STABILITY INDICATING FAST LC METHOD FOR DETERMINATION OF CEFTRIAXONE AND TAZOBACTAM FOR INJECTION RELATED SUBSTANCES IN BULK AND PHARMACEUTICAL FORMULATION

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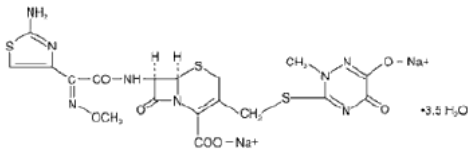
ABSTRACT: An LC method has been developed and subsequently validated for the determination of Ceftriaxone and Tazobactam for injection and its related substances in bulk and pharmaceutical formulation. Separation was achieved in isocratic mode using Kromasil, C18, 250 x 4.6 mm, 5 µm column with mobile phase A containing Potassium Dihydrogen Phosphate buffer (pH adjusted to 6.5±0.05 with Orthophosphoric acid),Citric acid buffer (pH adjusted to 5.0±0.05 with NaoH solution)and Acetonitrile and mobile phase B containing Tetradecyl ammonium bromide ,Tetraheptyl ammonium bromide and Acetonitrile at different time intervals as eluent at a flow rate 0.8ml/min. UV detection was performed at 230nm.The method is simple, selective and stability indicating .The described method is accurate and linear over a range of about 3.0289µg/mL to 9.0862µg/mL.The method precision for the determination of related impurities was below 5.0% RSD .The Percentage recoveries of known related impurities from dosage forms ranged from 87.3 to 112.5%. LOD and LOQ of all related impurities of Ceftriaxone and Tazobactam was established and ranged from 0.119µg/ml - 0.552µg/ml for LOD and 0.356µg/ml – 1.658µg/ml for LOQ .The method is useful in the quality control of bulk manufacturing and also in pharmaceutical formulations.

INTRODUCTION

Ceftriaxone and Tazobactam for injection is the combination of Ceftriaxone(3rdgeneration cephalosporin) and Tazobactam(beta-lactamase inhibitor) used for treatment of bacterial infections caused beta-lactam resistant pathogens.Ceftriaxone sodium is (6*R*,7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-[[(1,2,5,6-tetrahydro-2-methyl1-5,6-dioxo-as-triazin-3-yl)thio]methy1]-5-thia-1-azzbicyclo[4.2.0]oct-2-ene-2-carboxyalic acid7[2]-(Z)-(O-methyloxime),disodiumsalt,sesquaterhydrate.The empirical formula is $C_{18}H_{16}N_8Na_2O_7S_3 \cdot 3.5H_2O$ and its molecular weight is 661.59. Tazobactam sodium is (2S,3S,5R)-3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-4,4-dioxide The empirical formula is $C_{10}H_{11}N_4NaO_5S$ and its molecular weight is 322.3.It is a white-half white crystalline powder freely soluble in water , sparingly soluble in methanol and very slightly soluble in ethanol (Prakash et al, 2005, Bonomo et al, 1997, Nelson Lee et al, 2003) . The chemical structures are given below (Figure-1) (USP, 2002, Ph.Eur, 1997)



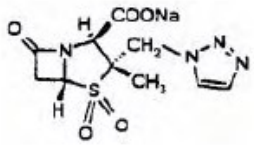
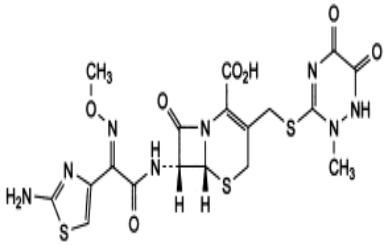


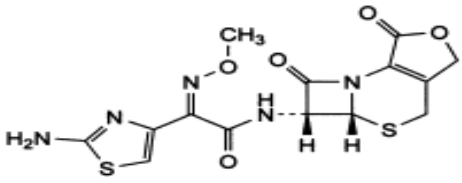
Figure-1 Ceftriaxone sodium Tazobactam sodium

Ceftriaxone and Tazobactam for injection is not official in any pharmacopoeia and liquid chromatography procedures have not been reported for the determination of Ceftriaxone and Tazobactam related substances in Bulk and Pharmaceutical dosage forms. However there are no publications concerning the analysis of combination product of Ceftriaxone and Tazobactam for injection in bulk and Pharmaceutical dosage forms. So it is felt necessary to develop a LC method which would serve as a reliable method for the determination of Ceftriaxone and Tazobactam in respective with related impurities [Figure-2] in bulk and pharmaceutical dosage forms. In the proposed method, related impurities were well separated and eluted before 30min.Finally the method was thoroughly validated for related substances of Ceftriaxone and Tazobactam for injection.

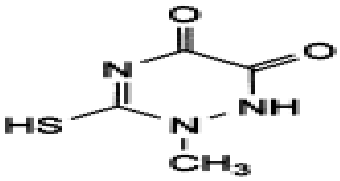
Figure-2: CEFTRIAXONE AND TAZOBACTAM RELATED IMPURITIES [5]



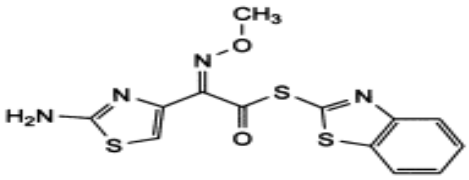
Ceftriaxone Impurity-A



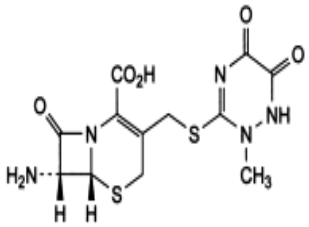
Ceftriaxone Impurity-B



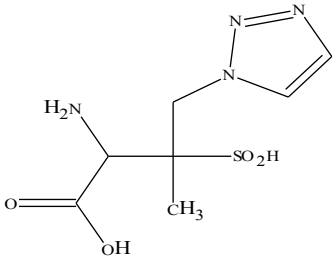
Ceftriaxone Impurity-C



Ceftriaxone Impurity-D



Ceftriaxone Impurity-E



Tazobactam Impurity-A

Chemical names of Ceftriaxone and Tazobactam related impurities:

1) Ceftriaxone Impurity-A: (6*R*,7*R*)-7-[[(2*E*)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]-3-[[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)sulphanyl]methyl]-8-oxo-5-thia-1- azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid ((*E*)-isomer),

2) Ceftriaxone Impurity-B: (5a*R*,6*R*)-6-[[(2*Z*)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]-5a,6-dihydro-3*H*,7*H*-azeto[2,1-*b*]furo[3,4-*d*][1,3]thiazine-1,7(4*H*)-dione,

3) Ceftriaxone Impurity-C: 2-methyl-3-sulphanyl-1,2-dihydro-1,2,4-triazine-5,6-dione

4) Ceftriaxone Impurity-D: *S*-benzothiazol-2-yl (2*Z*)-(2-aminothiazol-4-yl)(methoxyimino)thioacetate,

5) Ceftriaxone Impurity-E: (6*R*, 7R)-7-amino-3-[[(2-methyl-5, 6-dioxo-1, 2,5,6-tetrahydro-1,2,4-triazin-3-yl)sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

6) Tazobactam Impurity-A: 2-Amino-3-methyl-3-sulfino-4-(1H-1,2,3-triazol-1-yl)butyric acid.

EXPERIMENTAL

Instrumentation: Agilent 1200 series equipped with binary pump and DAD detector was used.
The output signal was monitored and integrated using waters Empower 2 software

Solutions:

Preparation of Mobile Phase A:

Preparation of pH 6.5 Buffer solution:

Accurately weighed and dissolved about 3.5g of Potassium dihydrogen orthophosphate anhydrous and 14.5g of disodiumhydrogen phosphate anhydrous in 1000 mL of milli-Q water, adjusted the pH to
6.5 ± 0.2 with dilute orthophosphoric acid and mixed well.

Preparation of pH 5.0 Buffer solution:

Accurately weighed and dissolve about 20.5g of Citric acid in 800mL of water; adjusted the pH to 5.0 ± 0.2 with NaoH solution and made up to 1000ml with water and mixed well.

Mobile Phase A:

Volume of milli-Q water, pH 6.5 buffer, pH 5.0 buffer and acetonitrile taken in the ratio 600:180:20:200 (v/v) and mixed well.
Preparation of Mobile Phase B:

Accurately weighed and dissolve about 4.0g of Tetra decyl ammonium bromide and 4.0g of Tetra heptyl ammonium bromide Bromide in 500 mL of Acetonitrile sonicated to dissolve and made up to 1000ml with Acetonitrile and mixed well

Preparation of 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 about 10 minutes.

Diluent:

Mobile phase is used as diluent.
Preparation of Resolution solution: About 60mg of Ceftriaxone sodium working standard and 6.25 mg of Tazobactam working standard was accurately weighed and transferred in to a 25mL volumetric flask and dissolved in 15ml of diluent and diluted to volume with diluent and mixed well and heat the solution at 70°c for 30minutes on water bath and cool to room temperature and filtered through 0.45µm nylon membrane prior to use. Resolution solution chromatogram is shown in Figure-4.
2.1.2 Preparation of Test Solution: About 260 mg of Ceftriaxone and Tazobactam powder Sample weighed accurately and transferred in to a 100mL volumetric flask 70ml of diluent was added and sonicated to dissolve and diluted to volume with diluent and mixed well. Filtered through 0.45µm nylon membrane filter prior to use.

Figure -3: TYPICAL CHROMATOGRAM OF CEFTRIAXONE AND TAZOBACTAM FOR INJECTION AND ITS RELATED IMPURITIES

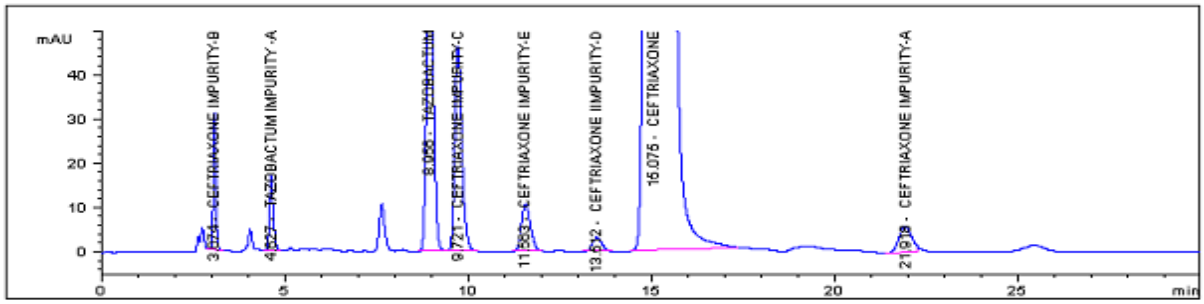
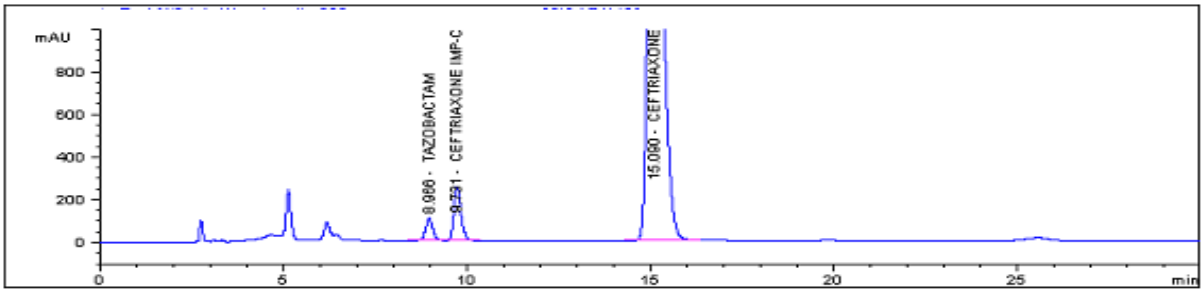


Figure-4: TYPICAL CHROMATOGRAM OF RESOLUTION SOLUTION



Preparation of Degradation samples for Specificity Study:

For Acid degradation Ceftriaxone and Tazobactam sample was refluxed with 0.01N HCl at 70ºC for 10 min. and then neutralized by adjusting pH to 7.0 with 0.01N NaOH .The Solution was further diluted to required concentration with diluent.
For basic degradation Ceftriaxone and Tazobactam sample was stressed with 0.01N NaOH for 5min
On bench top and then neutralized by adjusting pH to 7.0 with 0.01N HCl .The Solution was further diluted to required concentration with diluent.
For Oxidative degradation Ceftriaxone and Tazobactam sample was refluxed 0.1 %H2O2 for 5min on bench top .The Solution was further diluted to required concentration with diluent.
For Photolightic Stress the samples were exposed to UV at 254nm for 47hrs and visible light for 166hrs meeting the specification of ICH i.e. UV (200watt/m2) and Visible (1.2million Lux hours).
For Thermal Degradation Samples were Exposed to Temperature at 105ºC for 24hrs.
The above stressed samples i.e. Photolightic and Thermal stress samples solutions were prepared to required concentration with diluent.Specificity chromatograms for degradation products are shown in Figure-5.

Chromatographic Conditions:

Kromosil C18, (250 x 4.6mm; 5µm packing) column was used for analysis at column temperature 25 º C .The mobile phases was pumped through the column at a flow rate of 0.8mL/min.
The Sample injection Volume was 10µL.The photodiode array detector was set to a wavelength of 230nm.for the detection and run the chromatogram for 30 minutes.

RESULTS AND DISCUSSION

Method development (Haginaka, et al, 1984, Remington, 2000, Beckett, 1986, Mithal, 1991, Weibing, 2004)

Separation of Known degradant impurities

To develop a suitable and robust method for the determination of Ceftriaxone and Tazobactam for injection related impurities different mobile phases and columns were employed to achieve the best separation and resolution. The method development was started with a Prontosil 120-5-C18 H,250 x 4.6 mm,5 µm column using a mobile phase –A and mobile phase –B in the ratio 40:60 with 1.5 mL/min flow rate .In the above condition elution was very broad for Ceftriaxone peak little separation from Tazobactam peak and from impurities. Early elution with little separation was observed with mobile phase consisting of mobile phase –A and mobile phase –B in the ratio 60:40 using column Y.M.C pack,250 x 4.6 mm,5 µm with 1.2 mL/min flow rate. Finally the mobile phase consisting of mobile phase –A and mobile phase –B in the ratio 65:35 was found to be appropriate ,allowing good separation and symmetrical peak at a flow rate of 0.8mL/min using Kromosil, C18, 250 x 4.6 mm, 5 µm.. The Chromatogram of Ceftriaxone and Tazobactam sample spiked with the related impurities using the proposed method is shown in Fig.2.In the proposed method the resolution is more than 2 between the Tazobactam and Ceftriaxone impurity -C and resolution is more than 2 between the Ceftriaxone and Ceftriaxone impurity -D .System suitability results of the method are presented in Table 1. Ceftriaxone and Tazobactam and its related impurities show significant UV absorbance at Wavelength 230 nm .Hence this wavelength has been chosen for detection in the analysis of Ceftriaxone and Tazobactam for injection.

TABLE-1 SYSTEM SUITABILITY REPORT

System suitability parameters	Observed value	Acceptance criteria
The Resolution between Ceftriaxone impurity-C and Ceftriaxone	10.7	NLT 5.0
The Resolution between Tazobactam and Ceftriaxone impurity-C	2.3	NLT 1.5
Compound	**Tailing Factor [a]**	**Theoretical Plates [a]**
Ceftriaxone	1.2	7967
Tazobactam	1.1	10494

[a] Number of samples analyzed are six.

Column Selection (Merck, 2003, 1996)

Based on the retention time and separation of the impurities Kromosil,(250 x 4.6 mm, 5 µm) column was selected as suitable column for the analysis of Ceftriaxone and Tazobactam for injection.

Method Validation (ICH, 2003, 1995)

The developed LC method of Ceftriaxone and Tazobactam for injection is extensively validated for Ceftriaxone and Tazobactam and its related impurities using the following parameters.

SPECIFICITY:

INTERFERENCE FROM DEGRADATION PRODUCTS:

A study was conducted to demonstrate the effective separation of degradants from Ceftriaxone and Tazobactam peak. Separate portions of Drug product were exposed to following stress conditions to induce degradation.Stressed samples were injected into the RRLC system with diode array detector by following test method conditions. All degradant peaks were resolved from Ceftriaxone and Tazobactam peaks in the chromatograms of all samples. The chromatograms of the stressed samples were evaluated for peak purity of Ceftriaxone and Tazobactam using Empower software. In all forced degradation samples, Ceftriaxone and Tazobactam peaks Purity angle is less than purity threshold. The results are given under Table-2. From the above results it is clear that the method can be used for determining the stability of Ceftriaxone and Tazobactam for injection related substances in bulk and pharmaceutical formulations.

TABE -2 TABLE RESULTS FOR SPECIFICITY [INTERFERENCE FROM DEGRADATION PRODUCT]

Stress Condition	% Degradation	Purity Angle		Purity Threshold		Purity Flag	
		*I	*II	*I	*II	*I	*II
Acid Stress	25.54	0.156	1.863	2.002	2.043	No	No
Base Stress	0.36	0.287	0.181	2.001	2.057	No	No
Oxidation Stress	0.05	0.797	0.586	1.001	1.086	No	No
Photolightic Stress	1.06	0.869	0.363	2.009	2.217	No	No
Thermal Stress	0.06	0.255	0.219	2.001	2.057	No	No
Water Stress	4.55	0.278	1.331	2.002	2.060	No	No
Humidity Stress	1.73	0.166	0.263	2.002	2.096	No	No

*** I =Ceftriaxone *II =Tazobactam**

LIMIT OF DETECTION AND LIMIT OF QUANTITATION:

A study to establish the Limit of detection and limit of quantitation of Ceftriaxone and Tazobactam related impurities were conducted.

Limit of detection and limit of quantitation were established based on signal to noise ratio. A series of solutions having Ceftriaxone and Tazobactam related impurities were injected. Limit of detection for related impurities were established by identifying the concentration which gives signal to noise ratio about 3. Limit of quantitation was established by identifying the concentration which gives signal to noise ratio about 10.

Precision of Ceftriaxone and Tazobactam related impurities at about Limit of Quantitation were conducted. Six test preparations of Ceftriaxone and Tazobactam having related impurities at about Limit of quantitation was prepared and injected into the RRLC system. The %RSD at LOQ level was calculated for all known impurities and found to be less than 5.0%.

Accuracy of Ceftriaxone and Tazobactam related impurities at about Limit of Quantitation was conducted. Test solutions spiked with related impurities at about Limit of Quantitation was prepared in triplicate and injected into RRLC system and calculated the % recovery. The mean recovery of Ceftriaxone and Tazobactam related impurities at about Limit of Quantitation was ranged from 95.5 to 101.2% .The results are given under Table-3

TABLE-3 TABLE RESULTS FOR LOD AND LOQ OF CEFTRIAXONE AND TAZOBACTAM RELATED IMPURITIES

IMPURITY	Limit of detection	Limit of Quantitation	%RSD*	% Recovery
	Conc.µg/mL	Conc.µg/mL		
Ceftriaxone Impurity-A	0.247	0.803	4.2	95.5
Ceftriaxone Impurity-B	0.119	0.356	3.7	97.2
Ceftriaxone Impurity-C	0.181	0.482	4.8	101.2
Ceftriaxone Impurity-D	0.552	1.656	3.2	95.9
Ceftriaxone Impurity-E	0.366	1.098	4.6	96.8
Tazobactam Impurity-A	0.408	1.167	4.7	98.5

***Number of samples analyzed is six.**

Linearity of Detector Response:

a) Related impurities:

Linearity of detector response of all known Ceftriaxone and Tazobactam Related impurities is established by plotting a graph to concentration versus area of Ceftriaxone and Tazobactam related impurities and determining the correlation coefficient. A series of solutions of Ceftriaxone and Tazobactam related impurities in the concentration ranging from Limit of Quantitation level to about 150% of target concentration level of Ceftriaxone and Tazobactam known impurities were prepared and injected into the RRLC system.

The detector response was found to be linear from Limit of quantitation to 150% of target concentration level of Ceftriaxone and Tazobactam known Impurities. Linearity of detector response graph is shown in Figure-6.

Figure-6: LINEARITY OF DETECTOR RESPONSE GRAPH OF CEFTRIAXONE AND TAZOBACTAM RELATED IMPURITIES

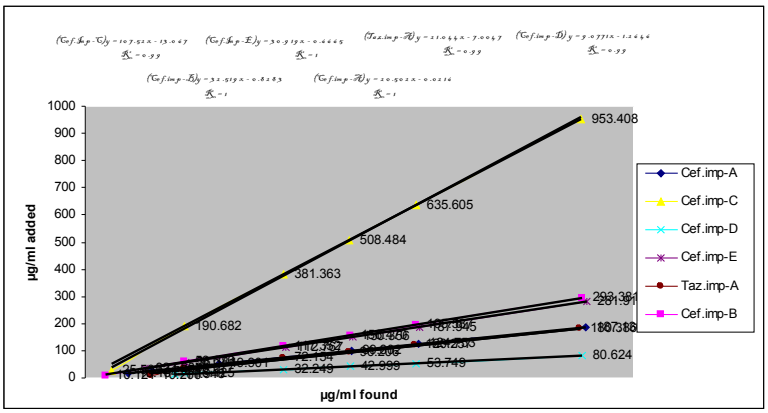
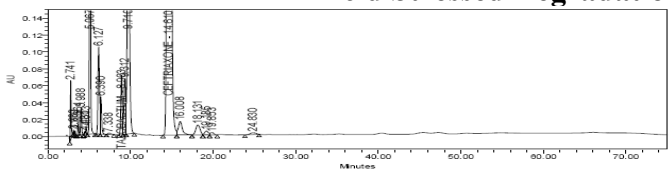
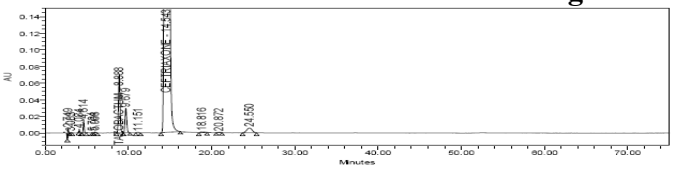


Figure-5: Specificity chromatograms of Ceftriaxone and Tazobactam and its degradation Products

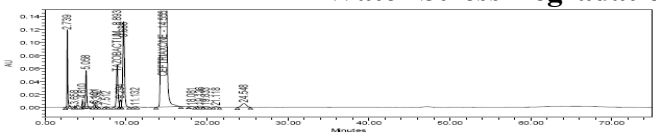
Acid Stressed Degradation



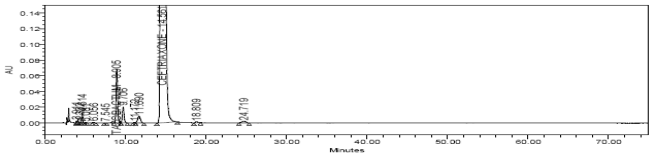
Alkali Stressed Degradation



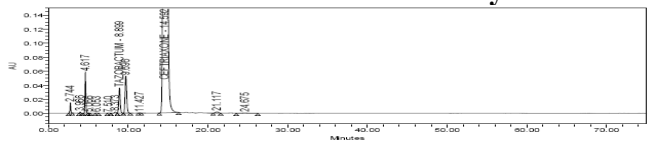
Water Stress Degradation



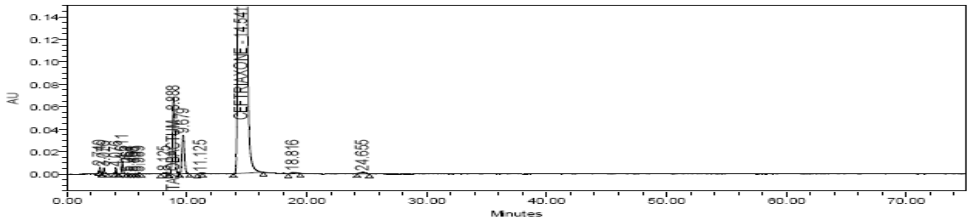
Oxidative Dégradation



Humidity Stressed Dégradation



Thermal Heat Stressed Dégradation



PRECISION OF TEST METHOD:

Related impurities:

The precision of test method of all known impurities of Ceftriaxone and Tazobactam was evaluated by spiking all known impurities at target concentration level on test preparation. The Relative standard deviations of all known impurities were calculated and found to be less than 5.0%. The results were given in Table-4.

TABLE-4 RESULTS FOR PRECISION OF TEST METHOD

Sample No	Ceftriaxone impurity-A	Ceftriaxone impurity -B	Ceftriaxone impurity -C	Ceftriaxone impurity -D	Ceftriaxone impurity -E	Tazobactam impurity -A
1	0.269	0.265	0.861	0.276	0.255	0.263
2	0.270	0.267	0.864	0.254	0.255	0.253
3	0.259	0.266	0.865	0.263	0.255	0.283
4	0.283	0.265	0.865	0.271	0.255	0.274
5	0.262	0.266	0.868	0.269	0.254	0.269
6	0.279	0.266	0.870	0.288	0.253	0.279
Average	0.270	0.266	0.866	0.270	0.255	0.270
%RSD	3.5	0.3	0.4	4.3	0.3	4.1

ACCURACY:

a) Related impurities:

A study of recovery of Ceftriaxone and Tazobactam related impurities in spiked samples of Ceftriaxone and Tazobactam test preparation was conducted. Samples were prepared in triplicate by spiking of all known impurities in test preparation at 50%, 75%, 100% and 150% of the target concentration level of known Impurities. The average %recovery for Ceftriaxone and Tazobactam Related Impurities was Calculated and given in Table-5. Ceftriaxone and Tazobactam related impurities from spiked were found to be in the range of 87.3-112.5%.

TABLE-5 ACCURACY IN THE DETERMINATION OF CEFTRIAXONE AND TAZOBACTAM RELATED IMPURITIES

Spike level	Ceftriaxone impurity-A			Ceftriaxone impurity-B			Ceftriaxone impurity-C		
	µg/ml added	**µg/ml found**	**Avg % Recovery**	**µg/ml added**	**µg/ml found**	**Avg % Recovery**	**µg/ml added**	**µg/ml found**	**Avg % Recovery**
50 %	3.0341	3.3180	109.3	3.0194	3.1120	103.1	3.0233	3.3013	109.2
75 %	4.5511	5.1213	112.5	4.5291	4.7413	104.7	4.5349	5.0540	111.4
100 %	6.0682	6.7533	111.3	6.0388	6.2593	103.6	6.0466	6.6807	110.5
150%	9.1022	10.2000	112.1	9.0582	9.3827	103.6	9.0698	9.2987	102.5
Correlation Coefficient	0.99			0.99			0.99		

Spike level	Ceftriaxone impurity-D			Ceftriaxone impurity-E			Tazobactam impurity-A		
	µg/ml added	**µg/ml found**	**Avg % Recovery**	**µg/ml added**	**µg/ml found**	**Avg % Recovery**	**µg/ml added**	**µg/ml found**	**Avg % Recovery**
50 %	3.0582	2.7027	88.4	3.0008	2.9893	99.6	3.0373	3.3687	110.9
75 %	4.5872	4.3547	94.9	4.5012	4.6500	103.3	4.5559	3.9907	87.6
100 %	6.1163	5.4687	89.4	6.0016	6.1593	102.6	6.0746	5.3047	87.3
150%	9.1745	8.3560	91.1	9.0024	9.1427	101.6	9.1118	8.3107	91.2
Correlation Coefficient	0.99			0.99			0.99		

[a] **Number of samples analyzed at each spike level are three.**

RUGGEDNESS:

A study to establish the stability of Ceftriaxone and Tazobactam in Test Solution was conducted on bench top and Refrigerator at Initial, 1 day. The % fall of impurities in test solutions was estimated against freshly prepared system suitability solution each time. The difference in % impurities of test solution from initial to 1 day was calculated and given in Table-6. From the above study, it was established that the Test Solution was not stable for a period of 1day on bench top and Refrigerator.

TABLE-6 STABILITY DATA OF TEST SOLUTIONS

BENCH TOP STABILITY

Time in days	CEF IMP-A	CEF IMP-B	CEF IMP-C	CEF IMP-D	CEF IMP-E	TAZ IMP-A	**%Total impurities**
Initial	0.256	0.235	0.838	0.277	0.252	0.271	2.240
1 day	0.244	0.238	1.349	0.000	0.235	0.290	3.516
%Difference	4.69	1.28	*60.98	*46.21	*50.79	*47.23	*23.04

REFRIGIRATOR STABILITY

Time in days	CEF IMP-A	CEF IMP-B	CEF IMP-C	CEF IMP-D	CEF IMP-E	TAZ IMP-A	**%Total impurities**
Initial	0.276	0.235	0.838	0.277	0.252	0.271	2.240
1 day	0.276	0.240	0.888	0.231	0.245	0.275	2.350
%Difference	0.00	2.13	5.97	*16.6	2.78	1.48	4.91

Robustness:

A study to establish the effect of variation in mobile phase composition, Flow, Temperature and pH of buffer in mobile phase was conducted. Resolution solution and test solutions spiked with known impurities of Ceftriaxone and Tazobactam prepared as per proposed method were injected into RRLC system. The System suitability parameters and RRT's of all individual known impurities were evaluated. From the above study the proposed method was found to be Robust.

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