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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD FOR SIMULTANEOUS ESTIMATION OF CEFTRIAXONE AND TAZOBACTAM INJECTION USING RP-UPLC METHOD

Vipul J. Panchal*, Hemant T. Desai and Ashwin G. Dave

Nirlife Healthcare (Healthcare Division of Nirma Limited), Sachana, Ahmedabad- 382 150, Gujarat, India

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Correspondence to Author:

Vipul Jayantilal Panchal

Senior Quality Assurance Officer, B-203, Ashirwad Residency, Nr. Navyug School, Opp. Shivdarshan Society, Naroda, Gujarat, India

E-mail: vipulj.2012@rediffmail.com

ABSTRACT: This research manuscript describes simple, sensitive, accurate, precise and repeatable RP- UPLC method for the simultaneous determination of Ceftriaxone (CEF) and Tazobactam (TAZ) Injection in combine dosage form. The sample was analyzed by reverse phase C18 column (Acquity UPLC BEH 100 × 2.1 mm ID, 1.7 µm) with mobile phase. In mobile phase, Solution A containing Potassium Dihydrogen Phosphate buffer (pH adjusted to 6.5±0.2 with Orthophosphoric acid), Citric acid buffer (pH adjusted to 5.0±0.2 with NaOH solution) and Acetonitrile and Solution B containing Tetradecyl ammonium bromide, Tetraheptyl ammonium bromide and Acetonitrile in the flow rate of 0.3 ml/min. Quantification was achieved 230 nm with PDA detector. The retention time for Ceftriaxone and Tazobactam was found to be 2.83 and 1.72 minute respectively. The linearity for Ceftriaxone and Tazobactam was obtained in the concentration range of 40-280 µg/ml and 5-35 µg/ml respectively. Ceftriaxone and Tazobactam API and market formulation were subjected to acid and alkali hydrolysis, oxidation, thermal and photolytic forced degradation. The peak purity of drug substance and drug product peak also confirmed the specificity of the methods with respect to the degradation products. In the forced degradation study Ceftriaxone and Tazobactam showed maximum degradation in base hydrolysis stress study followed by less degradation in thermal degradation. The developed method was simple, specific, sensitive, rapid, and economic and can be used for estimation of Ceftriaxone and Tazobactam in bulk and their combined dosage form for routine analysis and stability studies.

INTRODUCTION: Ceftriaxone Sodium (**Fig. 1**) is chemically known as 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid,7-[[2-amino-4-thiazolyl) (methoxyimino)acetyl]amino]-8–oxo-3[[(1,2,5,6-tetrahydro-2-methyl-5-,6-diaxo-1,2,4–triazin-3-yl)thio]methyl]-, disodium salt, [6R-[6α,7β (Z)]]-, hydrate, 2:7.

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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 (**Fig. 2**) is chemically known as (2S,3S,5R)-3-methyl-7-oxo-3- (1H-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo [3.2.0] heptanes-2-carboxylic acid 4,4-dioxide, Sodium salt. 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. Ceftriaxone Sodium is listed in Indian Pharmacopoeia [1], British Pharmacopoeia [2] and United State Pharmacopoeia[3].

Tazobactam Sodium is not official in any pharmacopoeia. Literatures survey reveals Spectroscopic [4]; ion-pair HPLC [5], and RP-HPLC [6, 7, 8] methods have been reported as a single as well as combination with other drugs. However, there is no work was reported for the simultaneous estimation of these drugs by RP-UPLC method. Hence, in the present study an attempt has been made to develop simple, and accurate, sensitive, precise and repeatable RP-UPLC method, for the simultaneous estimation of both drugs in dry powder for injection dosage form.

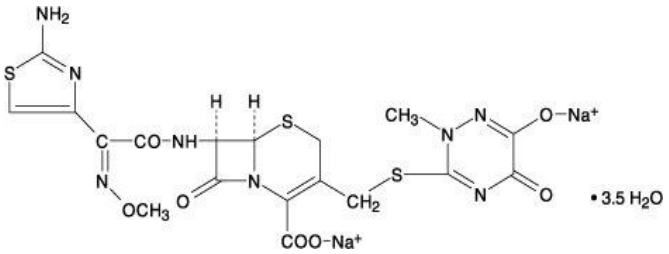

FIG. 1: STRUCTURE OF CEFTRIAXONE SODIUM

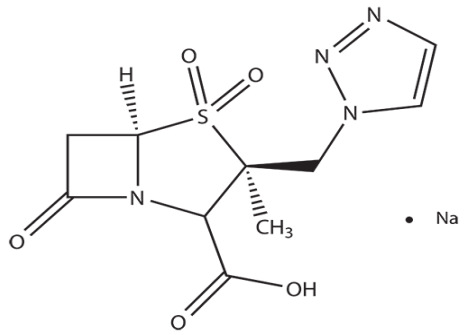

FIG. 2: STRUCTURE OF TAZOBACTAM SODIUM

MATERIALS & METHODS:

Apparatus: The chromatography was performed on a Waters (Acquity) RP-UPLC instrument equipped with PDA detector and Em-power 2 software, Acquity UPLC BEH C18 column (100 mm × 2.1 mm ID, 1.7 µm) was used as stationary phase. Mettler Toledo analytical balance (Germany), an ultrasonic cleaner (Frontline FS 4, Mumbai, India) and Whatmann filter paper No. 41 (Whatman International Ltd., England) were used in the study.

Reagents and materials: Ceftriaxone and Tazobactam bulk powder was obtained from Nirlife, Healthcare division of Nirma Ltd. Ahmedabad, India. The commercial fixed dose combination product was procured from the Nirlife. Acetonitrile (HPLC grade, Finar Reagent, Ahmedabad, India), Potassium di-hydrogen ortho-phosphate anhydrous (AR, Finar Reagent, Ahmedabad, India), Disodium hydrogen phosphate anhydrous (AR, Finar Reagent, Ahmedabad, India), Citric acid monohydrate (AR, Finar Reagent, Ahmedabad, India), Tetradecyl ammonium bromide (HPLC Grade, Molychem, Ahmedabad, India), Tetraheptyl ammonium bromide (HPLC grade, Finar Reagent, Ahmedabad, India), Sodium hydroxide (AR, Finar Reagent, Ahmedabad, India), Orthophosphoric acid (AR, Finar Reagent, Ahmedabad, India), used were of HPLC grade was used in the study.

Chromatographic condition: In this work, we used reverse phase Acquity UPLC BEH C18 column (100 mm × 2.1 mm ID, 1.7 µm), Waters) as stationary phase and using a mobile phase. In Mobile phase, Solution A containing Potassium Dihydrogen Phosphate buffer (pH adjusted to 6.5±0.2 with Orthophosphoric acid), Citric acid buffer (pH adjusted to 5.0±0.2 with NaoH solution) and Acetonitrile and Solution B containing Tetradecyl ammonium bromide, Tetraheptyl ammonium bromide and Acetonitrile. Volume of solution A and Solution B taken in the ratio 65:35 (v/v) for mobile phase, in the flow rate of 0.3 ml/min.

Preparation of mobile phase:

- **Solution A:** Accurately weighed and dissolved about 3.5 gm of Potassium di-hydrogen ortho-phosphate anhydrous and 14.5 gm of Disodium hydrogen phosphate anhydrous in 1000 ml of water for pH 6.5 Buffer solution. pH of 6.5±0.2 was adjusted by using diluted orthophosphoric acid. Accurately weighed and dissolved about 20.5 gm of Citric acid in 1000 ml of water for pH 5.0 Buffer solution. pH of 5.0±0.2 was adjusted by using NaOH solution. Water, pH 6.5 buffer, pH 5.0 buffer and acetonitrile taken in the ratio 600:180:20:200 (v/v) and mix well.

- **Solution B:** Accurately weighed and dissolved 4.0 gm of Tetradecyl ammonium bromide and 4.0 gm of Tetraheptyl ammonium bromide in 500 ml of acetonitrile sonicated to dissolve and made up to 1000 ml with acetonitrile and mix well.

- **Mobile phase:** Volume of solution (A) and Solution (B) taken in the ratio 65:35 (v/v) and mixed well and filter through 0.45 µm membrane filter and degas for 10 minutes.

Preparation of standard stock solutions: An accurately weighed Ceftriaxone (40 mg) and Tazobactam (5 mg) were transferred to 100 ml volumetric flask, dissolved in 50 ml with Mobile phase and diluted up to mark with Mobile phase to get 400 µg/ml solution of Ceftriaxone and 50 µg/ml solution of Tazobactam.

Method Validation: The method was validated in compliance with ICH guidelines [9].

Preparation of calibration curve: Aliquots (of 1,2,3,4,5,6,7 ml) of mixed standard working solutions (equivalent to 40,80,120,160,200,240,280 ppm of Ceftriaxone and 5,10,15,20,25,30,35 ppm of Tazobactam) were transferred in a series of 10 ml volumetric flasks, and the volume was made up to the mark with Mobile phase. Each solution was injected under the operating chromatographic condition as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentration, and the regression equations were calculated (**Table 1** and **Table 2**) and (**Fig. 3** and **Fig. 4**). Each response was average of three determinations.

TABLE 1: LINEARITY OF CEFTRIAXONE

Concentration (ppm)	Average Area	SD	% RSD
40	390859	641.9	0.164
80	782138	867.8	0.111
120	1172548	1189.4	0.101
160	1563607	1827.0	0.117
200	1955229	2143.1	0.110
240	2346153	3222.5	0.137
280	2713442	4001.7	0.147

TABLE 2: LINEARITY OF TAZOBACTAM

Concentration (ppm)	Average Area	SD	% RSD
5	4242	10.3	0.242
10	8549	30.3	0.354
15	12648	45.9	0.363
20	16932	20.3	0.120
25	21183	47.9	0.226
30	25284	30.8	0.122
35	29070	10.0	0.034

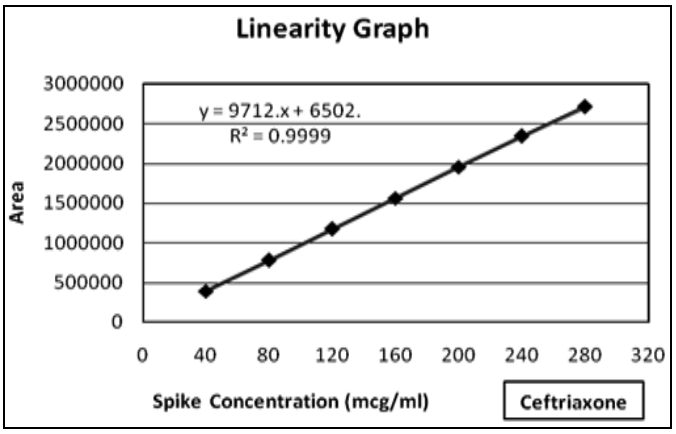

FIG. 3: LINEARITY OF CEFTRIAXONE

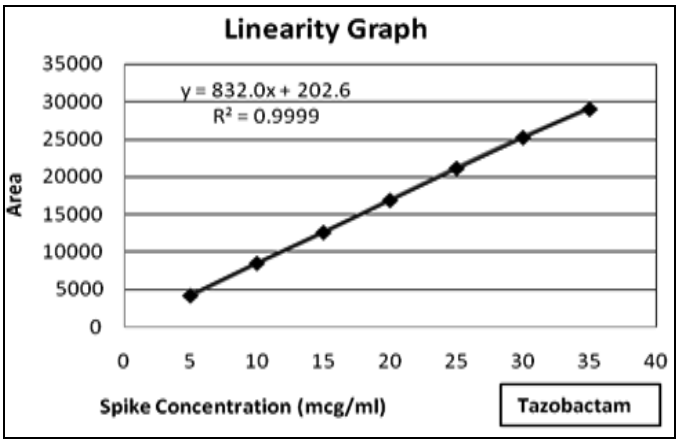

FIG. 4: LINEARITY OF TAZOBACTAM

Accuracy (recovery study): The accuracy of the method was determined by calculating the recoveries of Ceftriaxone and Tazobactam by the standard addition method. Known amounts of standard solutions of Ceftriaxone and Tazobactam were at added at 80, 100 and 120 % level to pre-quantified sample solutions of Ceftriaxone sodium equivalent to Ceftriaxone 400 µg/ml and Tazobactam 50 µg/ml. The amounts of Ceftriaxone and Tazobactam were estimated by applying obtained values to the respective regression line equations (**Table 3**).

Method precision (Repeatability): The precision of the instrument was checked by repeatedly injecting (n=6) solutions of Ceftriaxone and Tazobactam (400 µg/ml and 50 µg/ml respectively) without changing the parameters (**Table 3**).

Intermediate precision (reproducibility): The intraday and inter day precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week

for 3 different concentrations of standard solutions of Ceftriaxone sodium equivalent to Ceftriaxone (200, 400, and 600 µg/ml) and Tazobactam (25, 50 and 75 µg/ml). The results were reported in terms of relative standard deviation (% RSD) (**Table 3**).

TABLE 3: SUMMARY OF VALIDATION PARAMETER FOR CEF AND TAZ

Parameters	RP-UPLC method	
	Ceftriaxone	Tazobactam
Concentration range (ppm)	40-280	5-35
Slope	9712	832
Intercept	6502	202.6
Correlation coefficient	0.9999	0.9999
LODa (µg/ml)	0.005	0.125
LOQb (µg/ml)	0.017	0.416
Repeatability (% RSDd, n=6)	0.258	0.210
Precision (% RSD)		
Inter day (n=3)	0.080-0.345	0.045-0.321
Intraday (n=3)	0.055-0.136	0.055-0.090
Accuracy (% RSDd)	0.045-0.075	0.033-0.085

a= Limit of detection, b= Limit of quantification, n= number of determinations, d= Relative standard deviation

System suitability: The parameters used in system suitability test were asymmetry of the chromatographic peak, peak resolution and theoretical plates, as % RSD of peak area for replicate injections (**Table 4**).

TABLE 4: SYSTEM SUITABILITY TEST PARAMETERS FOR CEF AND TAZ

Parameters	CEF ± % RSD	TAZ ± % RSD
Retention Time (min)	2.828±0.112	1.718±0.074
Tailing Factor	1.48±0.509	1.19±0.462
Theoretical Plates	8681±0.428	11738±0.492
Resolution	11.20±0.087	

Preparation of Marketed sample solution for Assay: For determination of the content of Ceftriaxone and Tazobactam in dry powder for injection; take about 55 mg (Ceftriaxone sodium equivalent to Ceftriaxone 40 mg and Tazobactam sodium equivalent to Tazobactam 5 mg) of powder and transferred to 100 ml volumetric flask, dissolved in Mobile phase (50 ml) sonicated for 30 min and dilute up to the mark with Mobile phase. The solution was filtered through Whatmann filter paper No. 41 and residue was washed with Mobile phase. The solution was diluted up to the mark with Mobile phase to get final working concentration of Ceftriaxone sodium equivalent to Ceftriaxone (400 µg/ml) and Tazobactam sodium equivalent to Tazobactam (50 µg/ml). A sample solution was injected under the operating chromatographic condition as described above and responses were recorded (**Fig. 5**) and (**Table 5**). The analysis procedure was repeated three times with dry powder for injection formulation.

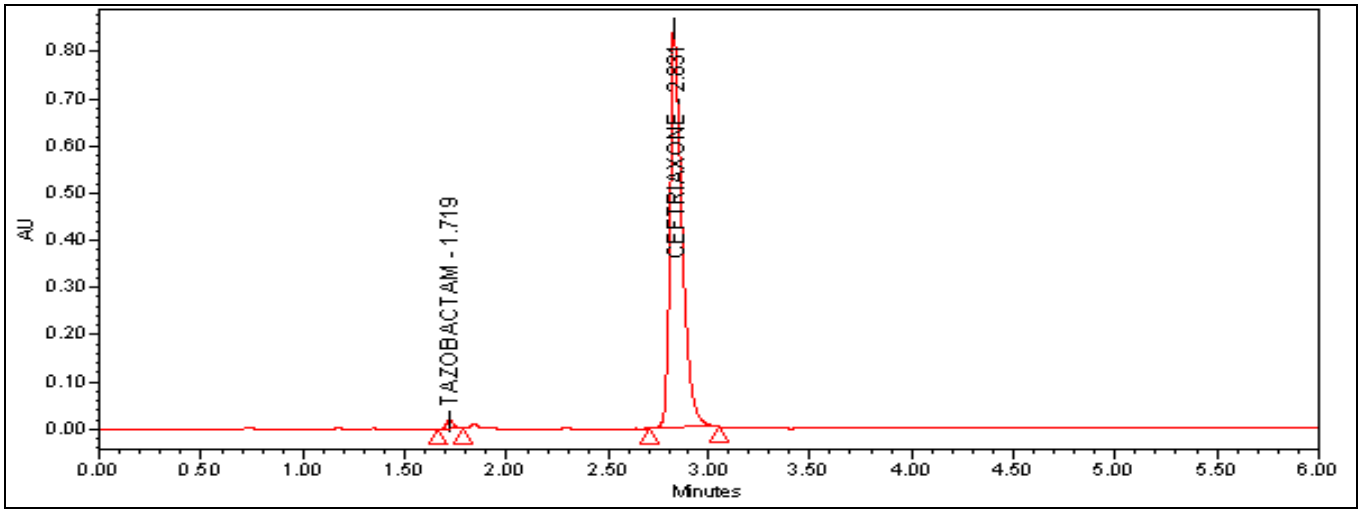

FIG. 5: OPTIMIZED CONDITION CHROMATOGRAM OF ASSAY OF DRUG

TABLE 5: ANALYSIS OF MARKETED FORMULATION OF CEF AND TAZ

Injection	Label Claim 1125 mg/Vial		Amount Found		% Label Claim ± % RSD (n=3)	
	CEF	TAZ	CEF	TAZ	CEF	TAZ
1	1000 MG	125 MG	999.6	124.9	99.96 ±0.015	99.92 ±0.031

RESULTS AND DISCUSSION: To optimize the RP-UPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Ceftriaxone and Tazobactam were obtained with a mobile phase. In moble phase, Solution A containing Potassium Dihydrogen Phosphate buffer (pH adjusted to 6.5±0.2 with Orthophosphoric acid), Citric acid buffer (pH adjusted to 5.0±0.2 with NaoH solution) and Acetonitrile and Solution B containing Tetradecyl ammonium bromide, Tetraheptyl ammonium bromide and Acetonitrile at a flow rate of 0.3 ml/min to get better reproducibility and repeatability.

Quantification was achieved with PDA detection at 230 nm based on peak area. The retention time for Ceftriaxone and Tazobactam were found to be 2.83 and 1.72 min, respectively (**Fig. 5**). Linear correlation was obtained between peak area versus concentrations of Ceftriaxone and Tazobactam in the concentration ranges of concentration range of 40-280 µg/ml and 5-35 µg/ml are r^2=0.9999 and r^2=0.9999 and mean accuracies 99.96 ± 0.015 % and 99.92 ± 0.031 % for Ceftriaxone and Tazobactam (**Table 5**), which indicates accuracy of the proposed method.

The % RSD values for Ceftriaxone and Tazobactam were found to be < 2 %, which indicates that the proposed method is repeatable. The low % RSD values of repeatability of assay (0.258-0.210 %), inter day (0.080-0.345 % and 0.045-0.321 %) and intraday (0.055-0.136 % and 0.055-0.090 %) variations for Ceftriaxone and Tazobactam, respectively, reveal that the proposed method is precise. LOD values for Ceftriaxone and Tazobactam were found to be 0.005 µg/ml and 0.125 µg/ml, respectively and LOQ values for Ceftriaxone and Tazobactam were found to be 0.017 µg/ml and 0.416 µg/ml, respectively (**Table 3**). These data show that the proposed method is sensitive for the determination of Ceftriaxone and Tazobactam. The results of system suitability testing are given in (**Table 4**).

Degradation study of Ceftriaxone and Tazobactam in 0.1N HCl at 70°C for 4 hours in reflux condition: Ceftriaxone and Tazobactam peak was observed at retention time 2.831 min and 1.719 min respectively (**Fig. 6**). The % drug degradation observed of Ceftriaxone and Tazobactam was 24.00 % and 11.62 % respectively (**Table 6**). From this it is observed that Ceftriaxone showed maximum degradation in Acid hydrolysis degradation condition.

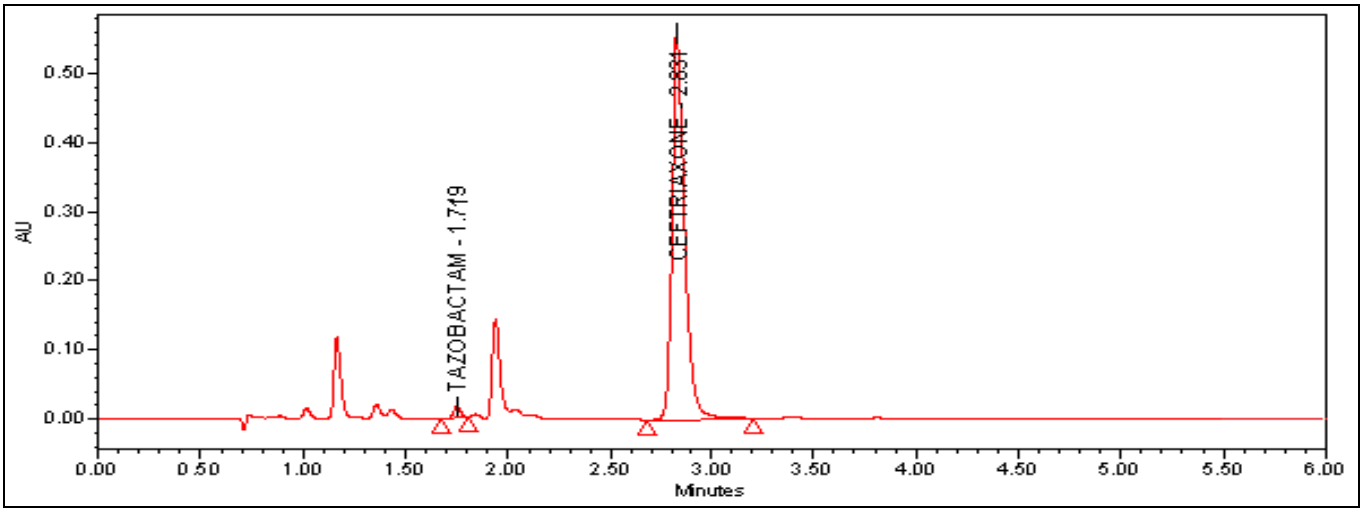

FIG. 6: ACID HYDROLYSIS OF CEFTRIAXONE AND TAZOBACTAM

Degradation study of Ceftriaxone and Tazobactam in 0.1N NaOH at 70°C for 4 hours in reflux condition: Ceftriaxone and Tazobactam peak was observed at retention time 2.739 min and 1.711 min respectively (**Fig. 7**). The % drug degradation observed of Ceftriaxone and Tazobactam was 16.84 % and 27.60 % respectively (**Table 6**). From this it is observed that Tazobactam showed maximum degradation in base hydrolysis degradation condition.

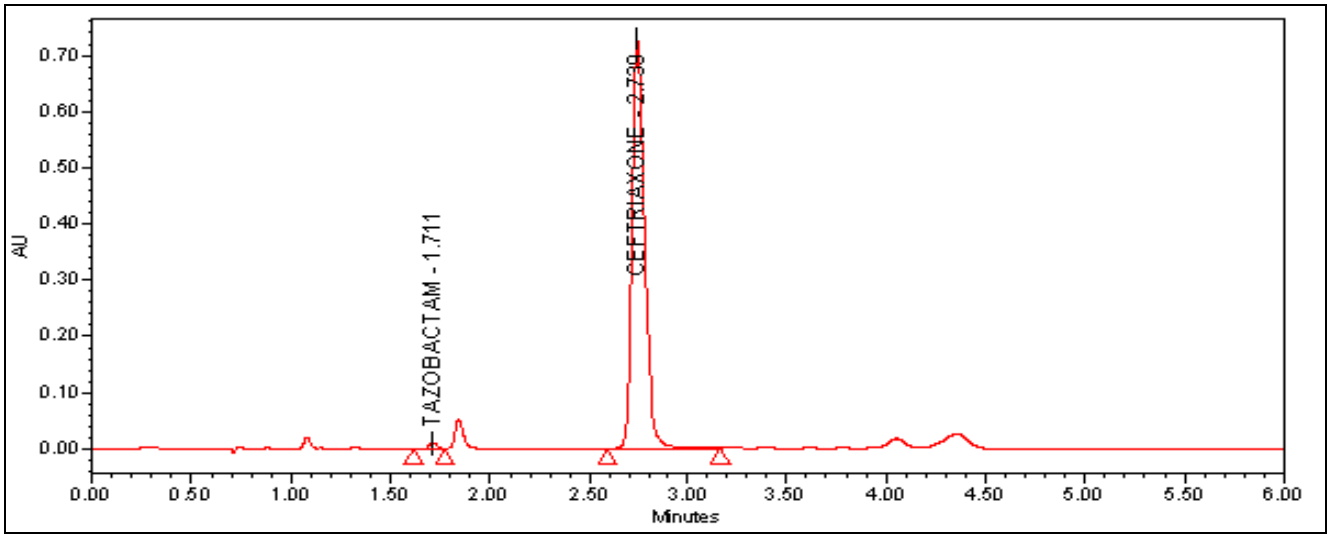

FIG. 7: BASE HYDROLYSIS OF CEFTRIAXONE AND TAZOBACTAM

Oxidation degradation study of Ceftriaxone and Tazobactam in 2 % H_2O_2 at 70°C for about 1 hour in reflux condition: Sample and drug substances were treated with 2 % solution of hydrogen peroxide and kept in water bath at 70°C in reflux condition for about 1 hour. It showed a peak of degradation product. Ceftriaxone and Tazobactam peak was observed at retention time 2.739 min and 1.711 min respectively (**Fig. 8**). The % degradation observed of Ceftriaxone and Tazobactam was 13.40 % and 7.78 % respectively (**Table 6**).

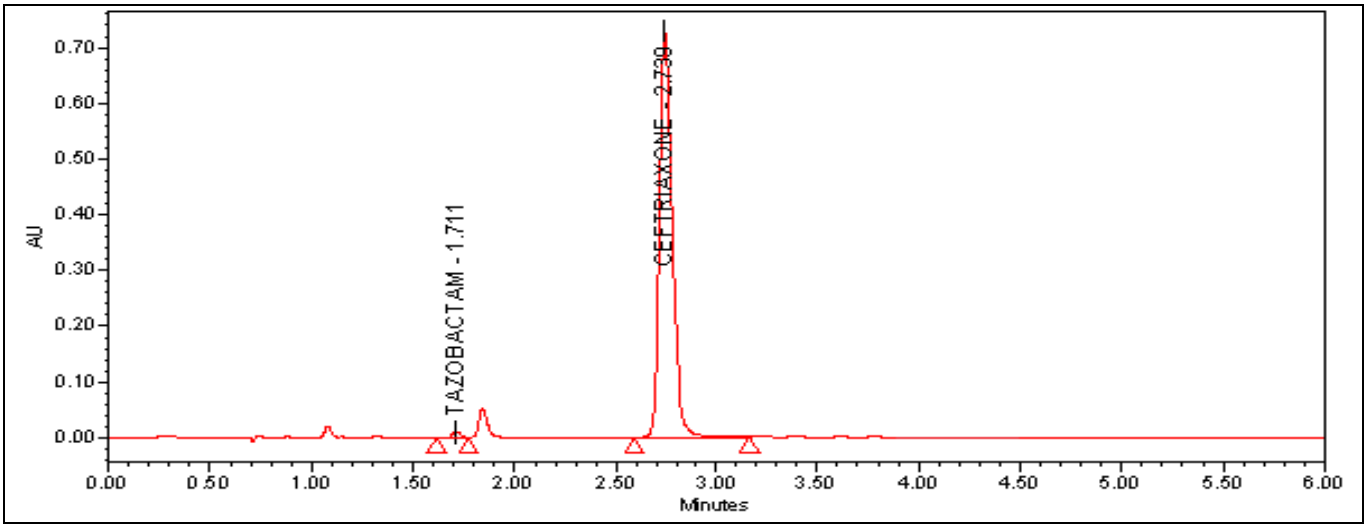

FIG. 8: OXIDATION OF CEFTRIAXONE AND TAZOBACTAM

Thermal Degradation study of Ceftriaxone and Tazobactam at 60°C for about 24 hrs: Thermal degradation of Ceftriaxone and Tazobactam at 60°C for about 24 hrs in hot air oven was carried out. There was no degradation peak found in thermal degradation chromatogram because there was lower degradation found in thermal degradation study. %Degradation of Ceftriaxone and Tazobactam was found to be 0.71 % and 0.53 % respectively (**Fig. 9** and **Table 6**).

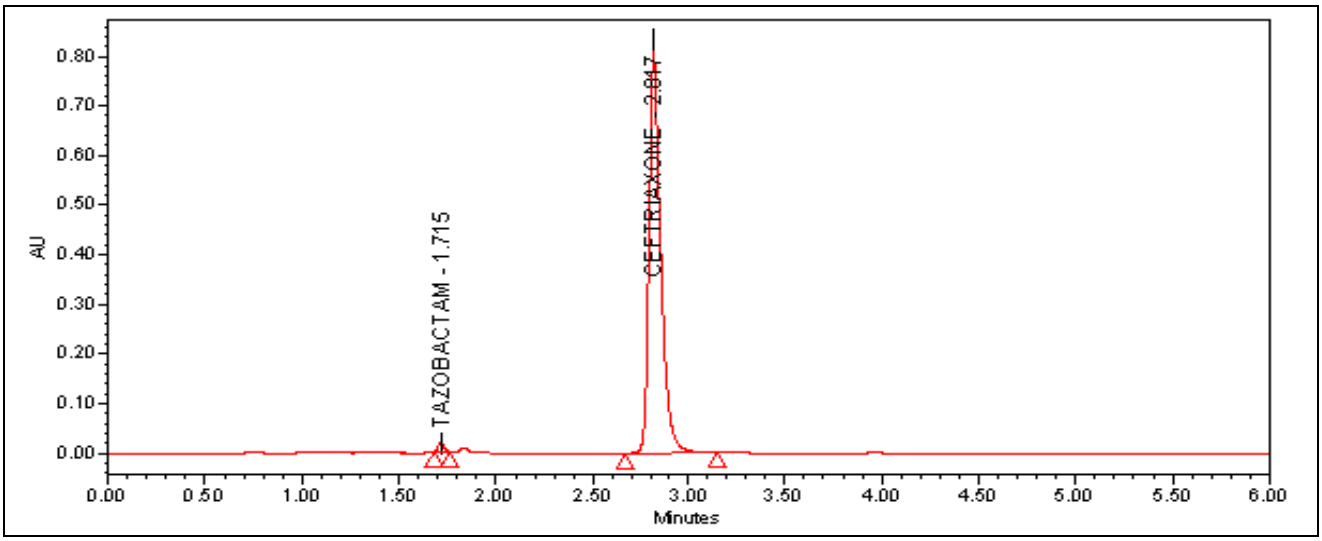

FIG. 9: THERMAL DEGRADATION OF CEFTRIAXONE AND TAZOBACTAM

Photolytic Degradation study of Ceftriaxone and Tazobactam: Sample and drug substances were exposed to energy of 1.2 million lux hrs fluorescent light and 200 w/m^2 of UV for about 7 days. % degradation of Ceftriaxone and Tazobactam was found to be 6.47 % and 3.35 % respectively (**Fig. 10** and **Table 6**).

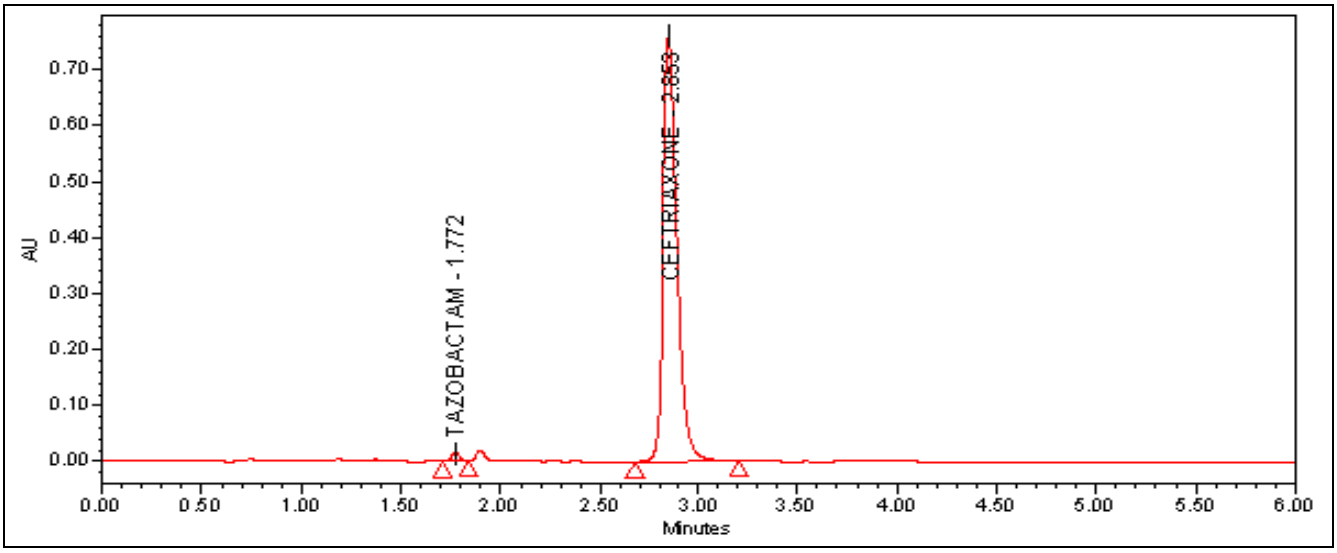

FIG. 10: PHOTO STABILITY OF CEFTRIAXONE AND TAZOBACTAM

TABLE 6: %DEGRADATION OF CEFTRIAXONE AND TAZOBACTAM IN DIFFERENT CONDITIONS

Degradation condition	Area		Concentration In mcg/ml		% Potency		% Degradation	
	CEF	TAZ	CEF	TAZ	CEF	TAZ	CEF	TAZ
Acidic/ 0.1N HCl/ 70°C/Reflux /4hr/ Solution	3946757	42940	399.84	49.96	99.96	99.92	24.00	11.62
	2945456	35918	303.84	44.15	75.96	88.30		
Alkaline/0.1N NaOH/70°C/Reflux/4 hr/Solution	3946757	42940	399.84	49.96	99.96	99.92	16.84	27.60
	3223288	29412	332.48	36.16	83.12	72.32		
Oxidative/2% H_2O_2/70°C /Reflux/ 1hr/ Solution	3946757	42940	399.84	49.96	99.96	99.92	13.40	7.78
	3356452	37473	346.24	46.07	86.56	92.14		
Thermal/60°C/24 hr/ Solid	3978274	43271	399.92	49.94	99.98	99.88	0.71	0.53
	3880191	40718	397.08	49.67	99.27	99.35		
Photo/1.2 million lux hrs fluorescent light/200w/m^2 of UV/7 days	3978274	43271	399.92	49.94	99.98	99.88	6.47	3.35
	3654856	39564	374.04	48.26	93.51	96.53		

CONCLUSION: Stability indicating RP-UPLC methods for estimation of Ceftriaxone and Tazobactam in their combine dosage form was established and validated as per the ICH guidelines. The forced degradation study and peak purity data confirmed that there was no merging between peaks of active ingredients and any other degradation products as well as other additives. Hence the specificity of the proposed method was established.

The linearity of developed method was achieved in the range of 40-280 µg/ml for Ceftriaxone (r^2=0.9999) and 5-35 µg/ml for Tazobactam (r^2=0.9999). The percentage recovery of drug was achieved in the range of 98-101 % which was within the acceptance criteria. The percentage RSD was NMT 2 % which proved the precision of the developed method. Different degradation products were found for drug product in acidic, alkaline, oxidative, thermal and photolytic force degradation.

Peak of Degraded products were not interfering with the main drug peak of Ceftriaxone and Tazobactam. Thus, these degradation products have not been identified.

The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust. Hence it can be used for the routine analysis of Ceftriaxone and Tazobactam in their bulk and combine dosage form in quality control laboratory and stability studies.

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