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VALIDATED ION PAIR HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFTRIAXONE SODIUM AND TAZOBACTUM SODIUM IN DOSAGE FORM

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ABSTRACT

A simple, sensitive and rapid ionpair high performance liquid chromatographic methodwas developed for the estimation ceftriaxone sodium (CS) and tazobactum sodium (TS) in pharmaceutical dosage forms. Lichrocart[R]100-RP18e5µm-C18 column was used with a mobile phase containing mixture of 0.012M tetra butyl ammonium hydroxide in 0.01M potassium dihydrogen phosphate : acetonitrile in the ratio of 70:30 % v/v. The flow rate was 0.8ml/min and effluents were monitored at 220nm and eluted at 4.5 and 6.7 min for tazobactum sodium (TS) and ceftriaxone sodium (CS) respectively. Calibration curve was plotted with a range from 2 to 12 µg/ml (CS) and 0.26 to 1.56 (TS) µg/ml. The assay was validated for the parameters like accuracy, precision, robustness and syste m suitability parameters. The proposed method can be useful in the routine analysis for the determination of ceftriaxone sodium and tazobactum sodium in pharmaceutical dosage forms.

KEY WORDS

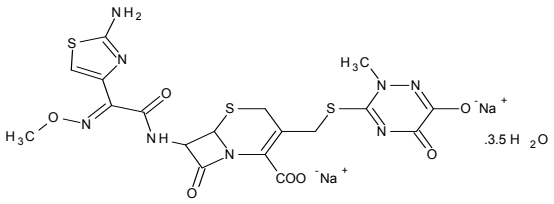
Ceftriaxone sodium, tazobactum sodium,ion-pair HPLC, Pharmaceutical dosage forms

INTRODUCTION

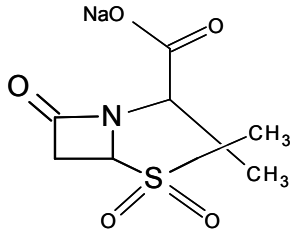
Ceftriaxone Sodium[1] is chemically known as 5-Thia-1-azabicyclo [4.2.0]oct-2-ene-2carboxylicacid, 7-[2-amino- 4thiazolyl) (methoxyimino) acetyl]amino]-8–oxo-3-3[[(1,2,5,6-tetrahydro-2methyl-5,6diaxo–1,2,4– triazin -3- yl) thio] methyl] - di sodium salt, [6R-[6α ,7β (2)]] – hydrate,2:7 (Fig 1). It is 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 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-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide (Fig 2). 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. Literature 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]. The objective of the present work was to develop a rapid and accurate HPLC method with UV detection in dosage forms. The developed method is simple, precise, sensitive and very useful for t he determination of ceftriaxone sodium and tazobactum sodium in pharmaceutical dosage forms.

Fig,1: Structure of Ceftriaxone Sodium



Fig,2:Structure of Tazobactum Sodium



MATERIALS AND METHODS

(i) Reagents : ceftriaxone sodium and tazobactum sodium obtained as gift sample from Orchid laboratories ltd, Chennai. Commercial powder for injection formulations zubacef (lyka labs ltd)containing 1g of ceftriaxone sodium and 125mg of tazobactum sodium were procured from local market.
HPLC grade acetonitrile, water was purchase d from Merck, Mumbai, India. Tetra butyl ammonium hydroxide (TBAH) from sigma Aldrich Bangalore, India. All other chemicals used were of HPLC grade.

(ii) Chromatographic equipment :
The HPLC system consisted of a Shimadzu C lass LC-10AT VP and LC-20AD pumps connected with SPD-10AV PDA detector. The data acquisition was performed by Spincotech 1.7 software.

(iii) Chromatographic conditions

Analysis was carried out at 220nm using a Lich rocart^{R}100RP18e,5µm,C18 at ambient temper ature. The mobile phase containing mixture of 0.012M tetra butyl ammonium hydroxide in 0.01M potassium dihydrogen phosphate : acetonitrile in the ratio of 70:30 % v/v, it was set at a flow rate of 0.8ml/min.
Thecolumn performance parameters for the method have been summarized in Table 1.

Table 1

Column performance parameters

Parameter	Result
Retention Time	4.5 (TS), 6.7(CS)
Resolution	3.5
Tailing factor	1.2 (TS), 1.4(CS)
No of theoretical plates	6724(CS), 8409(TS)

(iv)
Preparation of stock and sample solutions

The standard stock solution of the ceftriaxone sodium and tazobactum sodium was prepared using water in the concentration of 1 mg/ml of ceftriaxone sodium and 0.130 mg/ml of tazobactum sodium. The working standard solution of CS and TS was prepared by taking suitable aliquots of drug solution from the standard solution and the volume was made up to 10 ml with mobile phase to get concentrations of 2-12µg/ml and 0.26 to 1.56µg/mlrespectively.The solutions were filtered through 0.45µm membrane filter and then 20 µL of filtrate was injected into the column at flow rate of 0.8mL/min. Evaluation of drug was performed with PDA detector at 220nm.

Peak areas were recorded for all peaks. A plot of peak area versus the respective concentration gives the calibration curve. The regression of drug concentration over the peak area was computed. The regression was used to estimate the amount of CS and TS in powder for injection. A quantity of powder for injection (each contain 1000mg of ceftriaxone and 125mg of tazobactum) equivalent to 10mg of ceftriaxone sodium was weighed accurately. The formulation was dissolved in water and from this suitable aliquots of formulation solutions were prepared by using mobile phase.

Each of the solutions 20 µL was injected six times into the column. From thepeak areas, the drug content in the powder for injection was quantified using the regression equation obtained from pure sample

(v) Method validation

The method was validated by International Conference on Harmonization (ICH) guidelines for linearity, range, precision, specificity and accuracy parameters.

RESULTS AND DISCUSSION

An ionpair procedure was proposed as a suitable method for the determination of ceftriaxone sodium and tazobactum sodium from dosage form. The formulation chromatograms wereobtained by using the abovementioned mobile phase from 20 µL of the assay preparation and the retention time for ceftriaxone sodium and tazobactum sodium was 6.7 and 4.5min. The linearity of the method was tested from 2 to 12 µg/ml and 0.26 to 1.56 µg/ml.

Linearity solutions were injected in triplicate and the calibration graphs were plotted as peak area of the analyte aganist concentrations of the drug in µg/ml. They are shown in Table 2 below.

Table 2
Calibration Data Of Ceftriaxone

Concentration (µg/ml)	Peak Area
2	175221
4	211322
6	260664
8	321277
10	390574
12	460806

Table 3
Calibration Data Of Tazobactum

Concentration (µg/ml)	Peak Area
0.26	124826
0.52	244516
0.78	318424
1.04	407428
1.3	504954
1.56	603844

The calibration graph was found to be linear in the mentioned concentrations and the correlation coefficients for the regression line was 0.998 and 0.993 for CS and TS respectively The accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drug to the placebo. The recovery was determined at 100% of the selected concentration.

The recovery values ranged from 98-100% a

nd %RSD values are shown in Table 4.

Table 4
Recovery studies

Drug	% Recovery	%RSD
Ceftriaxone sodium	98.75	0.97
Tazobactum Sodium	99.82	1.28

The precision (repeatability and intermediate pr ecision) of the method was determined from on e lot of dosage form. Intra and Inter day studies were performed in six application of two concentrations. The results are shown in Table 5. The limit of detection (LOD) were found to be 50 ng/ml and 30 ng/ml for ceftriaxone sodium and tazobactum sodium and limit of quantitation (LOQ) was 500ng/ml and 100ng/ml respectively.

Table 5

Precision data of Ceftriaxone sodium Tazobactum sodium

Drug	Precision (% RSD*)		
	Intra day	Inter Day	Repeatability Of injection
Ceftriaxone sodium	0.978	0.751	0.197
Tazobactum sodium	0.36	0.392	0.150

Application of the method to pharmaceutical dosage forms

The method is sensitive and specific for the quantitative determination of ceftriaxone sodium and tazobactum sodium and also subjected to validation for different parameters hence has been applied for the estimation of drug in pharmaceutical dosage forms. Powder for Injection from manufacturers were evaluated for the amount of CS and TS present in the formulations. Each sample was i njected six times as mentioned above in experi mental section.The amount of CS

TS was found to be within the range of 99.6%-

100.1%and the results were shown in Table 6.

Table 6
Results of analysis of formulation

Drug	Amount (mg/vial)		%Label Claim	%RSD
	Labeled	Estimated		
Ceftriaxone sodium	1000	1001.52	100.15	0.87
Tazobactum sodium	125	124.52	99.62	0.53

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for deter mination of ceftriaxone sodium and tazobactum sodium from pure and in pharmaceutical dosage forms. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggest

ed non-interference of formulation excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of ceftriaxone sodium and tazobactum sodium from dosage forms and can also be used for dissolution
or similar studies.

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