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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CEFOPERAZONE AND TAZOBACTAM BY USING RP-HPLC

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Abstract:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Cefoperazone and Tazobactam in Tablet dosage form. Chromatogram was run through Kromasil C18 250 x 4.6 mm, 5µ. Mobile phase containing Buffer Perchloric acid: Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% Perchloric acid buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 230 nm. Retention time of Cefoperazone and Tazobactam were found to be 3.751 min and 2.568min. %RSD of the Cefoperazone and Tazobactam were and found to be 0.4% and 0.5% respectively. %Recovery was obtained as98.77% and 98.58% for Cefoperazone and Tazobactam respectively. LOD, LOQ values obtained from regression equations of Cefoperazone and Tazobactam were 0.10, 0.31 and 0.02, 0.05 respectively. Regression equation of Cefoperazone is y = 7979.x + 15373, and y = 19820x + 5778 of Tazobactam. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: *Cefoperazone, Tazobactam, RP-HPLC, Simultaneous estimation, Validation as per ICH guidelines, Stability studies.*

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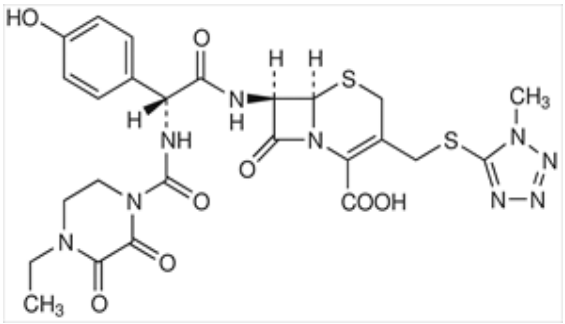
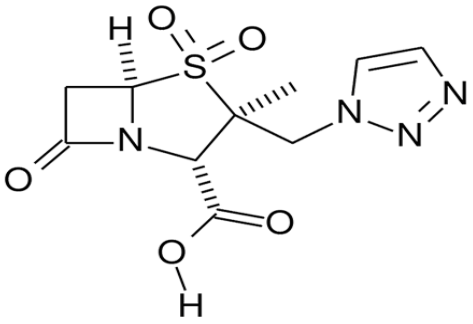
INTRODUCTION:

Cefoperazone is a third-generation cephalosporin antibiotic. Chemically, it is (6R,7R)-7-{2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl) amino]-2-(4-hydroxyphenyl) acetamido}-3-{[(1-methyl-1H-1,2,3,4-tetrazol-5-yl) sulfanyl] methyl}-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid. Like all beta-lactam antibiotics, cefoperazone binds to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins**.**

Tazobactam is a β-lactam antibiotic. Chemically, it is (2S,3S,5R)-3-methyl-4,4,7-trioxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4λ^6-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid. It broadens the spectrum of piperacillin by making it effective against organisms that express beta-lactamase and would normally degrade piperacillin. It is used in combination with piperacillin to broaden the spectrum of piperacillin antibacterial action [1-4].



a)



b)

Fig 1: Structure of (a) Cefoperazone (b) Tazobactam[5]

Though several methods are reported in literature for the estimation of Cefoperazone and Tazobactam individually, there are only few HPLC methods reported for the simultaneous estimation of Cefoperazone and Tazobactam combination. The objective of the present study is to develop and validate a new RP-HPLC method for simultaneous estimation of Cefoperazone and Tazobactam and its comparison with the earlier reported methods.

EXPERIMENTAL:

Materials and reagents

Acetonitrile (Rankem, avantor performance material India limited), HPLC water (Rankem, avantor performance material India limited), Methanol, Ortho phosphoric acid, Potassium dihydrogen ortho phosphate buffer are all from Rankem were used in the study. The working standards of Cefoperazone and Tazobactam were generous gift obtained from Natco Pharma., Hyderabad, India. Rampzone TZ tablet containing Cefoperazone 1000mg, Tazobactam 625mg was kindly supplied by Godrams lifeline.

Instrumentation

Chromatography was performed on a WATERS 2695 HPLC column (Alliance) with an auto sampler 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 cross inker, 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. Electronic balance (Denver), Ultrasonic bath (BVK enterprises), digital Ph meter (BVK enterprises) were used in the study.

Chromatography conditions

The chromatographic condition was performed on Kromosil C_{18} column (250 X 4.6mm,5µm particle size) at an ambient column temperature. The samples were eluted using 0.1% OPA: Acetonitrile(55:45v/v) as the mobile phase at a flow rate of 1 ml/min the mobile phase and samples were degassed by ultra-sonication 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 1.0 mL/min, and UV detection was carried out at 230 nm. All determinations were done at ambient column temperature (30°C). The chromatograms of the prepared standard stock solutions of Cefoperazone and Tazobactam were recorded under optimized chromatographic conditions (**Fig. 2**).

Diluent

Water and Acetonitrile in 50:50 v/v ratio.

Preparation of Standard Solutions

Stock solution of Cefoperazone

Standard stock solution of Cefoperazone was prepared by dissolving 50 mg of Cefoperazone in 3/4th of diluent (Water: Acetonitrile, 50:50v/v) in a 10ml clean dry volumetric flask and the solution was sonicated for 10 minutes and filter through 0.45µm nylon membrane filter and make up to the final volume with diluent to get the concentration of 5000µg/ml of Cefoperazone. The above standard stock solution was suitably diluted with diluent to obtain various concentrations of Cefoperazone.

Stock solution of Tazobactam

Standard stock solution of Tazobactam was prepared by dissolving 6.25mg mg of Tazobactam in 3/4th of diluent (Water: Acetonitrile, 50:50v/v) in a 10ml clean dry volumetric flask and the solution was sonicated for 10 minutes and filter through 0.45µm nylon membrane filter and make up to the final volume with diluent to get the concentration of 625µg/ml of Tazobactam. The above standard stock solution was suitably diluted with diluent to obtain various concentrations of Tazobactam.

Working Standard Solution

Working standard solutions of Cefoperazone and Tazobactam was prepared by taking 1ml of stock solutions of Cefoperazone and Tazobactam in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 500µg/ml of Cefoperazone and 62.5µg/ml of Tazobactam.

Preparation of Sample Solutions of Cefoperazone, Tazobactam

Five tablets were accurately weighed and calculate the average weight of each tablet then the weight equivalent to one tablet was transferred into 200ml volumetric flask, 100ml of diluent was added and sonicated for 25 minutes, further the volume made up with diluent and filtered. From the filtered solution 1 ml was pipetted out into a 10ml volumetric flask and made up with diluent.

RESULTS AND DISCUSSION:

Optimization of chromatographic conditions

Proper selection of the method depends upon the nature of the sample (ionic or ionizable or neutral molecule), its molecular weight and solubility. Cefoperazone and Tazobactam were dissolved in polar solvents, so the developed method of estimation was carried out on reverse phase high performance liquid chromatography. To develop a rugged and suitable HPLC method for the quantitative determination of Cefoperazone, Tazobactam, the analytical conditions were selected after the consideration of different parameters such as diluent, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition, and other chromatographic conditions. Preliminary trials were taken with different composition of buffer and organic phase of mobile phases with pH range of 2.5–5. The column selection has been done by backpressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution between Cefoperazone and Tazobactam peaks. After evaluating all these factors, a Kromasil C_{18} column was found to be giving satisfactory results. The selection of acetonitrile and buffer were based on chemical structure of both the drugs. The acidic pH range was found suitable for solubility, resolution, stability, theoretical plates, and peak shape of both components. Best results were obtained with 0.1% OPA pH adjusted to 2.0 that improved the peak shapes of Cefoperazone and Tazobactam. For the selection of organic constituent of mobile phase, acetonitrile was chosen to reduce the longer retention time and to attain good peak shape. Therefore, final mobile phase composition consisting of a mixture of buffer-pH 2.0 (0.1% OPA): Acetonitrile (55:45v/v). Flow rates between 0.5 to1.2ml/min were tried. Flow rate of 1ml/min was observed to be enough to get all the drugs eluted within less than 10min. The column temperature was set at 30^{O}C. Optimized method was providing good resolution and peak shape for Cefoperazone 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, various parameters were studied.

System suitability

The Retention time of Cefoperazone and Tazobactam using optimum conditions was 3.75min and 2.56min respectively. For all of them, the peak symmetries were <1.5 and the theoretical plates numbers were >2000 and %RSD of areas of six standard injections of Cefoperazone and Tazobactam were 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.**

Table 1: System suitability results of Cefoperazone and Tazobactam

Parameter	Cefoperazone	Tazobactam
Peak area	3865774	1198373
Theoretical plates	4053.6	4263.3
Retention time	3.75	2.56
Tailing factor	1.63	1.39

*RSD(%)

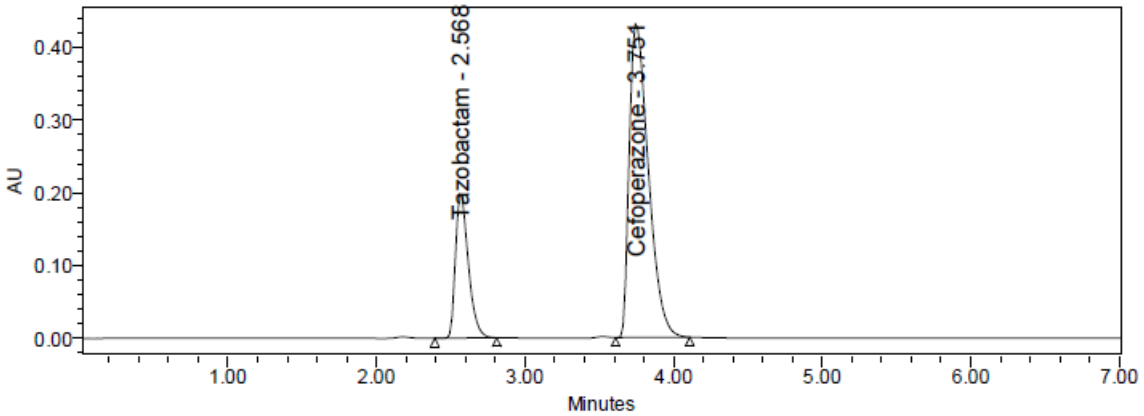


Fig 2: Optimized chromatogram of Cefoperazone and Tazobactam

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 Cefoperazone and Tazobactam is shown in **Fig. 2** clearly shows the ability of the method to assess the analyte in the presence of other excipients.

Linearity and Range

Linearity was assessed for all the four drugs at concentration ranges 125-750µg/ml for Cefoperazone, 16-94µg/ml for Tazobactam. The Chromatograms of level 1 and level 6 are shown in **Fig.3 and Fig.4**. 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.5 and Fig.6**). The results were tabulated in **Table 2.**

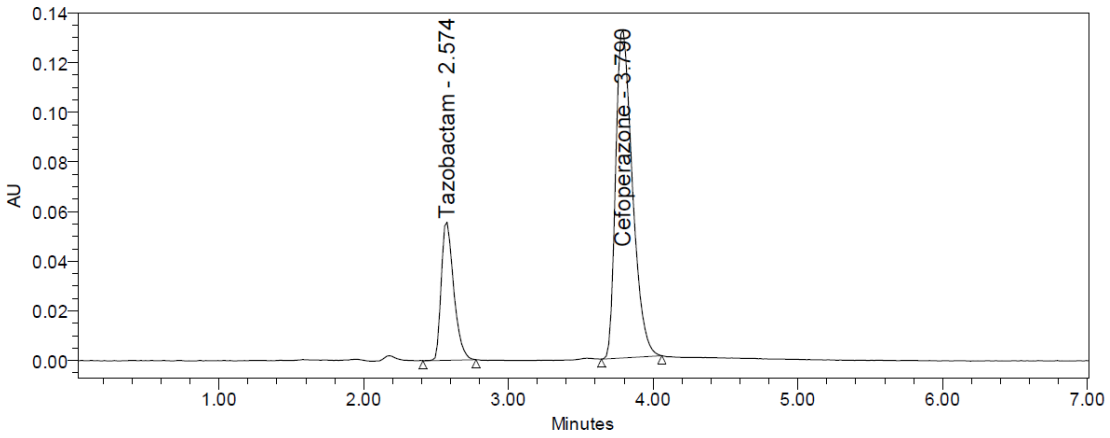


Fig 3: Chromatogram of Level 1

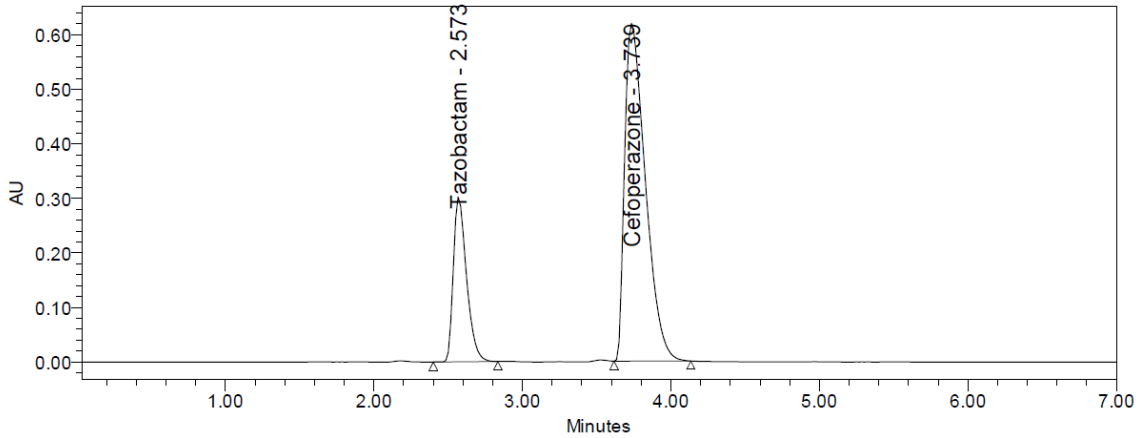


Fig.4 Chromatogram of Level 2

Table 2: Linearity data of Cefoperazone and Tazobactam

Level	Concentration of Cefoperazone(µg/ml)	Peak area	Concentration of Tazobactam (µg/ml)	Peak area
1	125	1060186	16	331025
2	250	1987537	31	620140
3	375	2998962	47	931832
4	500	3985706	63	1237229
5	625	5013924	78	1554724
6	750	6007576	94	1868907

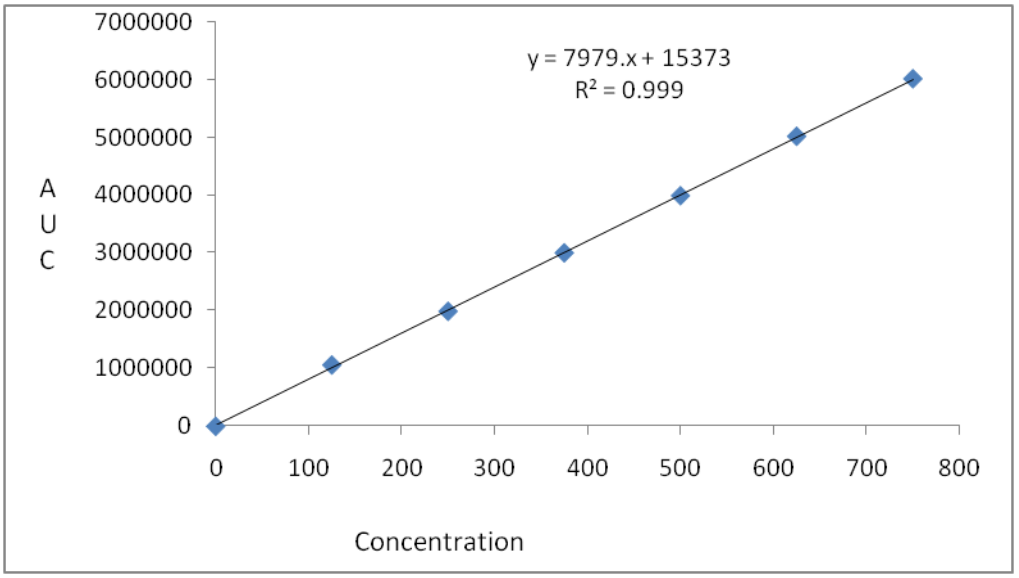


Fig 5: Calibration curve of Cefoperazone

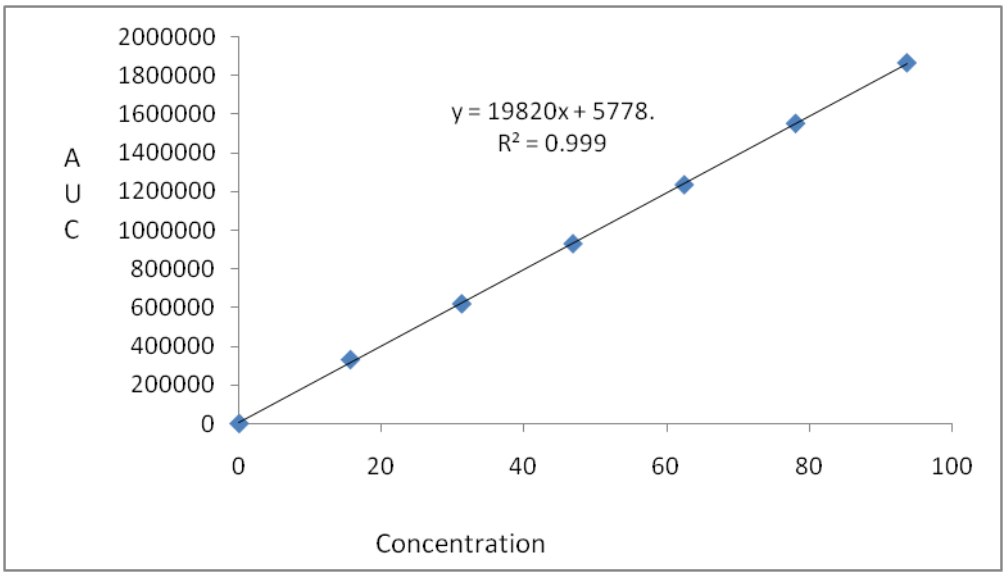


Fig 6: Calibration curve of Tazobactam

Limit of Detection (LOD)/Limit of Quantitation (LOQ)

The LOD was determined on the basis of signal to noise ratios and was determined using analytical response of three times the background noise. LOQ was determined as the lowest amount of analyte that was reproducibly quantified above the baseline noise following triplicate injections. Both LOQ and LOD were calculated on the peak area using the following equations:

LOQ= 10 x N/ B LOD= 3 x N/ B

The limit of detection and limit of quantification were evaluated by serial dilutions of Mupirocin and Fluticasone stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for Mupirocin and Fluticasone were found to be 0.38µg/ml, 0.02µg/ml respectively, and the LOQ values was found to be 1.16µg/ml, 0.05µg/ml respectively.

PRECISION System

Precision

System Precision was carried to ensure analytical system is working properly. One dilution of all 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 3** below.

Method Precision (Repeatability)

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 sample preparation of Cefoperazone and Tazobactam have been analyzed by injecting them into a HPLC column on the same day and on consecutive days. From the results obtained, %RSD was calculated and was found to be within the limits (<2). The results of precision are given in **Table 4**.

Table 3: System Precision data for Cefoperazone and Tazobactam

Cefoperazone				Tazobactam		
S. No	**Concentration (µg/ml)**	**Retention time (min)**	**Peak Area**	**Concentration (µg/ml)**	**Retention time (min)**	**Peak Area**
1	500	3.748	3854599	62.5	2.568	1196328
2	500	3.751	3853396	62.5	2.570	1200116
3	500	3.753	3852694	62.5	2.570	1195306
4	500	3.754	3875937	62.5	2.570	1193960
5	500	3.767	3869519	62.5	2.572	1205261
6	500	3.768	3888501	62.5	2.575	1199266
Average			3865774	Average		1198373
SD			14717.4	SD		4107.2
%RSD			0.4	%RSD		0.3

Table 4: Method Precision data for Cefoperazone and Tazobactam

Cefoperazone				Tazobactam		
S. No	**Concentration (µg/ml)**	**Peak Area**	**% Assay**	**Concentration (µg/ml)**	**Peak Area**	**% Assay**
1	500	3845245	99.27	62.5	1185658	98.74
2	500	3803714	98.20	62.5	1194699	99.49
3	500	3845972	99.29	62.5	1183867	98.59
4	500	3838125	99.09	62.5	1186147	98.78
5	500	3824099	98.72	62.5	1185646	98.74
6	500	3808939	98.33	62.5	1195454	99.56
Average		3827682	98.81	Average	1188579	98.98
SD		18388.3	0.47	SD	5098.5	0.42
%RSD		0.5	0.48	%RSD	0.4	0.42

Accuracy

The percentage recovery was calculated by preparing standard drug concentrations of Cefoperazone and Tazobactam with concentration levels of 50%, 100% and 150%. 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 Cefoperazone and Tazobactam was 98.77% and 95.58%. The results are given in **Tables 5 & 6** below.

Robustness

Robustness of the proposed analytical method is a measure of its capacity to remain unaffected, and it reflects the reliability of the analysis with respect to deliberate changes in the parameters such as flow rate (1.0 ± 0.2 mL), column temperature (30 ± 5°C), and mobile phase ratio of the mobile phase. The result of robustness study of the developed assay method was established in **Table 7**. 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.

Table 5: Recovery data of Cefoperazone

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Statistical Analysis
S1:50%	250	246.111	98.44	Mean=98.55
S2:50%	250	245.745	98.30	S.D=0.33
S3:50%	250	247.327	98.93	%RSD=0.33
S4:100%	500	497.152	99.43	Mean=99.49
S5:100%	500	496.833	99.37	S. D=0.15
S6:100%	500	498.347	99.67	%RSD=0.15
S7:150%	750	737.312	98.31	Mean=98.27
S8:150%	750	737.999	98.40	S. D=0.14
S9 :150%	750	735.925	98.12	%RSD=0.14

Table 6: Recovery data of Tazobactam

Sample Name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S1:50%	31	30.407	98.09	Mean=98.75
S2:50%	31	30.680	98.97	S.D=0.58
S3:50%	31	30.749	99.19	%RSD=0.58
S4:100%	63	61.842	98.16	Mean=98.72
S5:100%	63	62.459	99.14	S.D=0.50
S6:100%	63	62.285	98.87	%RSD=0.51
S7:150%	94	92.271	98.16	Mean=98.26
S8:150%	94	92.522	98.43	S.D=0.14
S9 :150%	94	92.300	98.19	%RSD=0.15

Table 7: Robustness data for Cefoperazone and Tazobactam.

S.no	Condition	%RSD of Cefoperazone	%RSD of Tazobactam
1	Flow rate (-) 0.9ml/min	0.0	0.1
2	Flow rate (+) 1.1ml/min	0.2	0.1
3	Mobile phase (-) 50B:50A	0.6	0.6
4	Mobile phase (+) 60B:40A	0.2	0.1
5	Temperature (-) 25°C	0.8	0.8
6	Temperature (+) 35°C	0.3	0.3

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 8 & 9.**

Table 8: Degradation Data of Cefoperazone

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.55	0.086	0.282
2	Alkali	2.97	0.100	0.297
3	Oxidation	1.92	0.087	0.288
4	Thermal	0.74	0.084	0.292
5	UV	0.89	0.083	0.286
6	Water	0.71	0.089	0.287

Table 9: Degradation Data of Tazobactam

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.63	0.088	0.267
2	Alkali	2.50	0.127	0.290
3	Oxidation	1.95	0.093	0.274
4	Thermal	0.55	0.098	0.271
5	UV	0.82	0.112	0.272

Table 10: Comparison of developed method with earlier reported methods[6-8]

Validation Parameters	Method developed		Reported Method 1		Reported Method 2		Reported Method 3	
	CEFO	TAZO	CEFO	TAZO	CEFO	TAZO	CEFO	TAZO
Range	125-750 µg/ml	16-94 µg/ml	24-72 µg/ml	3-9 µg/ml	25-50 µg/ml	3.125-9.37 µg/ml	20-60 µg/ml	2.5-7.5 µg/ml
Regression Coefficient	0.999	0.999			0.999	0.999	0.998	0.999
Slope (m)	7979	19820			29.16	89.63	1574	1464
Intercept (c)	15373	5778			16.38	7.009	1449	1440
Regression equation	Y=7979.x+ 15373	Y=19820.x+ 5778			Y=29.16.x- 16.38	Y=89.63.x- 7.009	Y=1574 .x+1449	y=1464 .x+1440
Assay	98.82%	98.98%			98.16%	98.04%	97.64%	97.13%
Retention time	3.75	2.56	3.3	5.1	3.24	4.83	3.86	7.57
System Precision (%RSD)	0.4	0.3			0.57	0.91	0.4	0.6
Method Precision (%RSD)	0.5	0.4			0.44	0.92	0.7	0.4
Accuracy	98.77%	95.58%			99.76%	99.64%	99.61%	99.01%
LOD	0.10	0.02			0.62	0.12	5.22	0.42
LOQ	0.31	0.05	0.87	0.04	1.87	0.38	16.02	14.15
Robustness	0.2-0.8%	0.1-0.8%			0.7-1.1%	0.5-1.3%	0.6-1.6%	0.8-1.1%

The retention time of Cefoperazone and Tazobactam were found to be 3.75min and 2.56 min respectively. Linearity was established for Cefoperazone and Tazobactam in the range of 125-750µg/ml for Cefoperazone, 16-94µg/ml for Tazobactam with correlation coefficients (r^2=0.999) and the percentage recoveries were 98.77% and 95.58% for Cefoperazone and Tazobactam respectively, which indicate accuracy of the proposed method. The % RSD values of accuracy for Diphenhydramine, Phenylephrine, Paracetamol, and Diazepam were found to be < 2%. The % RSD values of method precision are 0.97%, 0.49%, 0.64%, 0.60% for Diphenhydramine, Phenylephrine, Paracetamol, Diazepam respectively and % RSD values of system precision are 0.6% for Diphenhydramine, Paracetamol, Diazepam and 0.5% for Phenylephrine. LOD values for Diphenhydramine, Phenylephrine, Paracetamol and Diazepam were found to be 0.01µg/ml, 0.12µg/ml, 0.22µg/ml and 0.01µg/ml respectively and LOQ values for Diphenhydramine, Phenylephrine, Paracetamol and Diazepam were found to be 0.04µg/ml, 0.37µg/ml, 0.66µg/ml and 0.03µg/ml respectively was shown. The % RSD values of robustness studies were found to be < 2% reveal that the method is robust enough was shown in (Table 7). These data show that the proposed method is specific and sensitive for the determination of Cefoperazone and Tazobactam.

Table no 10: Comparison of developed method with earlier reported methods [6-8].

CONCLUSIONS:

1.RP-HPLC method for the simultaneous estimation of Cefoperazone and Tazobactam in their combine dosage form was developed and validated as per the ICH guidelines.

2.Linearity was observed in the range of 125-750µg/ml for Cefoperazone,16-94µg/ml for Tazobactam with correlation coefficients (r^2=0.999).

3.The percentage recoveries of Cefoperazone and Tazobactam were 98.77% and 95.58% Which was within the acceptance criteria.

4.The percentage RSD was NMT 2% which proved the precision of the developed method.

5.The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust.

6.The developed method was found superior in certain respects such as RT, LOD and the method was more economical when compared to others.

7.Accuracy and precision, ruggedness and robustness were similar to earlier reported methods

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REFERENCES:

1.www.wikipedia.com/cefoperazone/tazobactam

2.H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle. Instrumental Methods of Analysis, 7th edition CBS publishers and Distributors, New Delhi. 1986, P.518-521, 580-610.

3.D.A. Skoog. J. Holler, T.A. Nieman. Principle of Instrumental Analysis, 5th edition, Saunders College Publishing, 1998; P.778-787.

4.Connors Ka. A Textbook of Pharmaceutical Analysis, Wiley intersciences Inc; Delhi, 3rdEd, Pg 373-421, (1994).

5.www.drugbank.com/cefoperazone/tazobactam

6.Jatin R Gohil, et al., RP-HPLC method development and validation for the simultaneous estimation of Cefoperazone Sodium and Tazobactam Sodium in parenteral preparation in inventi journals.

7.Narendra Kumar et al., RP-HPLC method development and validation for simultaneous estimation of Cefoperazone and Tazobactam in their combined dosage form in world journal of pharmacy and pharmaceutical sciences, 2014;3(8): 1083-1093.

8.Amol R. Ranjale et al., development and validation of RP-HPLC method for simultaneous estimation of Cefoperazone and Tazobactam in marketed formulation in international journal of pharmacy and pharmaceutical sciences, 2014;6(8): 0975-1491.