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DEVELOPMENT OF A STABILITY INDICATING HPLC METHOD FORSIMULTANEOUS ESTIMATION OF CEFTOLOZANE AND TAZOBACTAMAND ITS VALIDATION AS PER ICH GUIDELINES

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Key Words

ABSTRACT

A new stability indicating RP HPLC method has been developed and validated for simultaneous estimation of Ceftolozane and Tazobactam in bulk and dosage forms. The method involves separation on XTerra C 18 column (150mm x 4.6mm x5µm particle size). The optimized mobile phase consists of 0.1%OPA and Acetonitrile (55:45v/v) with a flow rate of 1ml/min and UV detection at 260nm. Retention time was 2.14min (Ceftolozane), 3.19min (Tazobactam). Linearity range was 25-150ug/ml (Ceftolozane), 12.5-75ug/ml (Tazobactam). Accuracy was in the range of 99.59-100.55% for both drugs. Precision was 0.63% and 1.36 % for Ceftolozane and Tazobactam. LOD and LOQ are 0.63ug/ml and 1.89ug/ml for Ceftolazone, 0.09ug/ml and 0.27ug/ml for Tazobactam. The method developed is more sensitive, accurate and precise than the methods reported earlier. Retention time and run time were also less and hence the method is economical. When applied for tablet assay, drug content was within 100.12-101.27 % of labelled content. Forced degradation studies indicated the suitability of the method for stability studies.

INTRODUCTION

Ceftolozane is a semi-synthetic broad-spectrum fifth generation beta-lactam antibiotic.(1)It exhibits its bactericidal activities by interfering with bacterial cell wall synthesis.(2)The antibacterial activity of ceftolozane is also mediated through binding to penicillin-binding proteins (PBPs), which are required for peptidoglycan cross-linking for bacterial cell wall synthesis(3).Bacterial cells were killed due to inhibition of cell wall synthesis. Tazobactam also belongs to category of beta-lactamase inhibitor. Irreversible inhibition of beta-lactamase enzymes was promoted by binding to plasmid-mediated and chromosome-mediated beta-lactamase enzymes.(4). Ceftolozane and tazobactam is a novel antipseudomonal β-lactam/β-lactamase inhibitor combination that is currently approved by the United States Food and Drug Administration for the treatment of complicated intraabdominal infections (cIAI) and complicated urinary tract infections (cUTI).(5) Ceftolozane/tazobactam has activity against Pseudomonas aeruginosa, including drug-resistant strains, and other common gram-negative pathogens.(6). The literature review revealed that several analytical methods have been reported for estimation of ceftolozane and tazobactam by RP-HPLC (7-16), individually and in combination with other drugs. The present study reports simultaneous estimation of Ceftolozane and tazobactam by RP-HPLC in injection dosage form.

EXPERIMENTAL

Materials and reagents: HPLC grade Acetonitrile (Lichrosol**R**, Merck Lifesciences Pvt. Ltd., Mumbai, India), HPLC water (Lichrosolv**R** Merck Lifesciences Pvt.Ltd., Mumbai, India) and Ortho phosphoric acid (S D Fine –Chem. Ltd., Mumbai, India) were used in the study. The working standards of Ceftolazone and Tazobactam were generous gift obtained from HiQ Pharma Labs Pvt Ltd., Hyderabad, India. Zerbaxa injection was procured from local market.

Instrumentation: Chromatography was performed on a WATERS 2695 HPLC column (waters corporation, Mildord, USA) with an autosampler and equipped with a 2996 series of PDA detector with a spectral bandpass of 1.2nm. Components were detected using UV and that processing was achieved by Empower 2 software. A hot air oven was used for thermal degradation of the samples and a UV crossinker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300nm was selected for photolytic degradation. Ultrasonic bath (Toshcon by Toshniwal), digital P^H meter(Adwa – AD 1020), UV/VIS spectrophotometer (Labindia UV 3000) were used in the study.

Chromatography conditions: The chromatographic separation was performed on XTerra C_{18} (4.6 x 150mm, 5µm particle size) at an ambient column temperature. The samples were eluted using 0.1%OPAbuffer:Acetonitrile(60:40v/v) as the mobile phase at a flow rate of 1ml/min the mobile phase and samples were degassed by ultrasonication for 20 min and filtered through 0.45µm Nylon(N66)47mm membrane filter. The measurements were carried out with an injection volume of 10µL, flow rate was set to 1 mL/min, and UV detection was carried out at 260 nm. All determinations were done at ambient column temperature (30°C).

Preparation of Buffer and Mobile Phase:

Preparation of 0.1%OPAbuffer: 0.1ml of ortho phosphoric acid was taken in a 1000ml volumetric flask and solution was filtered by using 0.45 micron membrane filter and sonicated for 10 min.

Preparation of mobile phase: 550 ml (55%) of OPA buffer and 450 ml of Acetonitrile (45%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent: Mobile phase was used as diluent

Preparation of stock standard solutions: Accurately weighed and transfer25mg&12.5mg of Ceftolozane and Tazobactam working standards into a 25ml clean dry volumetric flask respectively, add 30ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Ceftolozane and Tazobactam.

Preparation of working standard solutions: Working standard solutions were prepared by taking 1ml of stock solutions of Ceftolozane and Tazobactam in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 100µg/ml of Ceftolozane and 50µg/ml Tazobactam.

Preparation of Sample Solutions of Ceftolozane and Tazobactam: One vial powder was weighed and powder equivalent to 850mgof ceftolozane and tazobactam was taken into 100 ml clean dry volumetric flask, diluent was added and sonicated to dissolve completely and volume was made up with the diluent. The above sample solution was filtered, 1ml of filtrate was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: During the optimization cycle, different columns with different lengths and internal diameters were tried namely, Waters C18 column, hypersil column, lichrosorb, and XTerracolumn but finally satisfactory separation was obtained on XTerra C 18 (4.6 x 150mm, 5µm) column. Methanol and acetonitrile were examined individually and simultaneously as organic modifiers and acetonitrile was found to be more suitable, individually, as it allowed better separation of

the analytes under investigation. Isocratic mode of elution with different ratios of organic to aqueous phases was tried in order to achieve proper separation of the cited analytes in a reasonable run time. The use of 0.1% OPA buffer was necessary in this method in order to influence the ionization of the analytes and to help in their co-elution. Different flow rates were studied and flow rate of 1 mL min-1 was found to be optimum. Quantitation was achieved with UV-detection at 260 nm. The column temperature was set at 30ºC. Optimized method was providing good resolution and peak shape for ceftolozane and tazobactam. Under above described experimental conditions, all the peaks were well defined and free from tailing. The concern of small deliberate changes in the mobile phase composition, flow rates, and column temperature on results were evaluated as a part of testing for methods robustness.

Validation of Method Developed: The proposed method was validated according to the ICH guidelines for system suitability, specificity, recovery, precision, linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied.

System suitability test: HPLC system was optimized as per the chromatographic conditions. 10 µl of standard solutions of drugs were injected in triplicate into the chromatographic system. To ascertain the system suitability for the proposed method, the parameters such as retention time, theoretical plates, and tailing factor were calculated.

Specificity: The specificity of the method was carried out to check whether there is any interference of any impurities with the retention time of analyte peaks. The specificity was performed by the injecting blank, Placebo and standard solutions of drugs.

Precision: Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of Ceftolazone (100 µg/mL) and Tazobactam(50µg/mL),have been analyzed by injecting them into a HPLC column on the same day. The intermediate precision was estimated by injecting samples prepared at the same concentrations on three different days by different operators. The peak area ratios of all injections were taken and standard deviation, % relative standard deviation (RSD), was calculated.

Accuracy: Accuracy is tested by the standard addition method at different levels : 50, 100 and 150%. A known amount of the standard drug was added to the blank sample at each level. The mean recovery of Ceftolazone and Tazobactam were calculated and accepted with 100±2%.

Linearity: Appropriate volumes of Ceftolazone and Tazobactam standard solutions were diluted with mobile phase to yield 25-150µg/mL of Ceftolazoneand12.5-75µg/mL Tazobactam respectively. Six replicates of each concentration were independently prepared and injected in to HPLC system. The linearity was determined by calculating a regression line from plot of peak area ratio of drug and IS versus concentration of the drug. Regression analysis were computed for Ceftolazone and Tazobactam. The method was evaluated by determination of correlation coefficient and intercept values according to ICH guidelines.

2.4.2. Limit of Detection and Limit of Quantification: Limit of detection(LOD)and limit of quantification(LOQ) of Ceftolazone and Tazobactam were determined by calibration curve method. Solutions of Ceftolazone and Tazobactam were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ was calculated by using the following equations:

$$LOD = 3 \times N / B$$

$$LOQ = 10 \times N / B$$

Where N is residual variance due to regression; B is the slope.

Table 1:System suitability results of Ceftolazone and Tazobactam

Parameter	Ceftolazone	Tazobactam
Peak area	924091(0.61%)*	22137 (0.34%)*
Theoretical plates	2599.3±0.861	5429.72±0.672
Retention time	2.14±0.027	3.19±0.057
Tailing factor	1.13±0.03	1.47±0.08

*RSD (%)

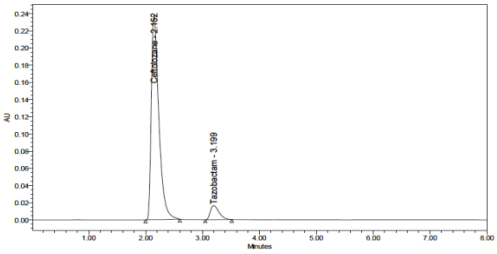

Fig. 1: Optimized Chromatogram of Ceftolazone and Tazobactam

Ceftolazone				Tazobactam		
S. No	Conc. (µg/ml)	Retention time (min)	Peak Area	Conc.(µg /ml)	Retention time (min)	Peak Area
1	100	2.147	918397	50	3.091	22137
2	100	2.144	926677	50	3.142	22575
3	100	2.143	928636	50	3.173	22575
4	100	2.148	919625	50	3.151	22563
5	100	2.146	933152	50	3.176	22673
6	100	2.141	921164	50	3.191	22484
Average		2.145	924609		3.15	22501.2
SD		0.003	5810		0.04	188.2
%RSD		0.123	0.6		1.13	0.8

Table 2: System Precision data for Ceftolazone and Tazobactam

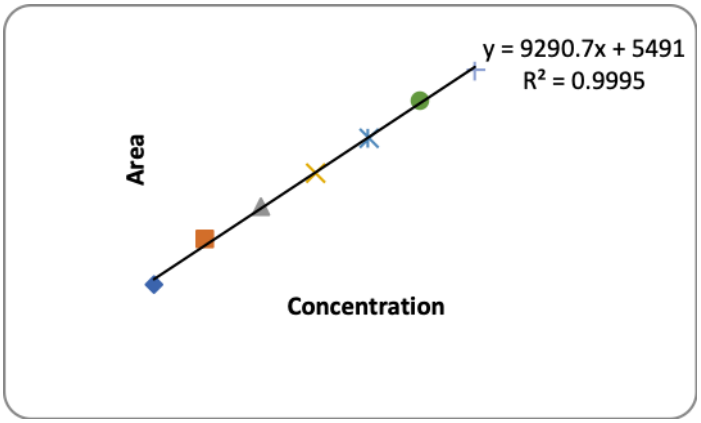

Fig.3. Linearity graph of Ceftolazone

Table 3: Method Precision data for Ceftolazone and Tazobactam

Ceftolazone				Tazobactam		
S. No	Concentration (µg/ml)	Peak Area	% Assay	Concentration (µg/ml)	Peak Area	% Assay
1	100	911508	99.3	50	22376	98.4
2	100	939016	100.2	50	21765	101.45
3	100	908096	100.4	50	21597	99.38
4	100	940019	99.4	50	21572	101.92
5	100	924217	100.9	50	21733	100.9
6	100	921693	99.6	50	22476	99.6
Average		924091.5	99.97		21919.8	100.28
SD		13389.5	0.63		400.4	1.36
%RSD		1.4	0.63		1.8	1.36

Table 4: Ruggedness Data for Ceftolazone

Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
Concentration (µg/ml)	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
100	99.45	97.25	98.25	99.47	102.08	101.08	102.38	101.51
100	98.50	99.27	101.27	100.30	101.87	100.26	100.18	100.18
100	97.09	96.91	99.22	99.19	99.38	100.71	101.61	100.51
100	99.48	98.18	99.40	98.42	101.90	99.78	100.39	101.81
100	99.34	100.13	97.08	99.28	100.20	99.23	101.82	101.47
100	100.24	98.09	100.24	101.08	100.29	100.78	101.27	101.29
Average	99.02	98.31	99.24	99.62	100.95	100.31	101.28	101.13
SD	1.09	1.22	1.47	0.93	1.14	0.70	0.85	0.64
%RSD	1.10	1.24	1.48	0.94	1.13	0.69	0.84	0.63

Table 5 : Ruggedness Data for Tazobactam

Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
Concentration (µg/ml)	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
100	101.83	102.54	100.21	101.34	100.21	98.37	100.21	98.35
100	100.18	101.29	99.81	100.65	100.61	101.67	99.47	100.52
100	100.38	100.51	101.3	99.78	99.4	98.02	101.82	102.78
100	100.39	101.81	100.61	101.81	100.39	98.42	100.74	99.58
100	100.65	101.72	100.8	101.72	97.56	99.28	101.47	102.55
100	101.27	101.29	99.79	100.27	101.27	100.69	102.27	99.79
Average	101	101.53	100	100.93	100	99	101	101
SD	0.6	0.68	0.6	0.82	1.3	1.5	1.1	1.8
%RSD	0.6	0.67	0.6	0.82	1.3	1.5	1.0	1.7

Table 6: Recovery data of Ceftolazone

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S1:50%	50	49.8	99.6	Mean=100.12%(n=3)
S2:50%	50	49.6	99.2	S.D=1.031
S3:50%	50	50.78	101.56	%RSD=1.030
S4:100%	100	100.56	100.56	Mean=100.53%(n=3)
S5:100%	100	100.45	100.45	S.D=0.060
S6:100%	100	100.59	100.59	%RSD=0.060
S7:150%	150	150.55	100.36	Mean=99.59%(n=3)
S8:150%	150	148.36	98.90	S.D=0.598
S9 :150%	150	149.29	99.52	%RSD=0.601

Table 7: Recovery data of Tazobactam

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S1:50%	25	25.65	102.6	Mean=101.11%(n=3)
S2:50%	25	24.77	99.08	S.D=1.48
S3:50%	25	25.41	101.64	%RSD=1.46
S4:100%	50	50.18	100.36	Mean=100.43%(n=3)
S5:100%	50	49.71	99.42	S.D=0.85
S6:100%	50	50.76	101.52	%RSD=0.85
S7:150%	75	76.25	101.66	Mean=100.40%(n=3)
S8:150%	75	75.44	100.58	S.D=1.11
S9 :150%	75	74.22	98.96	%RSD=1.10

TABLE 8: Linearity data of Ceftolazone and Tazobactam

Level	Concentration of Ceftolazone (µg/ml)	Peak area	Concentration of Tazobactam (µg/ml)	Peak area
1	25	365031	12.5	7362
2	50	590445	25	14723
3	75	824680	37.5	22084
4	100	938891	50	29512
5	125	1262631	62.5	36368
6	150	1482624	75	44237

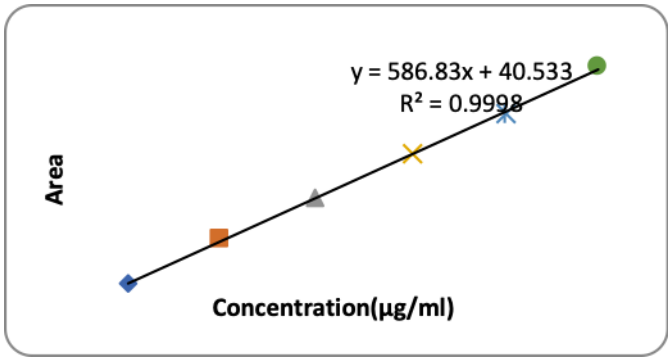

Fig.4. Linearity graph of Tazobactam

Table 9: Robustness (change in flow rate) for Ceftolazone and Tazobactam

Drug	Change in Flowrate (ml/min)	Change in flow Rate (0.8ml/min to 1.2 ml/min)		
		%Assay	SD	% RSD
Ceftolazone	0.8	98.2	1.2	1.34
	1	101.41	1.14	1.2
	1.2	99.26	1.6	1.64
Tazobactam	0.8	100.12	1.7	1.8
	1	98.46	0.79	0.8
	1.2	101.12	1.43	1.5

Table 10: Robustness (change in Mobile phase composition) for Ceftolazone and Tazobactam

Drug	Change in mobile phase	Change in Mobile phase (0.8ml/min to 1.2 ml/min)		
		%Assay	SD	% RSD
Ceftolazone	10% less organic phase	101.21	0.95	1.1
	Actual	99.42	1.28	1.3
	10% more organic phase	100.61	1.26	1.3
Tazobactam	10% less organic phase	100.81	1.43	1.5
	Actual	101.21	0.58	0.6
	10%more organic phase	99.41	1.4	1.5

Table 11: Robustness (change in column Temparature) for Ceftolazone and Tazobactam

Drug	Change in column temperature	Change in column temperature		
		%Assay	SD	% RSD
Ceftolazone	25°C	98.34	1.56	1.6
	30°C	101.42	1.26	1.3
	35°C	101.39	1.40	1.5
Tazobactam	25°C	101.45	1.58	1.6
	30°C	99.45	0.49	0.5
	35°C	99.81	0.61	0.7

Table 12: Forced Degradation studies of Ceftolazone

Sample Name	**Degradation (%)**	**Purity Angle**	**Purity Threshold**
Unstressed Sample	---	0.134	0.289
Thermal Stress Sample	0.7	0.109	0.371
Photolytic Stress Sample	0.2	0.276	0.784
Water Stress Sample	0.1	0.891	1.236
Acid Degradation	2.3	1.041	1.452
Alkali Degradation	1.4	1.209	2.983
Peroxide Degradation	1.1	1.26	2.853

Table 13: Degradation studies of Tazobactam

Sample Name	Degradation (%)	Purity Angle	Purity Threshold
Unstressed Sample	-----	1.092	2.011
Thermal Stress Sample	0.7	1.467	1.921
Photolytic Stress Sample	0.4	1.302	2.173
Water Stress Sample	0.6	1.910	2.492
Acid Degradation	1.24	1.562	2.310
Alkali Degradation	1.37	1.751	2.401
Peroxide Degradation	0.34	1.882	2.300

2.4.5. Robustness: HPLC conditions were slightly modified to evaluate the analytical method robustness. These changes included the flow rate, column temperature and the Acetonitrile proportion in the mobile phase.

2.4.6.Forced Degradation Study

Alkaline, acidic, oxidative stress, thermal, water and direct exposure to UV were carried out .No internal standard was added in the forced degradation study.

1) **Alkali Hydrolysis:** Ten mL of Ceftolazone and Tazobactam stock solution wasmixedinaflaskwith1Nsodium hydroxide (4mL)for1hr at 50°C.Before analysis, the solution was cooled at room temperature and neutralized with1N hydrochloric acid. The solution was completed with deionised water to reach the targeted concentration.

2)**Acid Hydrolysis:** Ten mL of Ceftolazone and Tazobactam stock solution was mixed in a flask with1N hydrochloric acid(4mL)for1hr at50°C.Beforeanalysis, the solution was cooled at room temperature and neutralized with1N sodium hydroxide. The solution was completed with deionised water to reach a targeted concentration.

(3)**Oxidative Stress:** Ten mL of the Ceftolazone and Tazobactam stock solution was mixed with 1mL of 3% hydrogenperoxide and stored at 50°C for 1hr. The solution was cooled and completed with deionised water until the volumetric flask mark to reach a targeted concentration.

(4)**Sunlight Degradation:** Ten mL of the Ceftolazone and Tazobactam stock solution was transferred into a 200mL volumetric flask and exposed to direct sunlight for 5days at room temperature. The solution was completed to the mark with deionised water to reach a targeted concentration.

(5)**Thermal Degradation :**Ten mL of Ceftolazone and Tazobactam stock solution was transferred into volumetric flask (200mL)and kept in airdry oven at105°C for 5h.Then, the solution was cooled and completed to the flask mark with deionised water to reach a targeted concentration.

(6)**HydrolyticDegradation:** Ten mL of Ceftolazone and Tazobactam solution was transferred into a volumetric flask and mixed with 10mL of deionised water. The solution was heated on water bath for 1hr.Then,thesolutionwascooledandcompleted to the flask mark with deionised water to reach a targeted concentration.

3.ResultsandDiscussion

Validation of Method Developed:

The proposed method was validated according to the ICH guidelines for system suitability, specificity, recovery, precision, linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied.

System suitability: The Retention time of Ceftolazone and Tazobactam using optimum

conditions was 2.14min and 3.19min respectively. For two of them, the peak symmetries were <1.5 and the theoretical plates numbers were >2000 and %RSD of areas of six standard injections of Ceftolazone and Tazobactam was less than 2. These values are within the acceptable range of United States pharmacopoeia definition and the chromatographic conditions. The results obtained are shown in **Table 1.**

Specificity: The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. Optimized Chromatogram of Ceftolazone and Tazobactam is shown in **Fig. 1** clearly shows the ability of the method to assess the analyte in the presence of other excipients.

Precision: System Precision: One dilution of both the drugs in six replicates was injected into HPLC system & was analyzed and the results were found within the acceptance limits (RSD<2) as shown in the **Table 2** below**.**

Method Precision(Repeatability): Six replicate injections of a known concentration of sample preparation of Saxagliptine (40 µg/mL) and Dapagliflozin (80 µg/mL) have been analyzed by injecting them into a HPLC column on the same day. From the results obtained, %RSD was calculated and was found to be within the limits (<2). The results of precision are given in **Table 3**.

Ruggedness: Intermediate precision was accessed injecting sample preparation of Saxagliptine (40 µg/mL) and Dapagliflozin (80 µg/mL) in six replicates in to HPLC column on the same day and on consecutive days and in different laboratories by different analysts . Results were found within the acceptance limits (RSD<2) as shown in the **Tables 4, 5** below.

Accuracy: A known amount of the standard drug was added to the blank sample at each level. Good recovery of the spiked drugs was obtained at each added concentration, and the mean percentage recovery of Ceftolazone and Tazobactam was achieved between 100.21–100.50 ± 0.148% and 99.99 –100.13±0.74.The results are given in Tables 6,7.

Linearity and Range: Linearity was assessed for the two oral anti diabetic drugs at concentration ranges 25-150µg/ml for Ceftolazone and 12.5-75µg/ml for Tazobactam. A linear relationship was established at these ranges between Area under the peak (AUP) and concentration. Good linearity was proved by high values of coefficient of determinations (**Fig.2 and Fig.3**). The results were tabulated in **Table 8**

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The limit of detection and limit of quantification were evaluated by serial dilutions of Ceftolazone and Tazobactam stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for Ceftolazone and Tazobactam was found to be 0.63µg/mL and 0.09µg/mL, respectively, and the LOQ value 1.89µg/mL and 0.27 µg/mL, respectively.

Robustness: The result of robustness study of the developed assay method was established in **Tables 9,10,11**. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

Forced degradation studies: The assay method was used to test the drug stability by conducting forced degradation studies for the drug substances under various stress conditions. Stress degradation studies were carried out for acid hydrolysis (1M HCl heated for 30 min at 60°C), alkali hydrolysis (2 N NaOH heated for 30 min at 60°C), oxidative degradation (20%H2O2 heated at 60°C for 30 min) and thermal degradation (samples placed in an oven at 105°C for 6 h). For photolytic stress studies, samples were exposed to UV light by keeping them in a UV chamber for 7 days. Results are shown in Tables 12,13.

The retention time of Ceftolazone and Tazobactam was found to be 2.14 min and 3.19min respectively with resolution of 3.45. Linearity was established for Ceftolazone and Tazobactam in the range of 25-150µg/ml for

Ceftolazone and 12.5-75µg/ml for Tazobactam with correlation coefficients(r^2=0.999) and the percentage recoveries were between 99.59% to 100.53% and 100.40 to101.11% for Ceftolazone and Tazobactam respectively, which indicate accuracy of the proposed method. The% RSD values of accuracy for Ceftolazone and Tazobactam were found to be<2%.The%RSD values of method precision are 0.630% and1.36% for Ceftolazone and Tazobactam respectively and %RSD values of system precisionare0.6%and 0.8%forCeftolazone and Tazobactam. The %RSD values of reproducibility for Ceftolazone and Tazobactam were found to be<2%,revealthattheproposedmethodisprecise . LOD values for Ceftolazone and Tazobactam were found to be 0.63µg/ml and 0.09µg/ml respectively and LOQ values for Ceftolazone and Tazobactam were found to be 1.89µg/ml and 0.27µg/ml respectively. The %RSD values of robustness studies were found to be<2% reveal that the method is robust enough was shown in(Table9). These data show that the proposed method is specific and sensitive for the determination of Ceftolazone and Tazobactam**.**

CONCLUSIONS

1. RP-HPLC method for the simultaneous estimation of Ceftolazone and Tazobactam in their combine dosage form was developed and validated as per the ICH guidelines.
2. The percentage recoveries of Ceftolazone and Tazobactam were in the range of 99.99-100.50% which was with in the acceptance criteria.
3. The percentage RSD was NMT 2% which proved the precision of the developed method.
4. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust.

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