRIAXONE AND SULBACTAM IN INJECTION DOSAGE FORM

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ABSTRACT

Simple, selective and highly sensitive spectrophotometric methods were developed for accurate quantification of ceftriaxone and sulbactam in injection dosage form by UV- Visible spectrophotometry using chromogenic reagents [Methylene blue and Bromocresol green]. The method involves formation of stable blue and green coloured ion-pair complexes at 672nm and 668nm respectively. The composition of the ion-pairs was found to be 1: 1 by Job's method. The method was validated according to ICH guidelines for different analytical parameters. Calibration curve was found to be linear over the concentration range of 2-20µg/ml with correlation coefficients of 0.999 and 0.999 respectively. Percent RSD of precision was found to be less than 2 for both drugs. Percentage recovery was found to be 98.8% & 100.4% for ceftriaxone and sulbactam respectively.

INTRODUCTION

Ceftriaxone, a cephalosporin β-lactum antibiotic used in treatment of bacterial infections that are caused by gram positive organism [1]. Ceftriaxone a derivative of 7-amino cephalosporic acid exhibits its antibacterial activity by inhibiting cell wall synthesis [2, 3]. Sulbactum, an irreversible β-lactamase inhibitor, a derivative of penicillin [4] is indicated for treatment of bacterial infections that are resistant to β-lactamase inhibitors [5]. Addition of β-lactamase inhibitor i.e., sulbactam to β-lactam antibiotic inhibits β-lactamase there by enhancing therapeutic value of this combination [6]. A Literature survey reveals that only few Spectrophotometric methods [7-13], High performance liquid chromatography [14-22] and High performance thin layer chromatography [23] methods are reported for determination of ceftriaxone and sulbactum alone and in combination. Sridharan et al proposed a UV spectrophotometric method for simultaneous determination of ceftriaxone and sulbactam, however the reported method has narrow linearity range with lower sensitivity and the method is not fully validated. Till now no methods are reported for the estimation of these drugs using chromogenic reagents by visible spectrophotometry. Therefore the present work describes a simple, economic and sensitive visible spectrophotometric method for simultaneous estimation of

both drugs using chromogenic reagents. The method was found linear over the concentration range of 2-20 µg/ml for ceftriaxone and 1-10 µg/ml for sulbactum respectively.

MATERIAL AND METHODS

Chemicals and reagents: 0.4% Methylene blue (Nice chemicals, Cochin) was prepared by adding 400mg of methylene blue to 100ml of water. 0.4% Bromocresol green (Nice chemicals, Cochin) prepared by adding 400mg of Bromocresol green to 100ml of water.

Instrument specifications: A double beam UV-Visible spectrophotometer (LAB INDIA-3000) with 1cm matched quartz cells were used for the spectral & absorbance measurements using UV-WIN software over the range of 400-800nm.

Preparation of standard solutions: Ceftriaxone and sulbactum were procured from SimsonPharma, Mumbai. Stock solution containing 1mg/ml was prepared by dissolving in methanol. Working standard solution equivalent to 100µg/ml of ceftriaxone and sulbactum were obtained by appropriate dilutions of stock solution with methanol.

Preparation of working solutions:

Method –A: Aliquots of pure drug solution (0.5-2.5) were transferred to a series of 5 ml volumetric flask. To each flask 0.5ml of methylene blue is added followed by 2 ml acetonitrile and the volume was made up with methanol. The contents were mixed with occasional shaking and the flask was kept aside for 15min. Solutions were scanned in spectrum mode against blank solution from 400-800nm.

Method-B: To a set of 5ml volumetric flask (0.5-2.5ml) aliquots of pure drug solution was added, followed by addition of 1.5ml of Bromocresol green solution and 2 ml of acetonitrile and the volume was made up with methanol. The contents were mixed with occasional shaking and the flask was kept aside for 15min. Solutions were scanned in spectrum mode against blank solution from 400-800nm.

2.5 Assay of sterile powder for injection dosage form: MONOZEN-SB® containing 250mg of Ceftriaxone Sodium and 125mg of Sulbactam Sodium were analyzed by this method. Weighed accurately 40mg of MONOZEN-SB® and transferred to a 100ml volumetric flask, add 50 ml of methanol and make up to the mark with same solvent. Filter through whatmann NO. 42 filter paper.

Optimization of the experimental conditions: The experimental parameters affecting the formation of CEF-MB ion pair complex & SUL-BCG ion pair complex were studied extensively and maintained throughout the experiments.

Effect of organic solvent: The solvent plays an important role as it facilitates the proton transfer and stabilization. The reaction of ceftriaxone with methylene blue (method A) and sulbactum with Bromocresolgreen (method B) was tested in different solvents (chloroform, isopropanol, acetonitrile, carbon tetra chloride, and methanol). The stability and sensitivity of product was good with acetonitrile as solvent. Therefore, 1.5ml of acetonitrile was chosen as optimum value (Figure. 3).

Effect of MB concentration (Method A)

The effect of methylene blue concentration on its reaction with ceftriaxone was investigated by adding various volumes (0.5-2ml) of 0.4% methylene blue to a fixed concentration of ceftriaxone (100µg/ml). The rate of formation of the CEF-MB ion pair complex was increased and it was observed that 1ml of 0.4% methylene blue solution was sufficient to obtain the maximum and reproducible absorbance values. (Figure. 4).

Effect of BCG concentration (Method B) The optimum Bromo cresol green concentration on its reaction with sulbactam was studied by adding various volumes (0.5-2ml) of 0.4%Bromocresol green to a fixed concentration of sulbactum(100µg/ml).The addition of 1.5ml of 0.4% Bromocresol green solution was sufficient to obtain the maximum and reproducible absorbance values (Figure .5)

Effect of time: Reaction time was determined by monitoring colour development at room temperature for both methods. However the colour was found instantly but the reaction was allowed to proceed for 10 min for higher precision measurements.

Association constant and the free energy changes of the complexes: The association constant of complexwas determined by employing the Benesi–Hildebrand method, besides the association constant was calculated byusingthe following equation:

$$[Ao]/A\lambda=1/\varepsilon+(1/Kc.\varepsilon).1/[Do]$$

Where,
[Do] = Concentrationof the drug,
[Ao]= Concentration of the reagent,
Aλ= Absorbance of the complex at 445nm,
ε= Molar absorptivity of the complex at 445nm,
Kc=Association constant of the complex.
The ΔG° (the standard free energy of complexation) and the association constant Kc are related by the following equation[24]

$$\Delta G^\circ=-2.303RT\log Kc$$

Where,
ΔG°=Free energy change of the complex, R=Gas constant (1.987calmol^{-1}degree^{-1}), T = Temperature in Kelvin, K= Association constant (Lmol^{-1}) of the drug-reagent complex.

Stoichiometry of the reaction (Method A& B): The drug–dye stoichiometric ratios were entrenched by Job's method of continuous variation. Equimolar solutions of drugs and reagents are prepared in varying volume ratios in acetonitrile such that total volume of each mixture was same. The solutions were kept at room temperature for 10 min .The absorbance of each solution was measured and plotted against the mole fraction of the drug. This procedure showed the formation of 1: 1 ion-pair.

Method validation: The developed spectrophotometric method was validated as per the guidelines set by ICH. Calibration curve was constructed by plotting the absorbance vs concentration. Estimation of linearity was done at six concentration levels. Accuracy & precision was determined by performing five replicate analysis at three different concentrations for same day and intra- and inter-day studies, respectively. Standard addition method was performed for recovery studies by giving the standard solution of drug at three different concentration levels (50, 100 and 150% of the labelled claim). Robustness of the method was established by minor deliberate changes in experimental parameters.

Linearity: For ceftriaxone and sulbactum determination calibration curve was plotted and the linearity was studied in the concentration range from 2-20µg/ml & 1-10µg/ml for ceftriaxone and sulbactam and regression co-efficient values are (> 0.99) indicates good linearity between the drug and reagent concentrations & linearity graphs shown in Figure 10& 11.

Results of analysis of sterile powder for injection

Drug	Ceftriaxone				Sulbactum			
Monozen SB inj	Label claim (mg)	Amount found ± SD	% Assay	% RSD	Label claim (mg)	Amount found ± SD	% Assay	% RSD
	250	250.49±1.12	100.19	0.44	125	124.39±0.72	99.51	0.58

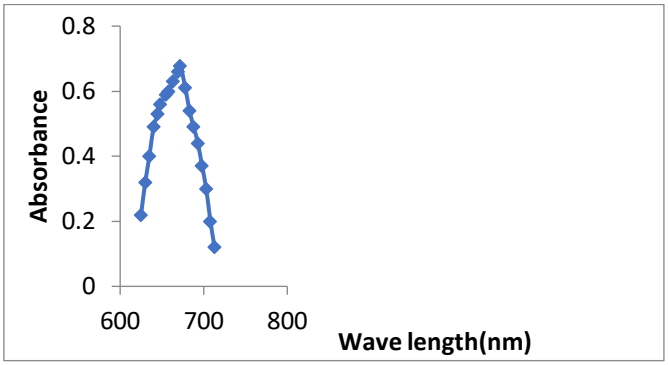

Figure 1: Absorption spectra of CEF-MB ion pair complex

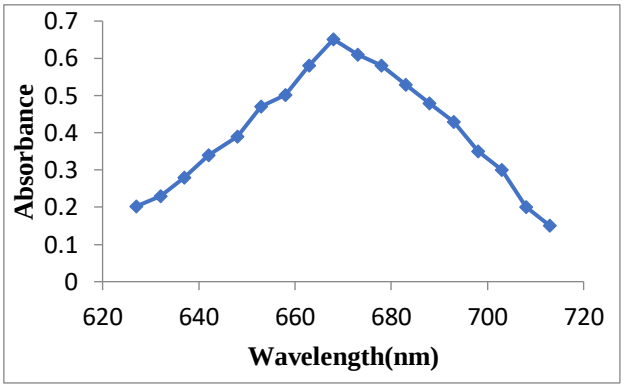

Figure 2: Absorption spectra of SUL-BCG ion pair complex

Table 1: Evaluation of intra-day accuracy and precision for the studied drugs with MB and BCG

Method	Drug	Drug taken (µg/ml)	Drug found (µg/ml)	SD	% RSD	% Recovery	%R.E
MB	Ceftriaxone	5	4.83	0.27	0.05	98.8	1.2
		10	9.9	0.12	0.012	99.5	0.5
		20	19.8	0.22	0.011	98.3	1.7
BCG	Sulbactum	2.5	2.35	0.17	0.074	99.8	0.2
		5	4.85	0.17	0.036	99.9	0.1
		10	9.85	0.17	0.017	98.6	1.4

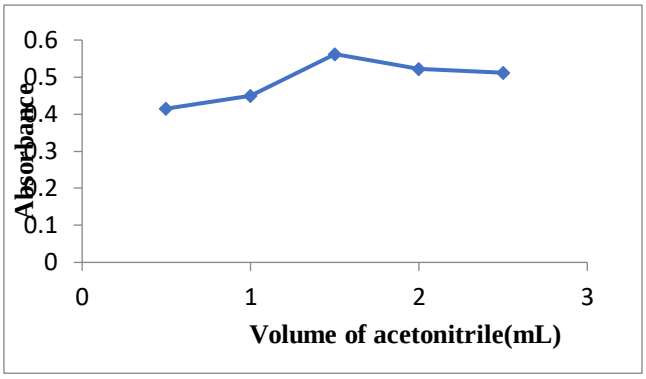

Figure 3: Effect of volume of acetonitrile

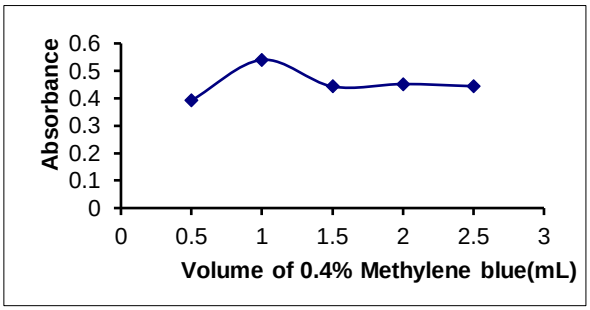

Figure 4: Effect of Methylene blue concentration on the formation of CEF-MB ion-pair complex (method A).

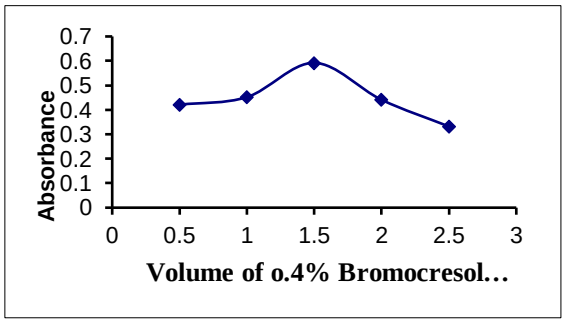

Figure 5: Effect of Bromocresolgreen concentration on the formation of SUL-BCG ion-pair complex (method B).

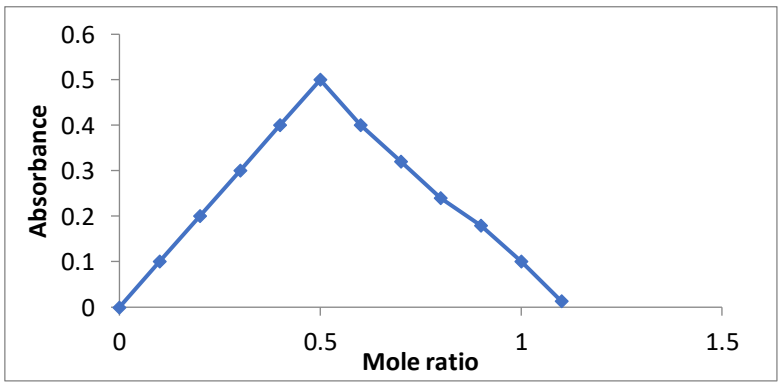

Figure 6: Job's continuous variation plot for method A.

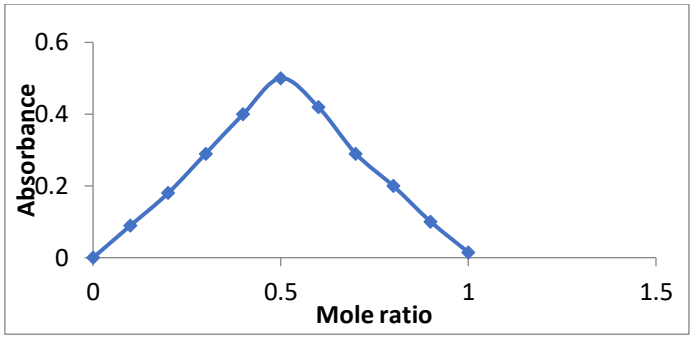

Figure 7: Job's continuous variation plot for method B

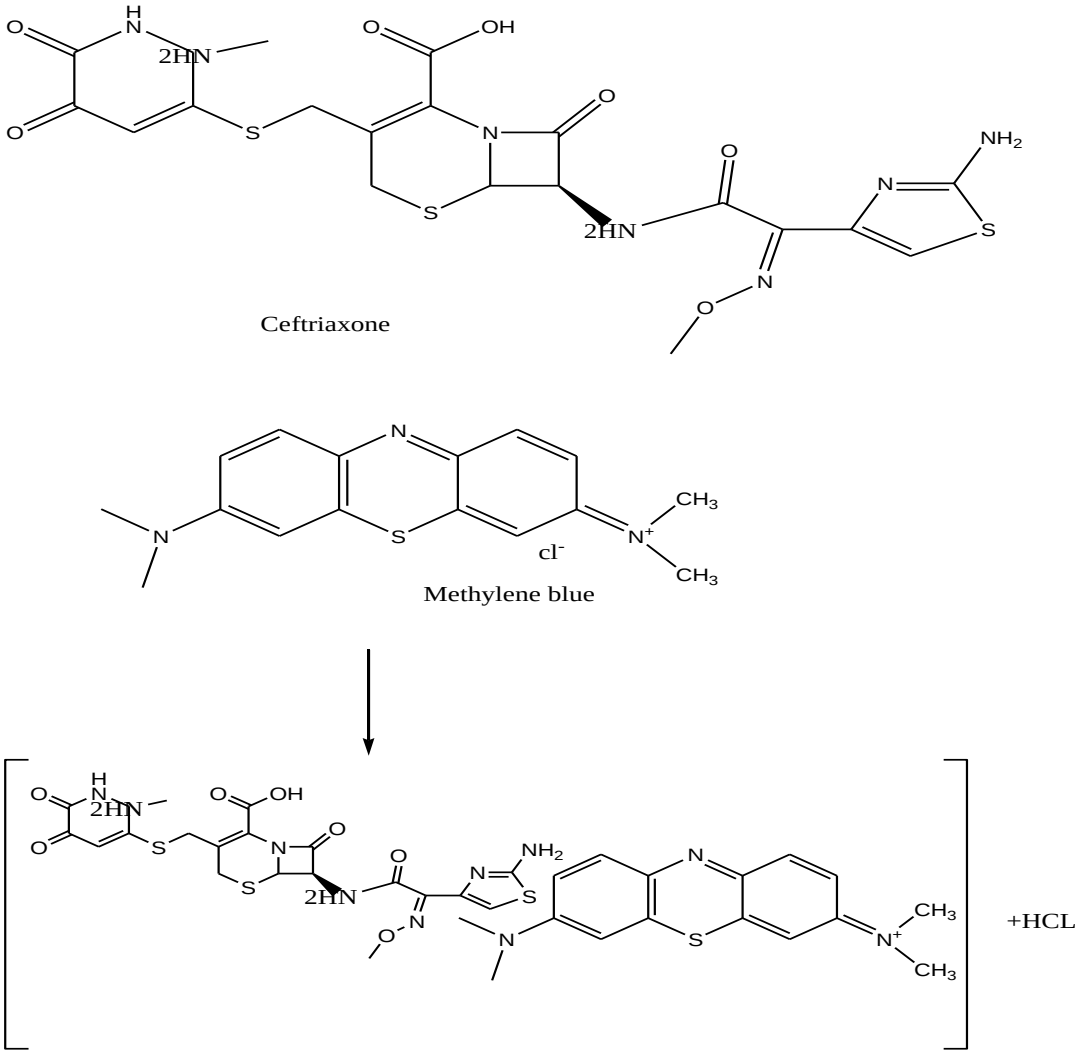

Figure 8: Ion pair complexation of ceftriaxone with methylene blue

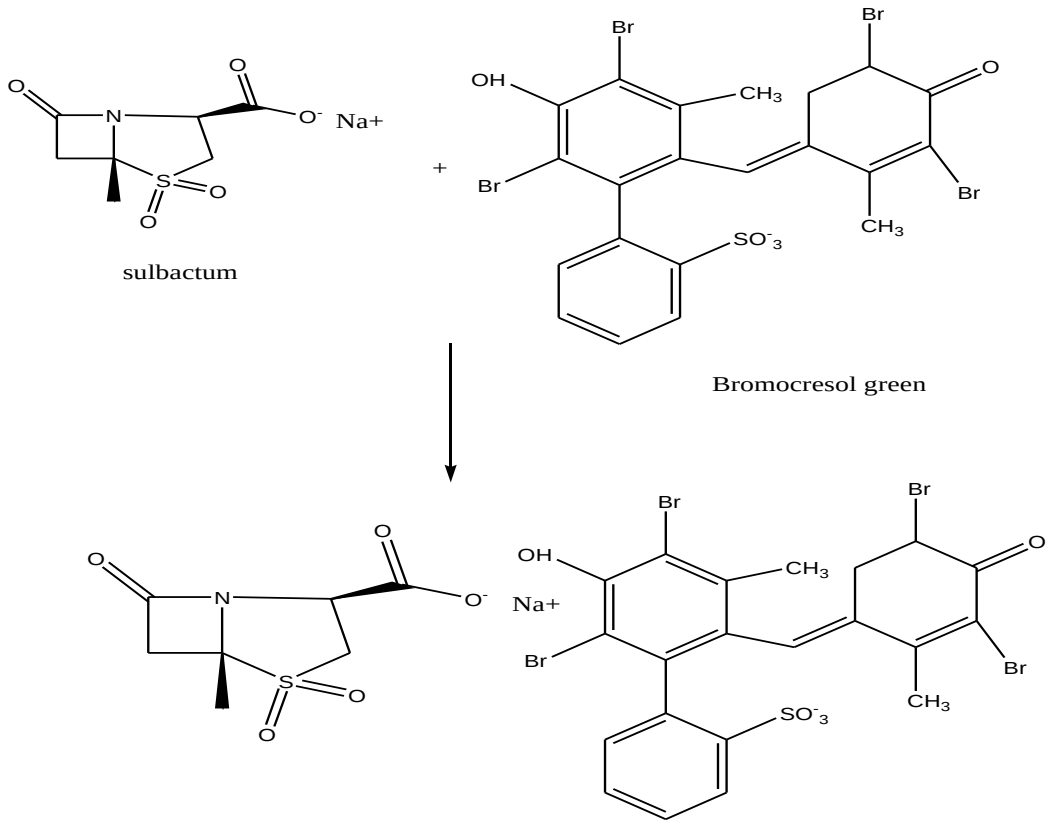

Figure 9: Ion pair complexation of sulbactum with Bromocresol green

Accuracy and Precision: Accuracy &precision of the proposed methods by determined by performing (intra-day& inter-day) precision and accuracy. In order to determine fixed concentration of drug solution (with in working limit) is prepared at three different concentration levels and they were analyzed in five replicates on the same day (intraday precision & accuracy) & inter day precision &accuracy. Accuracy values are expressed as Relative error and percent recovery. The precision values are expressed as standard deviation and percent relative standard deviation. Results confess with low values of RSD& mean recoveries indicated the high precision and accuracy & the results are presented in Table 1& 2.

Table 2: Evaluation of inter-day accuracy and precision for the studied drugs with MB and BCG

Method	Drug	Drug taken (µg/ml)	Drug found (µg/ml)	SD	% RSD	% Recovery	%R.E
MB	Ceftriaxone	5	4.8	0.22	0.047	99.1	0.9
		10	9.85	0.21	0.022	98.2	1.8
		20	19.75	0.30	0.015	99.6	0.4
BCG	Sulbactum	2.5	2.42	0.08	0.03	99.9	0.1
		5	4.9	0.12	0.02	99.4	0.6
		10	9.81	0.21	0.02	97.3	2.7

Table 3: Summary of validation parameters obtained for proposed UV

Parameter	Result	
	ceftriaxone	**sulbactam**
Beer's law limit (µg/mL)	2-20	1-10
Regression Equation(y=mx+c)	Y=0.0482x+0.007	Y= 0.0795x+0.0024
Slope (m)	0.0482	0.0795
Intercept (x)	0.007	0.0024
Molar Absorptivity	1.97×10^4	1.84×10^3
Regression coefficient (r^2)	0.999	0.999
Sandell's sensitivity ($\mu g\ cm^{-2}$/0.001 Absorbance unit)	0.028004	0.027001
LOD (µg/mL)	3	0.1
LOQ (µg/mL)	9	0.3
Association constant (L $mole^{-1}$)	3.5×10^{-7}	3.9×10^{-4}
Free energychange	-6.4×10^3	-5.8×10^4

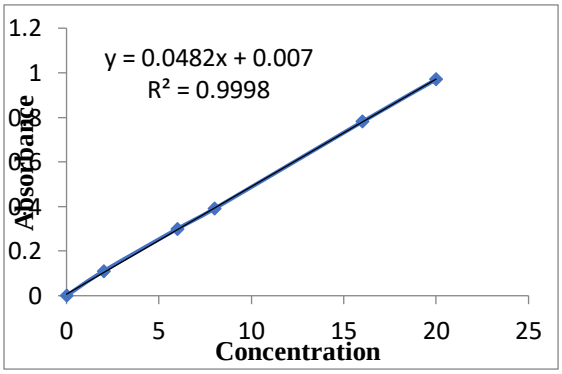

Figure 10: Linearity curve for method-A

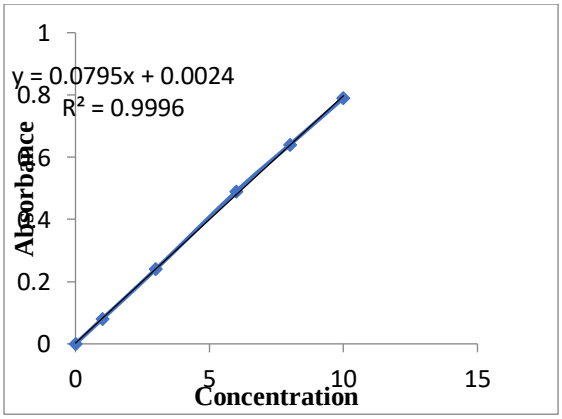

Figure 11: Linearity curve for method-B

Sensitivity: According to the ICH guidelines, the sensitivity parameters like molar absorptivity, Sandell's sensitivity, Limit of Detection and Limit of Quantification were calculated and summarized in Table 3.

Stability of the colored species: Stability of the colored species can be revealed by keeping the solutions at room temperature and by measuring the absorbance of the coloured solution at their corresponding wavelength at regular intervals of time. Absorption intensities values of the colored products were stable for at least 8hrs for both the reagents. Then we can proceed for measuring large batches of sample within the period of time.

Recovery: The accuracy of the proposed methods was further validated by standard addition technique. For this purpose addition of known amount of Ceftriaxone and sulbactum to preanalysed solution of injection at three different concentration levels (50%, 100% and 150%) and the nominal value of drug was estimated. The results are reported as relative standard deviation and percent recovery and revealed that there is no interference from excipients and results are shown in Table 4.

Table 4: Recovery results for ceftriaxone and sulbactum

Name of drug	Spiked drug conc. (µg/ml)	Standard drug conc. (µg/ml)	Total drug conc. (µg/ml)	Total amount found (µg/ml)	% Recovery
Ceftriaxone	10	100	110	109.88	98.8
	15	100	115	114.79	98.65
	20	100	120	120.15	100.75
Sulbactum	5	50	55	55.05	101.0
	7.5	50	57.5	57.53	100.4
	10	50	60	60.2	102.0

Robustness: In this experiment, one parameter was changed whereas the others were kept unchanged. Minor changes in the experimental variables such as Change in the volume of acetonitrile & temperature in method I & change in the volume of 0.4% Bromocresol green in method II. Relative standard deviation and recovery were calculated at each time. Results are summarised in the table and revealed that minor changes in the method Will not affect the analytical performance of the proposed method. The robustness of the methods was assessed by analysing ceftriaxone and sulbactam at two different concentration levels (4 and20 µg/mL).The percent recovery and % RSD of the method (Table5)was foundtobe satisfactory, indicatingthat the method is robust.

Table 5: Robustness of proposed method

Method	Experimental Parameter	Volume(ml)	Taken(4µg/ ml) Absorbance	%Recovery	%RSD	Taken(20 µg/ml) Absorbance	%Recovery	%RSD
MB	0.4% MB	0.8 0.9 1	0.204 0.202 0.206	99.52 97.58 100	0.648 0.687 0.721	0.881 0.887 0.884	99.55 100.23 99.89	0.05 0.08 0.06
	Volume of acetonitrile (ml)	0.9 1.0 1.1	0.206 0.202 0.204	99.52 98.07 99.03	0.687 0.725 0.751	0.883 0.885 0.884	99.77 100 99.89	0.09 0.06 0.11
BCG	0.4% BCG	0.8 0.9 1	0.204 0.203 0.206	98.55 98.07 99.52	0.647 0.690 0.721	0.882 0.886 0.881	99.65 100.11 99.59	0.05 0.08 0.06
	Volume of acetonitrile (ml)	0.9 1.0 1.1	0.203 0.201 0.205	99.56 98.54 96.54	0.690 0.724 0.731	0.883 0.882 0.884	99.75 100.01 99.65	0.09 0.05 0.06

CONCLUSION

A sensitive visible spectrophotometric method for the determination of ceftriaxone and sulbactam have been developed and validated. The present Methods demonstrate that acetonitrile can be used for the quantitative determination of ceftriaxone and sulbactam in injection dosage forms. The reagents used in the developed method are cheap and readily

available. From the values of LOD and LOQ, it was observed that the new method is more sensitive than the reported methods. From the study of validation parameters, it was observed that the method is specific, accurate, precise, reproducible and rugged.

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Conflicts of interest: NIL

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