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Simultaneous Estimation and Validation of Ceftriaxone Sodium and Tazobactum Sodium from Pharmaceutical Dosage Using *Indigo carmine, Methyl orange dye*

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Abstract: A simple, sensitive and rapid UV Spectrophotometric method was developed for the estimation of ceftriaxone sodium (CS) and tazobactum sodium (TS) in pharmaceutical dosage forms. The present work deals with simple Spectrophotometric method development for simultaneous estimation of ceftriaxone sodium and tazobactum sodium in two components formulation. The method employed simultaneous estimation and first order derivative spectroscopy. Scanning individual drug samples in 200-400 nm range. Sampling wavelengths were 247 nm and 260 nm for Ceftriaxone sodium and tazobactum sodium, respectively in simultaneous estimation method. For this method equations generated were Y=-0.004372 +0.03162x (r^2=0.9997) and Y=-0.0651+0.0018x (0.9994) for Ceftriaxone sodium and tazobactum sodium, respectively, 294 nm and 250 nm for Ceftriaxone sodium and tazobactum sodium, respectively in Method B Derivative Spectroscopy. Linearity of proposed method was found to be 5-40 µg/ml for Ceftriaxone sodium and 5-25 µg/ml for tazobactum sodium. Limits of detection were found to be 0.265 and 0.036 µg/ml of Ceftriaxone sodium and tazobactum sodium, respectively. The recovery studies confirmed accuracy of proposed method and low values of standard deviation confirmed precision of method. The method is validated as per ICH guidelines.

Key words: Ceftriaxone sodium · Tazobactum sodium Simultaneous equation · Indigo Carmine · Methyl orange

INTRODUCTION

Ceftriaxone Sodium[1] is chemically known as 5-Thia-1-azabicyclo [4.2.0] oct-2-ene- 2 carboxylic acid, 7-[2-amino-4thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-3-3 [[(1,2,5,6-tetrahydro-2methyl-5,6diaxo-1,2,4-triazin -3- yl) thio] methyl] - di sodium salt, [6R- [6α,7β (2)]] - hydrate, 2:7. It is an antibacterial (Parenteral third generation cephalosporin antibiotic) inhibits bacterial cell wall synthesis of actively dividing cells by binding to one or more penicillin-binding proteins (PBPs). Tazobactum Sodium[2] is chemically known as (2*S*,3*S*,5*R*)-3-methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-4-thia-azabicyclo [3.2.0] heptane-2-carboxylic acid 4,4-dioxide. It is a penicillinate sulfone, structurally related to sulbactam. Being a betalactamase inhibitor, it is synergistic with many beta-lactamase labile drugs such as penicillins and cephalosporins. Literatures survey reveals that analytical methods were reported for the estimation of individual drugs or in combination with other drugs; however there was no method reported for simultaneous estimation of two drugs in combination [3-8]. Author of the article and his research team has developed a UV Method development different pharmaceutical dosage form using ferric chloride [9-23]. In this communication we report a new UV Spectrophotometric method using derivative spectroscopy. In this study, a Simultaneous equation, Derivative Spectrophotometry, method was developed and validated for determination of Ceftriaxone Sodium without any interference from the excipients that are normally present in this tablet formulation. Also, optimization of the choice of solvent and the spectral variables is included, in order to obtain precise and accurate results in the application of the proposed method for the determination of this drug.

MATERIALS AND METHODS

UV Visible spectrophotometer was employed with spectral bandwidth of 1 cm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). All chemicals and reagents

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used were of AR/HPLC grade, Chloroform, ammonia (SD'S) and methanol (A.R., Ranbaxy Ltd., New Delhi) were used for mobile phase preparation and as solvent. All chemicals used in this study were of analytical grade and used without further purification.Commercial powder for injection formulations zubacef (lyka labs ltd) containing 1g of ceftriaxone sodium and 125 mg of tazobactum sodium were procured from local market. Solubility test for the drug ceftriaxone sodium and tazobactum sodium was performed by using various solvents. The solvents include Water, Methanol, Ethanol, Acetonitrile, Hydrochloric Acid (HCl), Sodium Hydroxide (NaOH) and Chloroform.

Determination of Λ Max

Preparation of Stock Solution: Standard stock solution of ceftriaxone sodium and tazobactum sodium was prepared by dissolving 100mg of ceftriaxone sodium and 12.5 mg tazobactum sodium in 1 N NaOH: methanol (60:40) which gives 1000 µg/ml. One ml of this stock solution was taken and was diluted up to 10ml by using methanol and 1N NaOH (50:50) to produce a concentration of 100 µg/ml solution.

Preparation of Working Solution: From the above stock solution 2 ml was transferred into 10 ml volumetric flask and volume was made up to the mark with methanol to make 20µg/ml. Then the sample was scanned with UV Spectrophotometer in the range 200-400nm against 1 N NaOH: methanol (60:40) as blank and the wavelength corresponding to maximum absorbance was noted which is its λmax i.e. at 289 nm.

Reagent Preparation: One gram of methyl orange was weighed and transferred into a 100 ml standard flask and the volume was made up to the mark to get the required concentration (0.5%w/v). 50mg of Indigo Carmine was weighed and transferred into a 100 ml standard flask and the volume was made up to the mark to get the required concentration (0.1%w/v).Aliquots of solution (500 µg mL^{-1}) were transferred into a series of 10 mL calibrated flasks and the total volume was adjusted to 4.0 mL with water. To each flask were added 5 mL of 0.1M Acetic acid followed by 2 mL of bromate-bromide mixture (10 µg mL^{-1} in $KBrO_3$). The content was mixed well and the flasks were set aside for 10 min with occasional shaking. Finally, 10 mL of 1000 µg mL^{-1} methyl orange solution was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 683.7 nm against reagent blank after 10 min.

Method-A Simultaneous Equation Method: Simultaneous equation method [24-25] of analysis is based on the absorption of drugs (ceftriaxone sodium and tazobactum sodium) at the wavelength maximum of the each other. Two wavelengths were selected for the developments of the simultaneous equations were 247 nm and 260 nm, λmax of ceftriaxone sodium and tazobactum sodium, respectively. The absorptivity values E (1%, 1cm) determined for ceftriaxone sodium at 288 nm and 297 nm were 235 and 272nm, while respective values for tazobactum sodium were 214 and 488. These values were means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in following equations to obtain the concentration of both drugs.

$$CCES = \frac{(A_2 \times 247\text{-}A_1 \times 260)}{7621} \quad (1)$$

$$CTS = \frac{(A_1 \times 214\text{-}A_2 \times 488)}{7621} \quad (2)$$

Where C_{CES} and C_{TS} are concentrations of ceftriaxone sodium and tazobactum sodium respectively in g/10mL. A_1 and A_2 are the absorbance of the mixture at 247 nm and 288 nm respectively.

Method B: Derivative Spectrophotometry [24-25]: Stock solutions were prepared separately in 100 ml 1 N NaOH: methanol (60:40) to obtain 100 µg/ml of all drugs. The five working mixed standards were prepared by dilution of stock solution in same solvent system in concentration range 5-40 µg/ml of ceftriaxone sodium and 5-25 µg/ml for tazobactum sodium. Ceftriaxone sodium and tazobactum sodium initially scanned for determining sampling wavelength in range 200-400 nm. Sampling wavelengths were 294 nm for Ceftriaxone sodium where tazobactum sodium showed zero crossing point and 250 nm for tazobactum sodium where Ceftriaxone sodium showed zero crossing point. Calibration graphs were constructed from the absorbances at respective wavelength.

Analysis of Commercial Formulation: Content of powder equivalent to 250 mg of Ceftriaxone sodium and 50 mg tazobactum sodium taken and added in 1000 ml of solvent system sonicated for 10 min after sonication volume was made up to 100 ml. 1ml of this stock solution was diluted to 10 ml to get concentration equal to 10 µg/ml of Ceftriaxone sodium and 5 µg/ml of tazobactum sodium. This solution is scanned in range 200-400 nm taking solvent system as blank. The spectra obtained were

converted Simultaneous equation method and first order derivative spectra absorbances were noted and concentrations were determined from regression equations generated from calibration graph.

Validation of Methods: The developed methods for simultaneous estimation of Sparfloxacin validated as per ICH guidelines [26]. To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments was carried out by standard addition method. From that total amount of drug found and percentage recovery was calculated. The results were reported in Table 1. Precision check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Five samples of the formulations were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated. The results were reported in Table 3.The intra and inter-day precision was calculated by assay of the sample solution on the same day and on different days at different time intervals respectively. The results are presented in Table 3.Short-term stability after 6 and 24 hrs at room temperature was studied to verify if analyses degrade over the course of analyses. Short-term stability can be evaluated by analyzing either working solutions or matrix-based samples added to working solutions and kept at room temperature before the extraction step. The working solutions were left at room temperature for at least 1h. The matrix-based samples added to working solutions were left at room temperature for 24h.Long-term stability was studied in order to be sure that analytes present in samples ("real" samples) do not degrade in the storage conditions before being analyzed.

RESULTS AND DISCUSSION

The effect of Indigo Carmine and methyl orange concentration on the reaction was checked out at room temperature and away from direct sunlight. This method utilizes the active analogue principle that lies at the spectroscopic method [22]. The reaction of Ceftriaxone sodium and tazobactum sodium was dependent on the concentration of dye used. A concentration of 0.5% (w/v) was selected as the optimum reagent concentration. Higher concentrations caused a distinct decrease in the absorbance.The absorbance of the solution was measured after 50 minutes after adding reagent and up to 2.5 hrs. The reactions was slow and the formed colour was stable up to 6 hrs.The developed method was found to be precise as the % RSD values for intraday and inter-day were found to be less than 2% . Recoveries (100.09 % to 101.1 %) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the % recoveries ranging from 99.87 % - 101.43 %. Sampling wavelengths were determined from scanning individual drug samples in 200-400 nm range. Sampling wavelengths were 247 nm and 260 nm for Ceftriaxone sodium and tazobactum sodium respectively in simultaneous estimation method. For this method equations generated were Y=-0.004372 +0.03162x (r^2=0.9997) and Y=-0.0651+0.0018x (0.9994) for Ceftriaxone sodium and tazobactum sodium respectively. 294 nm and 250 nm for Ceftriaxone sodium and tazobactum sodium respectively in Method B Derivative Spectroscopy. Linearity of proposed method was found to be 5-40 µg/ml for Ceftriaxone sodium and 5-25 µg/ml for tazobactum sodium. Limits of detection were found to be 0.265 and 0.036 µg/ml of Ceftriaxone sodium and tazobactum sodium respectively. Limits of quantitation were found to be 0.076 and 0.21 µg/ml for Ceftriaxone sodium and tazobactum sodium, respectively. Results of analysis were reported in Table 1, result of precision studies and recovery study reported in Tables 2 and 3, respectively. The statistical analysis of the experimental data the regression equation from the calibration graphs along with standard error of the slopes and intercepts and regression correlation coefficient for the ultraviolet spectroscopic method. The absorptivity were found approximately same for all the concentrations hence both drugs obeyed Beer's law in

Table 1: Optical Characteristics Data for Method-A and Method-B

	Ceftriaxone sodium		Tazobactum sodium	
Parameters / Working λ	247 nm	260 nm	294 nm	250 nm
Beer's law limit (µg/ml)	5-40	5-40	5-25	5-25
Absorptive E (1%,1cm)*	288	297	214	488
Molar absorptivity (l/mol.cm)*	8953	3771	6340	9842
Correlation coefficient*	0.9997	0.9999	0.9994	0.9988
Intercept*	0.0231	0.0216	0.0272	0.0761
Slope*	0.0564	0.0128	0.0287	0.0113

*Average of six estimation

Table 2: Analysis Data of Tablet Formulation, Statistical Validation and Recovery Studies

			Amt.found*					Amt. Added		
Method	Drug	Lab.claim (mg/tab)	mg/tab.	%	S.D.*	% COV	S.E*.	At (%)	mg/ml	% Rec.#
A	CS	1000	999.98	99.93	0.876	0.826	0.98	80	980	100.08
								100	1100	101.04
								120	1220	99.98
	TS	125	125.09	100.08	0.328	0.466	0.123	80	150	100.03
								100	280	99.22
								120	400	99.83
B	CS	1000	1000.12	100.08	0.0213	0.325	0.439	80	980	100.01
								100	1100	100.21
								120	1220	99.09
	TS	125	124.98	99.72	0.773	0.712	0.221	80	150	100.01
								100	280	100.41
								120	400	99.91

CS-Ceftriaxone sodium, TS- Tazobactum sodium S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation, # Average of three estimation at each level of recovery.

Table 3:Validation Parameters

				Precision (% COV)			
					Interday*		
Method	Drug	LOD*µg/ml	LOQ*µg/ml	Intraday n=6	First day	Second day	Third day
A	CS	0.2810	0.998	0.435	0.321	0.765	1.098
	TS	0.1832	1.832	1.143	1.124	0.987	0.772
B	CS	0.3281	0.329	0.321	0.488	0.694	1.126
	TS	0.2039	0.622	0.321	1.095	1.111	0.987

CS-Ceftriaxone sodium, TS- Tazobactum sodium S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation,

indicated concentration range. The high values of correlation coefficients (r^2) also indicate good linearity of calibration curve for both the drugs. The recoveries of Ceftriaxone sodium and tazobactum sodium from the standard mixture solution were found to be 101.21% and 99.89% respectively.

CONCLUSION

The proposed Spectrophotometric methods are indirect and are based on the determination of the residual bromine (*in situ* generated) after allowing the reaction between Ceftriaxone sodium and tazobactum sodium and a measured amount of bromine to be complete. The bromine was determined by reacting it with a fixed amount of methyl orange, indigo carmine dye. The methods make use of bleaching action of bromine on the dyes, the decolouration being caused by the oxidative destruction of the dyes. Ceftriaxone sodium and tazobactum sodium when added in increasing amounts to a fixed amount of *insitu* generated bromine consumes the latter proportionately and there occurs a concomitant falls in the amount of bromine. When a fixed amount of dye is added to decreasing amounts of bromine, a concomitant increase in the concentration of dye results.

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