IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 4, Issue 12 (December 2014), PP. 53-60

Development and Validation of Stability Indicating Method for Simultaneous Estimation of Cefepime and Tazobactam Injection using RP-UPLC Method

Panchal Vipul J*[1], Desai Hemant T[1], Patel Nirav B[1], Panchal Kalpesh B[1],
[1]*Nirlife Healthcare (Healthcare Division of Nirma Limited), Sachana, Ahmedabad, Gujarat, India-382150,*

ABSTRACT: *This research manuscript describes simple, sensitive, accurate, precise and repeatable RP-UPLC method for the simultaneous determination of Cefepime (CEFE) 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 Cefepime and Tazobactam was found to be 0.68 and 1.69 minute respectively. The linearity for Cefepime and Tazobactam was obtained in the concentration range of 40-280 µg/ml and 5-35 µg/ml respectively. Cefepime 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 Cefepime 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 Cefepime and Tazobactam in bulk and their combined dosage form for routine analysis and stability studies.*

Keywords: *Cefepime, Tazobactam, Method validation, RP-UPLC, Forced degradation.*

I. INTRODUCTION

Cefepime (Figure-1) is chemically 1-[[(6R,7R)-7-[2-(2-amino-4-thiazolyl)-glyoxylamido]- 2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride, 72-(Z)-(O-methyloxime), mono-hydrochloride, monohydrate. It is a fourth generation, β-lactamase resistant parenteral cephalosporin with broad spectrum of activity against many Gram-positive and Gram-negative bacteria. Tazobactam (Figure- 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. Cefepime Hydrochloride is listed in the Indian Pharmacopoeia [1], British Pharmacopoeia [2] and United State Pharmacopoeia [3]. Tazobactam Sodium is not official in any pharmacopoeia. Literatures survey reveals Spectroscopic [4] and HPLC [5, 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.

II. MATERIALS & METHODS

2.1 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.

2.2 Reagents and materials

Cefepime 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 market. 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.

2.3 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.

2.4 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.

2.5 Preparation of standard stock solutions

An accurately weighed Cefepime (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 Cefepime and 50 µg/ml solution of Tazobactam.

2.6 Method Validation

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

2.7 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 Cefepime 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 (Figure 3 and Figure 4). Each response was average of three determinations.

2.8 Accuracy (recovery study)

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

2.9 Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of Cefepime and Tazobactam (400 µg/ml and 50 µg/ml respectively) without changing the parameters.

2.10 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 Cefepime Hydrochloride equivalent to Cefepime (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).

2.11 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)

2.12 Preparation of Marketed sample solution for Assay

For determination of the content of Cefepime and Tazobactam in dry powder for injection; Take about 88 mg (Cefepime Hydrochloride equivalent to Cefepime 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 Cefepime Hydrochloride equivalent to Cefepime (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 (Figure 5) and (Table 5). The analysis procedure was repeated three times with dry powder for injection formulation.

III RESULTS AND DISCUSSION

To optimize the RP-UPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Cefepime and Tazobactam were obtained with 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 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 Cefepime and Tazobactam were found to be 0.68 and 1.69 min, respectively (Figure 5). Linear correlation was obtained between peak area versus concentrations of Cefepime 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 99.97 ± 0.017 % and 99.93 ± 0.030 % for Cefepime and Tazobactam (Table 5), which indicates accuracy of the proposed method. The % RSD values for Cefepime and Tazobactam were found to be < 2 %, which indicates that the proposed method is repeatable. The low % RSD values of repeatability of assay (0.215-0.675 %), inter day (0.041-0.253 % and 0.044-0.175 %) and intraday (0.040-0.171 % and 0.069-0.181 %) variations for Cefepime and Tazobactam, respectively, reveal that the proposed method is precise. LOD values for Cefepime and Tazobactam were found to be 0.010 µg/ml and 0.125 µg/ml, respectively and LOQ values for Cefepime and Tazobactam were found to be 0.033µg/ml and 0.416 µg/ml, respectively (Table 3). These data show that the proposed method is sensitive for the determination of Cefepime and Tazobactam. The results of system suitability testing are given in (Table 4).

3.1 Degradation study of Cefepime and Tazobactam in 0.1N HCl at 70°C for 4 hours in reflux condition.

Cefepime and Tazobactam peak was observed at retention time 0.680 min and 1.691 min respectively (Figure 6). The % drug degradation observed of Cefepime and Tazobactam was 27.36 % and 10.33 % respectively (Table 6). From this it is observed that Cefepime showed maximum degradation in Acid hydrolysis degradation condition.

3.2 Degradation study of Cefepime and Tazobactam in 0.1N NaOH at 70°C for 4 hours in reflux condition.

Cefepime and Tazobactam peak was observed at retention time 0.681 min and 1.695 min respectively (Figure 7). The % drug degradation observed of Cefepime and Tazobactam was 15.95 % and 26.49 % respectively (Table 6). From this it is observed that Tazobactam showed maximum degradation in base hydrolysis degradation condition.

3.3 Oxidation degradation study of Cefepime 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. Cefepime and Tazobactam peak was observed at retention time 0.694 min and 1.772 min respectively (Figure 8). The % degradation observed of Cefepime and Tazobactam was 12.50 % and 6.22 % respectively (Table 6).

3.4 Thermal Degradation study of Cefepime and Tazobactam at 60°C for about 24 hrs.

Thermal degradation of Cefepime and Tazobactam at 60°C for about 24 hrs in hot air oven was carried out. There was no degradation peak found because there was lower degradation found in thermal degradation study. % Degradation of Cefepime and Tazobactam was found to be 0.79 % and 0.50 % respectively (Figure 9 and Table 6).

3.5 Photolytic Degradation study of Cefepime 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 Cefepime and Tazobactam was found to be 8.04 % and 4.18 % respectively. (Figure 10 and Table 6).

IV CONCLUSION

Stability indicating RP-UPLC methods for estimation of Cefepime 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 Cefepime (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 Cefepime 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 Cefepime and Tazobactam in their bulk and combine dosage form in quality control laboratory and stability studies.

ACKNOWLEDGEMENT

The authors are thankful to Nirlife HealthCare, Ahmedabad, India for providing a Sample and facilities for research.

REFERENCES

[1]. *Indian Pharmacopeia,* (The Indian pharmacopeia commission, Indian pharmacopeia laboratory government of India, Ministry of Health & Family welfare, Sector 23, Raj nagar, Ghaziabad-201002, 2014) 1302-1305.

[2]. *British Pharmacopeia,* (British Pharmacopeia Commission, expert Advisory groups, panels of experts and working parties, 2014) 438-440.

[3]. *United State Pharmacopeia,* (United State Pharmacopeia Convention, Rockville, 2013) 2855-2858.

[4]. Navin K. Khare, Rabindra K. Nanda, Raymond M. Lawrence and Dipak A. Navathar, Development and validation of Spectrophotometric Methods for Simultaneous estimation of Cefepime and Tazobactam in combined dosage form by area under curve and Q-Analysis, *International Journal of Institutional Pharmacy and Life Sciences, 2(2)*, 2012, 1-8.

[5]. N. Sunitha, L. Sindhura, B. Thangabalan and S. Manohar Babu, Development and Validation of RP–HPLC Method for Simultaneous Estimation of Cefepime and Tazobactam in Injection Formulation, *Asian J. Pharm Ana, 3(4),* 2013, 131-137.

[6]. K. Neelima, Y. Rajendra Prasad, N. Appalraju, S. Selina and R. Nikhila, Analytical Method Development and Validation of Cefepime Hydrochloride and Tazobactam Sodium in Bulk and Sterile Dry Powder for Injection by Gradient RP-HPLC, *Indo American Journal of Pharmaceuticals Science, 3(10),* 2013, 8400-8407.

[7]. M. Bhavana, T. Ramamohana Reddy, M. Sandhya and V. Uma Maheswara Rao, RP-HPLC Method Development and Validation for Simultaneous estimation of Cefepime and Tazobactam in Marketed formulation, *International Journal of Pharmacy, 3(4),* 2013, 837-842.

[8]. Nanda Rabindra K. and Shelke Ashwini V, Development and Validation of RP-HPLC Method for The Simultaneous Estimation of Ceftazidime Sodium and Tazobactam Sodium in Marketed Formulation, *International Journal of PharmTech Research, 5(3),* 2013, 983-990.

[9]. ICH, Q2 (R1), Harmonised tripartite guideline, *Validation of analytical procedures: text and methodology*, International Conference on Harmonization ICH, Geneva, Nov 2005.

Table 1: Linearity of Cefepime

Concentration (ppm)	Average Area	SD	% RSD
40	314604	1871.2	0.595
80	628777	2664.9	0.424
120	947253	821.3	0.087
160	1245976	1124.3	0.090
200	1559023	5283.9	0.339
240	1885578	3313.4	0.176
280	2191689	4724.9	0.216

Table 2: Linearity of Tazobactam

Concentration (ppm)	Average Area	SD	% RSD
5	4957	45.1	0.910
10	9707	64.6	0.666
15	14928	49.6	0.333
20	19691	30.4	0.155
25	24980	50.6	0.205
30	30020	49.9	0.168
35	35119	101.2	0.288

Table 3: Summary of validation parameter for CEFE and TAZ

Parameters	RP-UPLC method	
	Cefepime	Tazobactam
Concentration range (ppm)	40-280	5-35
Slope	7818.4	1008.3
Intercept	2324.3	252.24
Correlation coefficient	0.9999	0.9999
LODa (µg/ml)	0.010	0.125
LOQb (µg/ml)	0.033	0.416
Repeatability (% RSDd, n=6)	0.215	0.675
Precision (% RSD)		
Inter day (n=3)	0.041-0.253	0.044-0.175
Intraday (n=3)	0.040-0.171	0.069-0.181
Accuracy (% RSDd)	0.066-0.085	0.038-0.079

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

Table 4: System suitability test parameters for CEFE and TAZ

Parameters	CEFE ± % RSD	TAZ ± % RSD
Retention Time (min)	0.680±0.080	1.697±0.030
Tailing Factor	1.68±0.243	1.21±0.455
Theoretical Plates	3119±0.184	11348±0.174
Resolution	17.51±0.087	

Table 5: Analysis of marketed formulation of Cefepime and Tazobactam

Injection	Label Claim		Amount Found		% Label Claim ± % RSD (n=3)	
	1125 mg/Vial					
	CEFE	TAZ	CEFE	TAZ	CEFE	TAZ
1	1000 MG	125 MG	999.7	124.9	99.97 ±0.017	99.93 ±0.030

Table 6: %Degradation of Cefepime and Tazobactam in different conditions

Degradation condition	Area		Concentration In mcg/ml		% Potency		% Degradation	
	CEFE	TAZ	CEFE	TAZ	CEFE	TAZ	CEFE	TAZ
Acidic/ 0.1N HCl/ 70°C/Reflux /4hr/ Solution	3131947	48946	399.88	49.96	99.97	99.93	27.36	10.33
	2232751	44387	290.44	44.80	72.61	89.60		
Alkaline/0.1N NaOH/70°C/Reflux/4 hr/ Solution	3131947	48946	399.88	49.96	99.97	99.93	15.95	26.49
	2552124	36383	336.08	36.72	84.02	73.44		
Oxidative/2% H_2O_2/Reflux/70°C /1hr/ Solution	3131947	48946	399.88	49.96	99.97	99.93	12.50	6.22
	2656899	46426	349.88	46.85	87.47	93.71		
Thermal/60°C/24 hr/ Solid	3148455	50366	399.92	49.94	99.98	99.88	0.79	0.50
	3028620	50084	396.76	49.69	99.19	99.38		
Photo/1.2 million lux hrs fluorescent light/200w/m^2 of UV/7 days	3148455	50366	399.92	49.94	99.98	99.88	8.04	4.18
	2807355	48230	367.76	47.85	91.94	95.70		

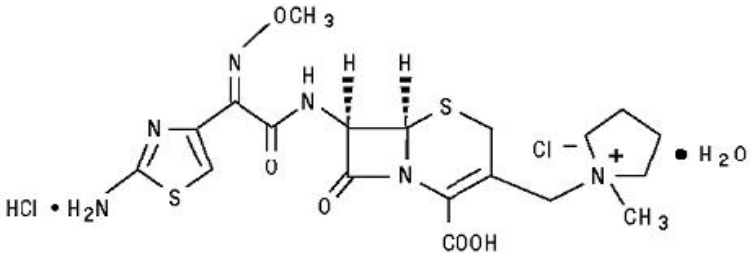

Figure 1: Structure of Cefepime Hydrochloride

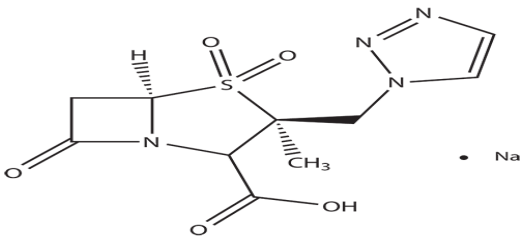

Figure 2: Structure of Tazobactam sodium

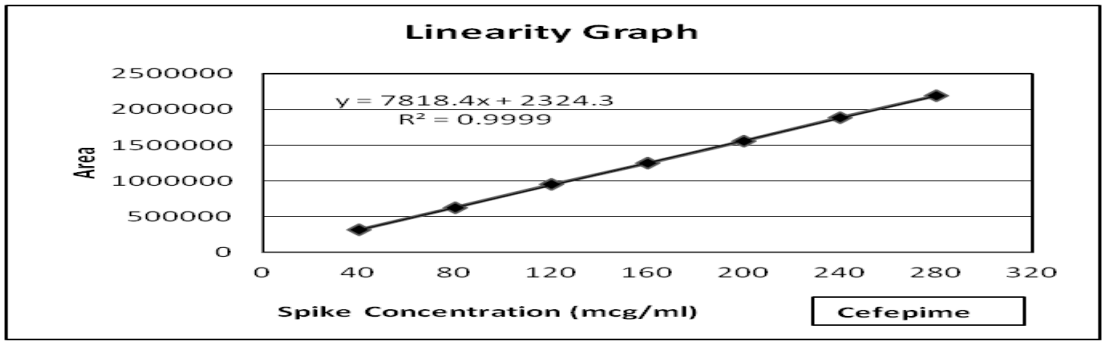

Figure 3: Linearity of Cefepime

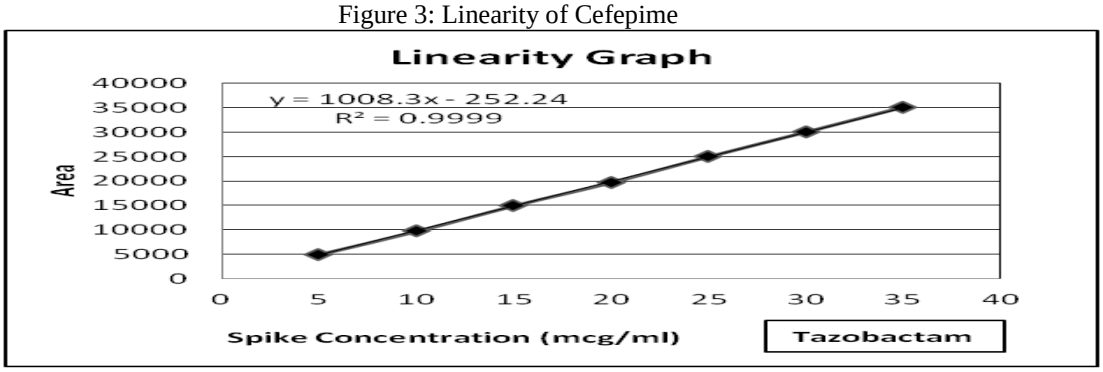

Figure 4: Linearity of Tazobactam

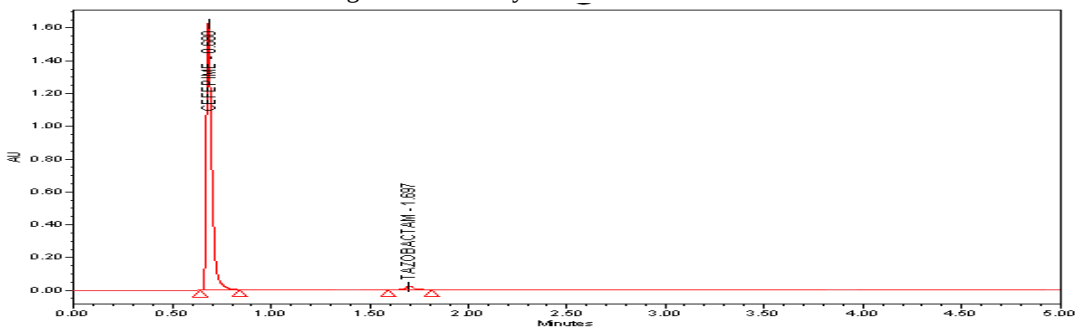

Figure 5: Optimized condition chromatogram of Assay of Drug

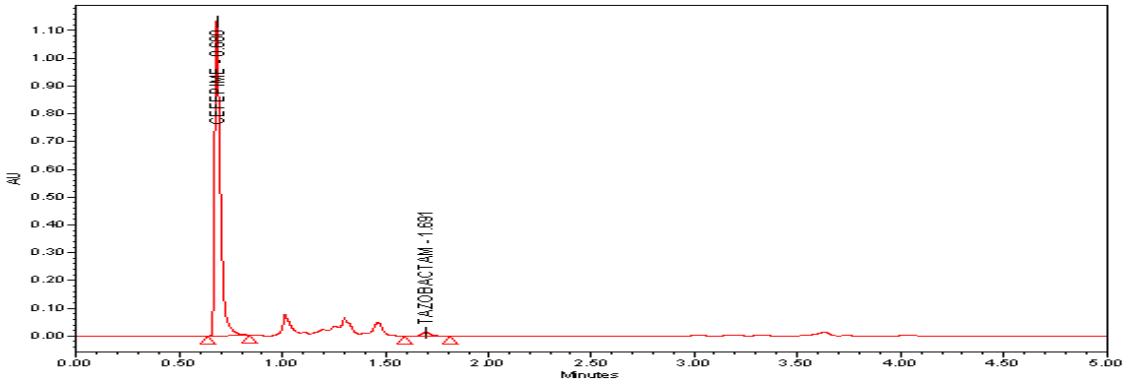

Figure 6: Acid hydrolysis of Cefepime and Tazobactam

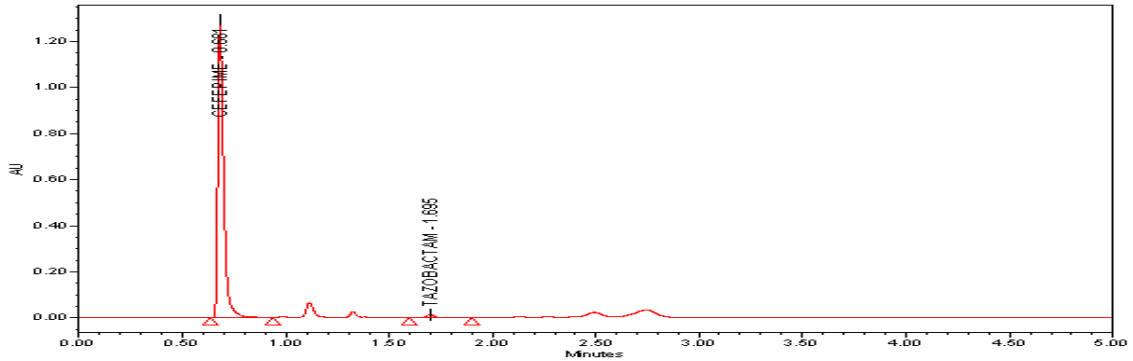

Figure 7: Base hydrolysis of Cefepime and Tazobactam

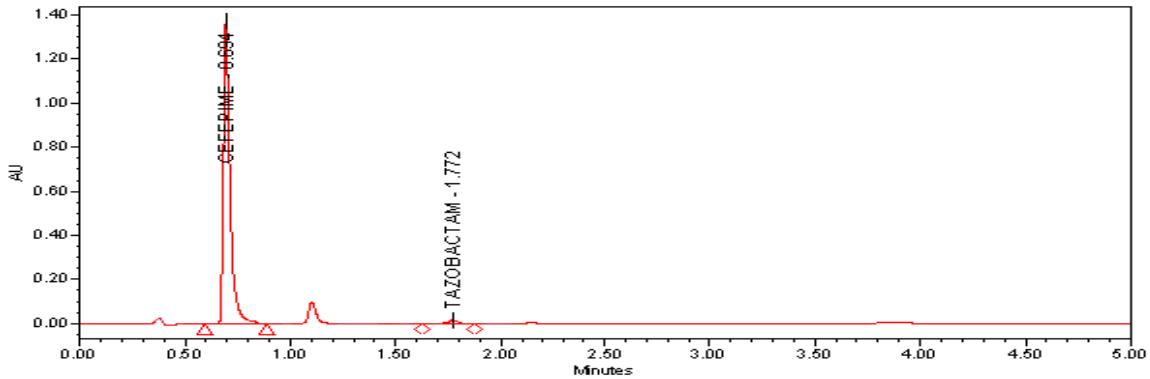

Figure 8: Oxidation of Cefepime and Tazobactam

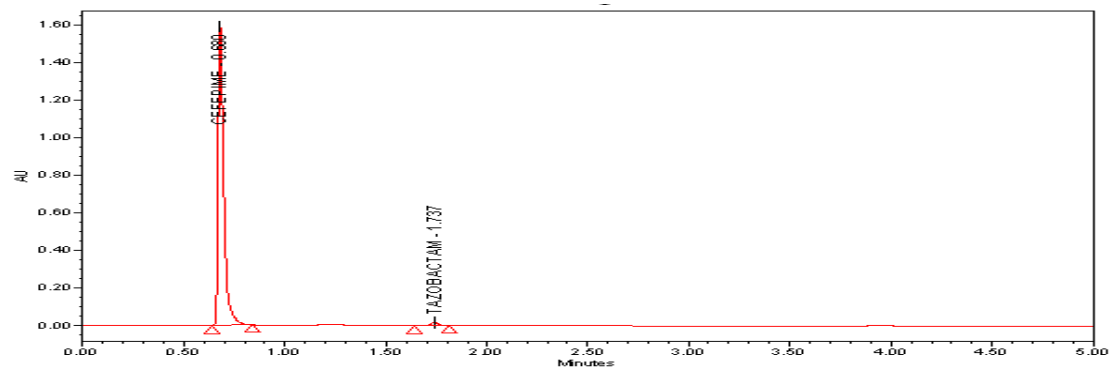

Figure 9: Thermal degradation of Cefepime and Tazobactam

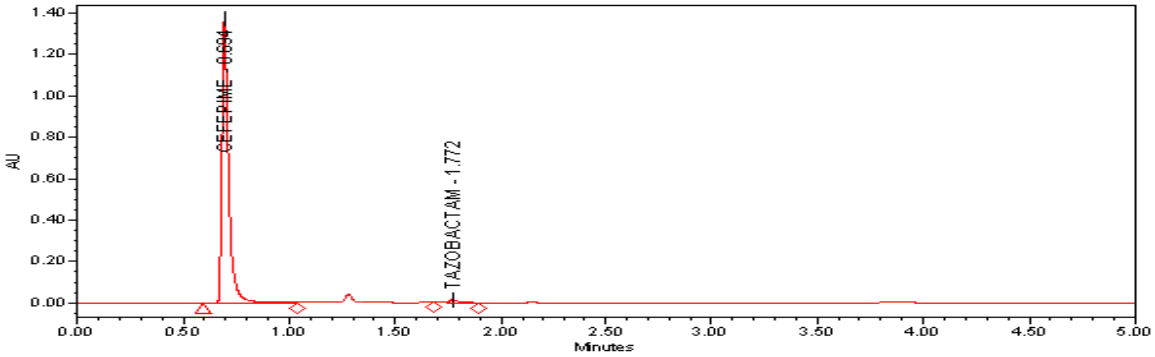

Figure 10: Photo stability of Cefepime and Tazobactam