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Development And Validation Of RP-HPLC Method For The Simultaneous Estimation Of Ceftazidime Sodium And Tazobactam Sodium In Marketed Formulation

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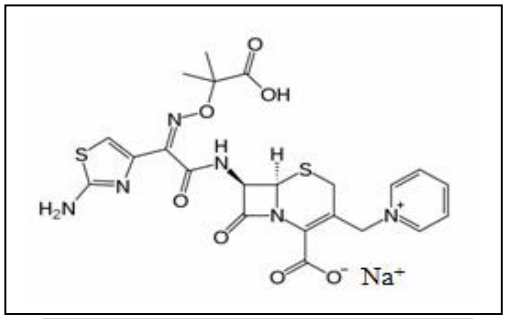
Abstract: A reverse phase high performance liquid chromatography (HPLC) method has been developed for the simultaneous determination of Ceftazidime Sodium and Tazobactam Sodium in pharmaceutical dosage form. HPLC was carried out on a BDS Hypersil C18 column (5 µm, 250 X 4.6 mm ID) using Acetonitrile: 0.02 M potassium dihydrogen phosphate buffer pH 3.5 with orthophosphoric acid (10% aqueous) with 2 drops of TEA in the ratio of 80:20 (v/v) as the mobile phase at a flow rate of 1.0 mL/min and eluents were monitored at 254 nm. The calibration curves were linear over the range of 50 – 200 µg/mL for Ceftazidime Sodium and 5-30 µg/mL for Tazobactam Sodium. The retention time of Ceftazidime Sodium and Tazobactam Sodium was found to be 3.0 min and 5.4 min respectively. The accuracy and precision of the methods were determined and validated statistically. All the methods showed good reproducibility and found to be rapid, specific, precise and accurate with % RSD less than 2. These methods can be successfully applied for the routine analysis of CEFTA and TAZO in bulk and combined dosage form.
Keywords: Reverse Phase High Performance Liquid Chromatography; Ceftazidime Sodium; Tazobactam Sodium.

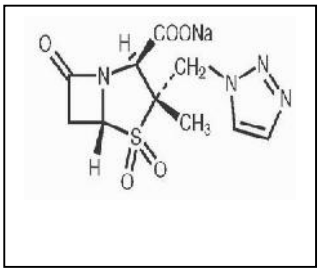
Introduction

Ceftazidime Sodium (CEFTA) is sodium salt of (1-{[(6R,7R)-7-[(2Z)-2-(2amino-1,3-thiazol-4-yl)-2-[(1-carboxy methylethoxy)imino]acetamido]-2-carboxylato-8-oxo-5-thia-1 azabicyclo[4.2.0] oct-2-en-3-yl]methyl} pyridin-1-ium).[1,2,3] It is official in IP,USP & BP. It is an approved semisynthetic, broad-spectrum antibacterial derived from cephaloridine and it is widely used especially for Pseudomonas and other gram-negative infections in debiliated patients [4]. It is used in the treatment of Biliary tract infection, Bone & joint infection, Endometriosis, GI infections, Intra-abdominal infection, Lower respiratory tract infection and Urinary tract infection. [5,6] Ceftazidime Sodium alone or in combination with other drugs has been reported by various spectrophotometric methods. [7, 8, 9, 10] Analysis has been carried out using RP-HPLC methods for single as well as in combination with other drugs. [11,12,13,14] Tazobactam Sodium (TAZO) is sodium salt of (2S,3S,5R)-3-methyl-4,4,7-trioxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4S/l{6}-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid [15]. TAZO is not official in any pharmacopoeia. Some Spectroscopic [16] as well as RP-HPLC methods [17, 18, 19, 20] have been reported for Tazobactam as single as well as in combination with other drugs. It is an antibacterial penicillin derivative which inhibits the action of bacterial beta-lactamases. Cephalosporins are destroyed by a family of enzymes called beta-lactamases, which hydrolyze the four member beta-lactam ring. Tazobactam inhibits these enzymes and shows synergistic antimicrobial effect. Various combinations of Ceftazidime &

Tazobactam are available in the market. The proposed method is optimized and validated as per the international conference on harmonization (ICH) guidelines (Q2B) [21]



(a) CEFTAZIDIME SODIUM



(b) TAZOBACTAM SODIUM

Fig.1 Chemical structure of CEFTA (a) and TAZO (b).

Experimental

Materials and Methods

Analytically pure samples of CEFTA (Hindustan Antibiotics Limited, Pimpri, Pune, India) and TAZO (Gensen Laboratories, Mumbai) were used in the study. The pharmaceutical Fixed dose combination dry powder injection vial containing 1000 mg CEFTA and 125mg TAZO (8:1) were procured from Abbott Healthcare Pvt. Ltd. Mumbai. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

Lachrom HPLC quaternary gradient system (Make: Merck-Hitachi) with L-7100 double reciprocating pump and L-7400 UV detector was used. Chromatographic data was acquired using Winchrome software. A reversed-phase BDS Hypersil C18 column (5 µm, 250 X 4.6 mm ID) was used for separation

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Selection of analytical wavelength

By using appropriate dilutions of the standard stock solutions with the mobile phase containing Acetonitrile and 0.02 M potassium dihydrogen phosphate solution (80:20 % v/v), various concentrations of CEFTA and TAZO were prepared separately and their overlain spectra were obtained using the Shimadzu UV visible spectrophotometer 1700, in the spectrum mode between the wavelength ranges of 400 nm to 200 nm. From the overlain spectra, it was observed that CEFTA and TAZO exhibited significant absorbance at about 254.0 nm which was selected as the analytical wavelength for further analysis.

Chromatographic conditions

Hypersil C18 column (250 mm X 4.6 mm i.d.) was used as stationary phase. Acetonitrile and 0.02 M potassium dihydrogen phosphate buffer in the ratio of 80:20 % v/v was used as mobile phase and was filtered before use through 0.4 µ membrane filter. A constant flow of 1.0 ml/min was maintained throughout the analysis. Detection was carried out using UV detector at 254 nm. To ascertain the suitability of the proposed chromatographic conditions, system suitability tests were carried out and the results are shown in Table 1. Chromatogram of standard solution containing CEFTA and TAZO is shown in Fig.2.

Table 1: System suitability parameters

Sr. No.	Parameter	CEFTA	TAZO
1	Retention time (min)	3.0	5.4
2	Tailing factor (T)	1.125	1.10
3	Resolution (R_s)	2.4	
4	No. of theoretical plates (N)	5760	2960

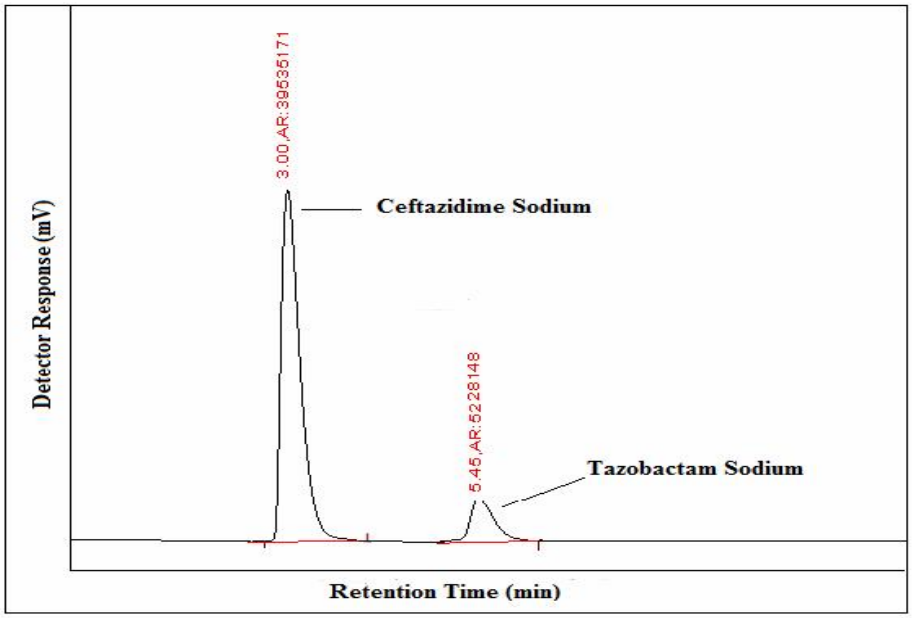

Fig.2: Typical chromatogram of standard CEFTA and TAZO

Preparation of standard stock solutions

About 20 mg of Ceftazidime Sodium and 10 mg Tazobactam Sodium weighed and transferred to 100 ml volumetric flasks respectively. It was dissolved in the mobile phase. Then, solutions were made up to mark with same mobile phase to obtain stock solutions of concentration 200 µg mL^{-1} of Ceftazidime Sodium and 100 µg mL^{-1} Tazobactam Sodium each.

Analysis of mixed standards

Pure standards of Ceftazidime Sodium and Tazobactam Sodium in the concentration ratio of (8:1) were prepared and analyzed under the optimized chromatographic conditions. The results of the analysis of pure mixed standards are given in Table 2.

Table 2: Results of analysis of the mixed standards by HPLC method

Sr. No.	Amount present (µg/ml)		Area under curve* (AUC)		% of drug found*	
	CEFTA	TAZO	CEFTA	TAZO	CEFTA	TAZO
1.	160	20	39535171	5236142	100.05	99.71

*Average of six determinations

Analysis of the marketed formulation

A powder sample equivalent to 16 mg of Ceftazidime Sodium was weighed, transferred to a 100 mL volumetric flask and dissolved in mobile phase.The solutions were sonicated for 20 mins to allow for dissolution of the active components and the volume was made up to the mark with the mobile phase. Solution was mixed and filtered through 0.2 µ filter paper. Appropriate dilutions were made and the concentrations of Ceftazidime Sodium and Tazobactam Sodium in the sample solutions were determined by the proposed method. The appropriate dilutions were prepared in triplicate, 20 µl volume of each sample solution was injected twice into the sample injector of RP-HPLC under the optimized chromatographic conditions. The area of each peak was measured at selected wavelength. The amount of each drug present in the injection sample was determined using the prepared standard calibration curves of Ceftazidime Sodium and Tazobactam Sodium Fig. No.3 represents the chromatogram of Ceftazidime Sodium and Tazobactam Sodium in injection formulation. The results of analysis of injection formulation are given in Table No.3.

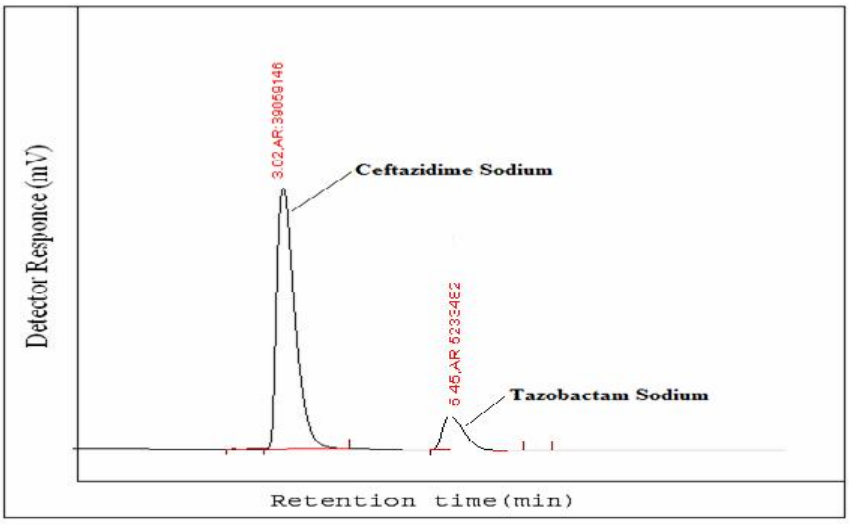

Fig.3: HPLC chromatogram of CEFTA and TAZO in Injection formulation

Table 3: Statistical validation of injection formulation for HPLC method

Drugs	Mean Content (%)*	S.D.*	% R.S.D.*
CEFTA	99.84	0.1233	0.123
TAZO	99.46	0.2581	0.259

Results And Discussion

For HPLC Method development initially various mobile phases were tried in an attempt to obtain the best separation and resolution between CEFTA and TAZO. The mobile phase consisting of Acetonitrile : 0.02 M potassium dihydrogen phosphate buffer in the ratio of 80:20 % v/v was selected which gave satisfactory separation and gave two well resolved peaks for CEFTA and TAZO as shown in Fig.2. CEFTA and TAZO exhibit significant absorbance at wavelength 254 nm. Hence, 254nm was selected as detection wavelength for their simultaneous determination. The retention times for CEFTA and TAZO were 3.0 min and 5.4 min respectively.

Method Validation

The developed method was validated as per ICH guidelines[17] for the simultaneous assay determination of CEFTA and TAZO using following parameters –

Linearity (Calibration Curve)

Aliquots of standard stock solutions were appropriately diluted with mobile phase to obtain concentration range of 50-200 µg/ml for CEFTA and 5-30 µg/ml for TAZO. The diluted standard solutions with varying concentrations were injected (in triplicate) into the HPLC system separately and chromatographed under above mentioned chromatographic conditions. Chromatographic peaks were recorded at 254 nm using UV detector. The calibration curves of mean peak area versus concentration were plotted. The regression coefficient (R^2) for calibration curve of CEFTA and TAZO was 0.998 and 0.999 respectively (Table 4).

Table 4: Regression analysis of the calibration curves for CEFTA and TAZO for the proposed HPLC method

Parameters	CEFTA	TAZO
Linearity Range (µg/ml)	50-200	5-30
Slope* ± S.D.	24752±2585.0	25442±822.11
Intercept* ± S.D.	32280±982.13	785.2±94.0
Regression Co-efficient (R^2)*	0.998	0.999
LOD (µg/ml)	0.1309	0.0121
LOQ (µg/ml)	0.3967	0.0369

*Average of six determinations

Accuracy (% Recovery)

Accuracy of the developed method was confirmed by doing a recovery study as per ICH guidelines at three different concentration levels (80%, 100% and 120%) by replicate analysis (n=3). Standard drug solutions were added to a preanalyzed sample solution, and then percentage of drug content was calculated. The results of the accuracy study are reported in Table 5. From the recovery study, it was clear that the method is very accurate for quantitative estimation of CEFTA and TAZO in injection formulation because all the statistical results were within the acceptance range (i.e., % RSD <2.0).

Table 5: Result of recovery data for HPLC method

Level of % Recovery	% Mean Recovery*		S. D. *		% R.S.D.*	
	CEFTA	TAZO	CEFTA	TAZO	CEFTA	TAZO
80	99.53	99.24	0.1724	1.096	0.1732	1.104
100	99.67	98.80	0.1803	0.400	0.1809	0.404
120	99.81	99.14	0.0900	0.5554	0.0901	0.5602

*Average of three determinations

Method Precision

Intermediate Precision (Reproducibility)

The Intra and inter-day precision were determined by repeating the assay three times for six replicated dilutions of the same concentrations after every two hours on the same day for intraday precision. The assay was performed with the same sample solutions after 24 hours to understand interday precision assay of the sample solution at different time intervals and on different days respectively. The results of the same are given in (Table 6).

Table 6: Result of precision studies for HPLC method

Drugs	Intra-day Precision*			Inter-day Precision*		
	Mean % content	S.D.	% R.S.D.	Mean % content	S.D.	% R.S.D.
CEFTA	99.74	0.105	0.105	99.86	0.079	0.079
TAZO	99.39	0.228	0.229	99.59	0.347	0.349

*Average of six determinations

LOD and LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.
LOD = 3.3 x [σ]/S
LOQ = 10 x [σ]/S
Where σ = the standard deviation of the response and S = the standard deviation of y- intercept of regression lines.

LOD for CEFTA and TAZO was found to be 0.1309 µg/ml and 0.0121 µg/ml and LOQ was found to be 0.3967 µg/ml and 0.0369 µg/ml respectively (Table 4)

Robustness

To evaluate the robustness of the method, each parameter selected was varied at different levels. The results presented in Table 7 indicate the selected factors.

Table 7: Results of robustness testing for HPLC method

Chromatographic changes						
Flow Rate (ml/min)	**Retention time**		**Tailing factor**		**% Content**	
	CEFTA	**TAZO**	**CEFTA**	**TAZO**	**CEFTA**	**TAZO**
0.9	3.7	5.9	1.21	1.12	98.91	100.10
1.0	3.0	5.6	1.125	1.1	99.82	99.68
1.1	2.6	5.1	1.25	1.15	98.92	99.98
Mean ± S.D.	3.1 ± 0.5568	5.53 ± 0.4041	1.195 ± 0.0638	1.123 ± 0.02517	99.21 ± 0.5224	99.92 ± 0.2163
% of ACN in the mobile phase (v/v)						
82	3.34	4.90	1.22	1.140	98.63	99.11
80	3.0	5.6	1.25	1.1	99.72	99.64
78	2.92	4.63	1.23	1.120	98.96	99.77
Mean ± S.D.	3.08 ± 0.2230	5.04 ± 0.5006	1.191 ± 0.0579	1.12 ± 0.020	99.10 ± 0.5590	99.30 ± 0.3496
pH of Phosphate Buffer solution						
3.3	2.95	5.49	1.241	1.120	99.81	99.64
3.5	3.0	5.6	1.125	1.1	99.91	99.89
3.7	2.97	4.90	1.22	1.130	99.61	99.73
Mean ± S.D.	2.97 ± 0.0251	5.33 ± 0.3764	1.194 ± 0.0618	1.116 ± 0.0153	99.77 ± 0.1528	99.75 ± 0.1266

Conclusion

As literature survey reveals that one RP-HPLC method[20] has been developed on the same combination. In the reported method, mobile phase was composed of 18% methanol: 82% Phosphate buffer with longer retention time for CEFTA (9.3 min) and TAZO (11.4 min).The proposed method has a different mobile phase

composition (80% Acetonitrile: 20% Phosphate buffer) and shorter retention times of the components CEFTA (Rt-3.0 min) and TAZO (Rt-5.4 min).

Hence the proposed chromatographic method has advantage over the previously reported method and is suitable fot separation and quantitation of CEFTA and TAZO with good resolution, peak shapes and minimum tailing. The peak areas of the drugs were reproducible as indicated by low relative standard deviation indicating the repeatability of the proposed method. Both the drugs were found to give linear detector response in the concentration range under study with correlation coefficient of 0.998 for CEFTA and 0.999 for TAZO. The sample recoveries from the formulation were in good agreement with their respective label claim. The method exhibited good selectivity and sensitivity. Percent recoveries for CEFTA and TAZO were 99.53-99.81 % and 98.80-99.24 %, respectively indicating accuracy of the proposed method. %RSD for injection analysis, recovery studies and intra-day & inter-day precision studies is less than 2. LOD for CEFTA and TAZO was found to be 0.1309 µg/ml and 0.0121 µg/ml and LOQ was found to be 0.3967 µg/ml and 0.0369 µg/ml respectively The results of robustness study also indicated that the method is robust and is unaffected by small deliberate variations in the method parameters. The proposed method was validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed method is low, indicating high degree of precision of the method. The results of the recovery studies performed show the high degree of accuracy of the proposed method.

Hence, it can be concluded that the developed RP-HPLC method is accurate, precise, selective and can be employed successfully for the estimation of CEFTA and TAZO in bulk and in pharmaceutical dosage form.

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