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STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF CEFTAZIDIME PENTAHYDRATE AND TAZOBACTAM SODIUM IN BULK AND DOSAGE FORMS

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ABSTRACT

A simple rapid, accurate, precise and reproducible validated reverse phase HPLC method was developed for the determination ofCeftazidimepentahydrate and Tazobactam sodium in bulk and pharmaceutical dosage forms. The quantification was carried out using Hypersil BDS C18(150 X 4.6mm, 5 µm) columnrun in isocratic way usingmobile phase comprising of phosphate buffer pH 3.0, acetonitrile, and tetrahydrofuran in the ratio of 60:30:10 with a detection wavelength of 205nm and injection volume of 20µL, with a flow rate of 1.0ml/min. The retention timesof the drugs were found to be 3.490min and 2.353min. The linearity ranges of the proposed method lies between 60-140mcg/ml and 7.5-17.5mcg/ml for Ceftazidimepentahydrate and Tazobactam sodium with correlation coefficient of r^2=0.999 for both. The assay of the proposed method was found to be 99.38% and 99.26%. The recovery studies were also carried out and mean % Recovery was and found to be 99.91% and 100.84%. The % RSD from reproducibility was found to be <2%.The proposed method was statistically evaluated and can be applied for routine quality control analysis of Ceftazidimepentahydrate and Tazobactam sodium in bulk and in Pharmaceutical dosage form.

Key Words: Ceftazidime, Tazobactam sodium, RP-HPLC, Hypersil BDS, Validation, Forced degradation studies.

INTRODUCTION

CeftazidimePentahydrateis(Z)-(7R)-7-[2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1 methylethoxyimino) acetamido]-3-(1-pyridiniomethyl)-3-cephem-4-carboxylate pentahydrate. The molecular weight is 636.7, molecular formula is $C_{22}H_{22}N_6O_7S_2 \cdot 5H_2O$.It is a third generation Cephalosporin with enhanced antibacterial activity against gram negative organisms. Ceftazidime is bactericidal in action, exerting its effect on target cell wall proteins and causing inhibition of cell wall synthesis. It is official in IP and BP. It is used in the treatment of biliary tract infections, lower respiratory tract infections, bone and joint infections and urinary tract infections.

Tazobactam is a potent and novel β-lactamases inhibitor which belongs to the class of penicillanic acid sulfones. The molecular formula is $C_{10}H_{11}N_4NaO_5S$, molecular weight is 322.3and the chemical name is (2*S*, 3S, 5R) - 3 - methyl -7- oxo - 3 - (1 *H* - 1, 2, 3 - triazol - 1 -ylmethyl) – 4 - thia-1-azabicyclo [3.2.0] heptanes – 2 - carboxylic acid4,4-dioxide. Tazobactam inhibits the action of bacterial beta lactamases. It broadens the spectrum of penicillin by making it effective against organisms that express beta-lactamases degrade piperacillin.Used to reduce the development of drug resistant bacteria.

Literature review reveals that several methods are reported for these drugs alone or in combination with other drugs. For combination of these drugs Spectroscopic method, HPTLC method are reported, there is no single work done for this combination by using RP-HPLC. Hence an attempt has been made for the development of HPLC method for the combination of drugs.

MATERIALS AND METHODS

UV-3000 LABINDIA double beam with UV win 5software UV-VISIBLE spectrophotometer with 1cm matched quartz cells. Schimadzu HPLC equipped with SPD 20A UV-VIS detector and the column used was HYPERSIL BDS C18 (150*4.6mm, 5µ). The data acquisition was performed by using LC solutions software.

Ceftazidime and Tazobactam pure sample was taken as a gift sample from local labs and dosage form "Combitaz" marketed by LUPIN LABS was purchased from local pharmacy. Other chemicals all are of HPLC grade.

Preparation of mobile phase: A suitable quantity of degassed mixture of pH 3.0 phosphate buffer, Acetonitrile, Tetra hydro furan in the ratio of 60:30:10 was prepared and filtered through 0.45µ filter under vacuum filtration.

Preparation of standard stock solution: About 100mg of Ceftazidime and 12.5mg of Tazobactam sodium were weighed and taken into 100ml, 50ml volumetric flasks. To each flask 25ml of diluents were added and sonicated for

15min to dissolve the drugs then made up to required volume with the diluents, to get a concentration of 1mg/ml solutions. From this solution 10ml was taken into a 100ml flask and made up to final volume with diluents to get a concentration of 100ppm filtered through 0.45µ filter under vacuum filtration. From this stock solution further dilutions were made for the validation of the method developed.

Preparation of the sample solution: The powder equivalent to 100mg of Ceftazidime and 12.5mg of Tazobactam sodium were weighed and taken into a 100ml, 50ml volumetric flask. To this 25ml of diluents was added and sonicated for 15min to dissolve the drugs then made up the volume to required volume with the diluents. From this solution 10ml was taken into a 100ml flask and made upto final volume with diluents to get a concentration of 100ppm filtered through 0.45µ filter under vacuum filtration. From this stock solution further dilutions were made by taking the two drugs in the ratio of 8:1 for the validation of the method developed. NANDA .R.K et al.., 2012.

Method development

Method optimization: The chromatographic separation was performed using Hypersil BDS C18 (150×4.6mm, 5µm) column. For selection of mobile phase, various mobile phase compositions were observedfor efficient elution and good resolution.The mobile phase consisting of pH 3.0 phosphate buffer, acetonitrile and Tetra hydro furan in the ratio of 60:30:10 was found to be the optimum composition for efficient elution of analyte. The mobile phase was injected to the column at a flow rate of 1.0 ml/min for 8min. The column temperature was maintained at 35 ± 1^0C. The analyte was monitored at 205 nm using UV-detector. The retention time of the drugs was found to be 3.490 and 2.353min. Mobile phase was used as diluent during the standard and test samples preparation.The optimized chromatographic conditions are mentioned in Table-1 and chromatogram for standard was shown in the figure no:1.

RESULTS

Method Validation:

Specificity: Specificity is the ability of analytical method to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample. The specificity of method was determined by spiking possible impurities at specific level to standard drug solution (100ppm). The diluent and placebo solutions were also injected to observe any interference with the drug peak. The results are tabulated in the table no-2 and the chromatogram was shown in the figure no- 2.

Linearity: Linearity is the ability of the method to produce results that is directly proportional to the concentration of the analyte in samples with given range. The linearity of Ceftazidime was in the concentration range of 60-140%, for Tazobactam sodium 7.5-17.5%. From the linearity studies calibration curve was plotted and concentrations were subjected to least square regression analysis to calculate regression equation. The regression coefficient was found to be 0.999 and shows good linearity for both the drugs. The results are tabulated in the table no-3 and the chromatogram was shown in the figure no-.3,4.

Precision: Precision is the degree of closeness of agreement among individual test results when the method is applied to multiple sampling of a homogeneous sample. Study was carried out by injecting six replicates of the same sample preparations at a concentration of 100ppm/ml. The results are tabulated in the table no-4 and the chromatogram was shown in the figure no-10.

Accuracy: Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. Accuracy of the method was evaluated by standard addition method. Recovery of the method was determined by spiking an amount of the pure drug (80%,100% ,120%) at three different concentration levels in its solution has been added to the pre analyzed working standard solution of the drug. The results are tabulated in the table no:4.

LOD & LOQ: LOD is the lowest concentration of analyte in a sample that can be detected but not quantified under experimental conditions. The LOD values were determined by the formulae LOD=3.3σ/s (where σ is the standard deviation of the responses and s is the mean of the slopes of the calibration curves).

LOQ is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under experimental conditions. It is a parameter of the quantitative determination of compounds in the mixtures. The LOQ values were determined by the formulae LOD=10σ/s. The results are tabulated in the table no: 4

FORCED DEGRADATION OF CEFTAZIDIME AND TAZOBACTAM SODIUM

Acid and Base degradation: Acid/base degradation was determined by taking 5ml of stock solution in 10ml volumetric flask and to this 2ml of 0.1N HCl/NaoH was

added and sonicate for 5min, kept aside for 12hrs at room temperature. After 12hrs the solution was neutralized with 2ml of 0.1N HCL/NaoH then diluted with diluents to get a concentration of 10µg/ml solution.

Oxidative degradation: Oxidative degradation was determined by taking 5ml of stock solution in 10ml volumetric flask and diluted up to the mark with 5% H2O2 and kept aside for 12hrs. After 12hrs the solution was diluted with diluents to get a concentration of 10µg/ml solution.

Thermal degradation: Sample powder equivalent to 100mg of Ceftazidime and 12.5mg of Tazobactam was taken and kept in a controlled temperature oven at 80^0_c for 12hrs. After 12hrs the powder was diluted with diluents to get a concentration of10µg/ml solution.

Photolytic degradation: The Ceftazidime and Tazobactam powder and solutions of both were prepared and exposed to light to determine the irradiation of light on the stability of solution and powder form of drugs. Approximately 100mg of drug powder and 1mg/ml solution were spread on a glass dish in a layer that was less than 2mm thickness and were placed in a light cabinet and exposed to UV light for 12hrs. After 12hrs the samples are removed and diluted with diluents to get a concentration of10µg/ml solution and then injected.

The summary of results is tabulated in table no: 5and figures are shown in figure no: 5,6,7,8,9.

Disscussion: Several trials has made until getting good peak resolution, acceptable plate count and tailing factor. Method was optimized and the retention times of Ceftazidime and Tazobactam was reported as 3.480 & 2.353.

Specificity: The Chromatograms of Standard and Sample are identical with nearly same Retention time. There is no interference with blank and placebo to the drugs. Hence the proposed method was found to be specific.

Linearity: From the Linearity data it was observed that the method was showing linearity in the concentration range of 60-140µg/ml for Ceftazidime and 7.5-17.5µg/ml for Tazobactam. Correlation coefficient was found to be 0.999 for both the compounds.

Accuracy: The recoveries of pure drug from the analyzed solution of formulation were 99.91 % for Ceftazidimepentahydrate and 100.84 % for Tazobactam sodium, which shows that the method was accurate.

Precision: The %RSD for the sample chromatograms of method precision were found to be 0.39 & 0.81 for Ceftazidime and 0.23 &0.64 for Tazobactam. Hence it passes method precision.

Robustness: All the system suitability parameters are within limits for variation in flow rate (±0.2 ml). Hence the allowable flow rate should be within 0.8 ml to 1.2 ml. All the system suitability parameters are within limits for variation (±2nm) in wavelength. Hence the allowable variation in wavelength is ± 2nm.

Ruggedness: Comparison of both the results obtained for two different Analysts shows that the method was rugged for Analyst-Analyst variability. The %RSD for intermediate precision for Ceftazidime was found to be 0.45 & 0.002 and for Tazobactam was found to be 0.62 & 0.012.

LOD & LOQ of Ceftazidime was found to be 0.03, 0.10and for Tazobactam was found to be 0.57, 1.72 respectively.

All the system suitability parameters are within in the limits when the drugs are subjected to stress conditions like acid, base peroxide, thermal and photolysis.The results obtained were satisfactory and good agreement as per the ICH guidelines.

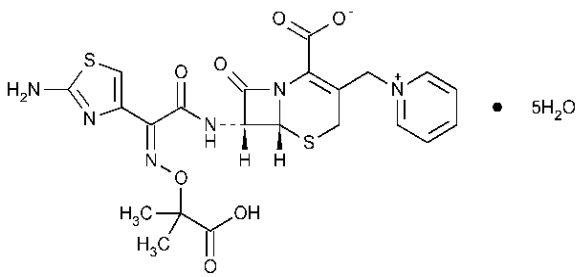

Fig No 1 Structure of Ceftazidimepentahydrate

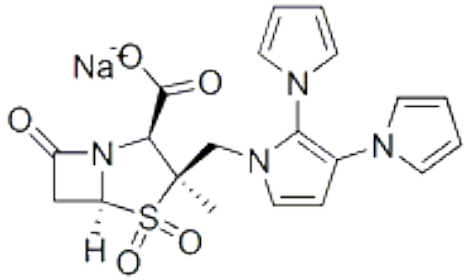

Fig No 2 Structure of Tazobactam Sodium

Table no: 1 Optimized chromatogram conditions for Ceftazidime and Tazobactam

Column	Hypersil BDS C18 (150*4.6mm,5µ)
Mobile phase	Phosphate Buffer pH 3.0:ACN:THF(60:35:05)
Flow rate	1.0 ml/ min
Wavelength	205 nm
Injection volume	20 µl
Column temperature	Ambient
Run time	8 min

Table No: 2 Specificity Data for Ceftazidime and Tazobactam

Standard Injection	**Retention time**	**Area**	**Theoretical Plates**	**Retention time**	**Area**	**Theoretical Plates**
	3.46	2910.42	3745	2.353	247.82	2254
	3.50	3091.18	3817	2.357	285.2	2137
	3.47	2943.91	3410	2.343	277.824	2210
Sample Injection	3.48	2984.39	3226	2.350	264.30	2125
	3.48	2996.31	3781	2.352	262.89	2248
	3.47	2989.06	3410	2.360	263.40	2143
Blank injection	-	-	-	-	-	-

Table.No: 3 Linearity data for Ceftazidime and Tazobactam

For Ceftazidime			**For Tazobactam**		
Mcg/ml	**Area**	**R_t**	**Mcg/ml**	**Area**	**R_t**
60	1847.225	3.470	7.5	163.031	2.337
80	2526.511	3.463	10	223.261	2.330
100	3072.796	3.490	12.5	276.082	2.353
120	3793.229	3.467	15	337.177	2.336
140	4341.245	3.453	17.5	394.041	2.323
Slope	31.27		Slope	23.037	
Correlation coefficient	0.99913		Correlation Coefficient	0.999811	
Intercept	11.17		Intercept	9.249	

Table No: 4 Summary of validation parameters

Parameter	**Ceftazidime**		**Tazobactam**	
Linearity	60-140µg/ml		7.5-17.5µg/ml	
Precision(% RSD)	0.39	0.81	0.23	0.64
Accuracy	99.91%		100.84%	
LOD & LOQ	0.03,0.10		0.57,1.72	
Assay	99.38%		99.26%	

Table No: 5 Summary of Forced degradation data for Ceftazidime and Tazobactam:

Stress Condition	Time(hrs)	Retention Time	Time(hrs)	Retention Time
As such	12hrs	3.490	12hrs	2.353
Acid Hydrolysis (0.1 N, at RT)	12hrs	3.457	12hrs	2.337
Base Hydrolysis (0.1N at RT)	12hrs	3.477	12hrs	2.360
Oxidation (5% H_2O_2 at RT)	12hrs	3.453	12hrs	2.353
Photolysis(UV Light and sunlight)	12hrs	3.457	12hrs	2.337
Thermal (at 80^0c)	12hrs	3.490	12hrs	2.353

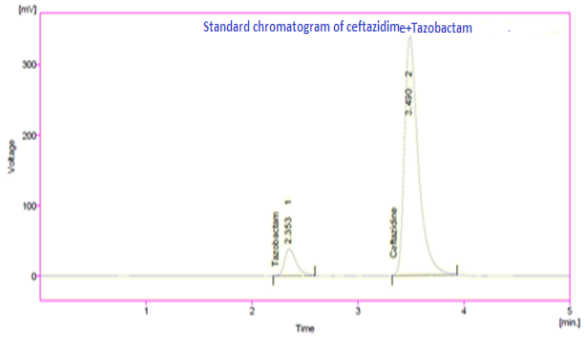

Fig: 1 Chromatogram of standard drug

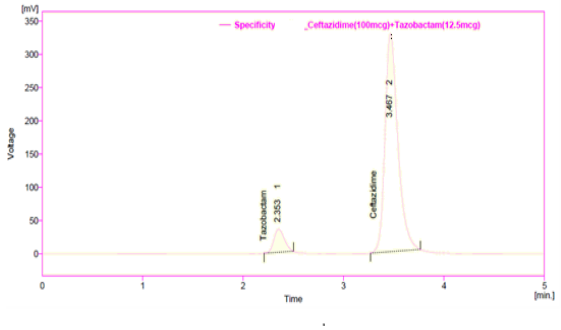

Fig: 2 Chromatogram for specificity

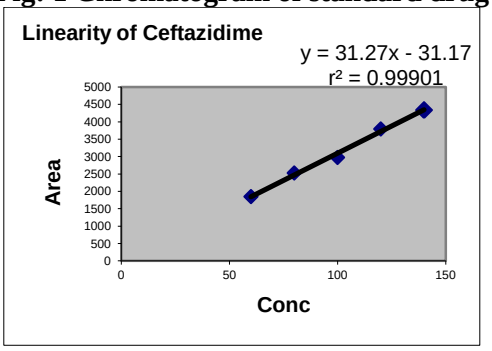

Fig: 3 linearity plot for Ceftazidime

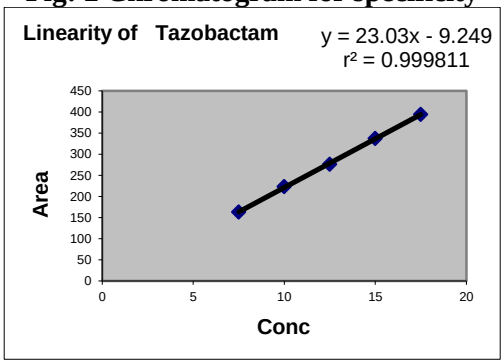

Fig: 4 linearity plot for Tazobactam

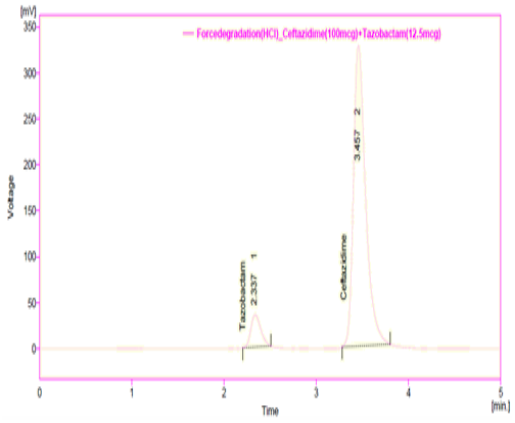

Fig: 5 Acid Degradation

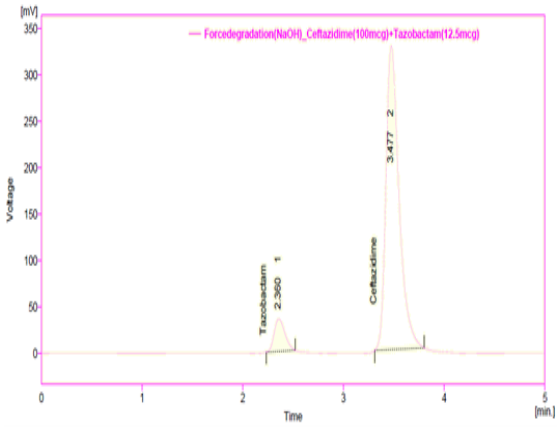

Fig: 6 Base Degradation

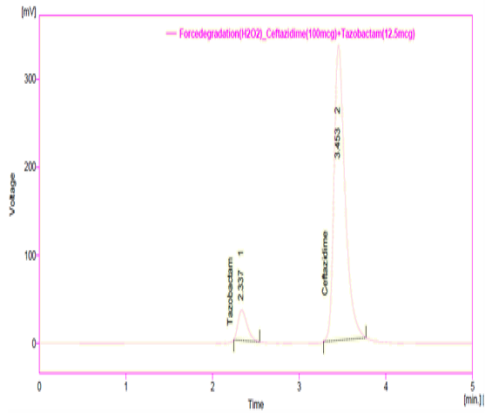

Fig: 7 Peroxide Degradation

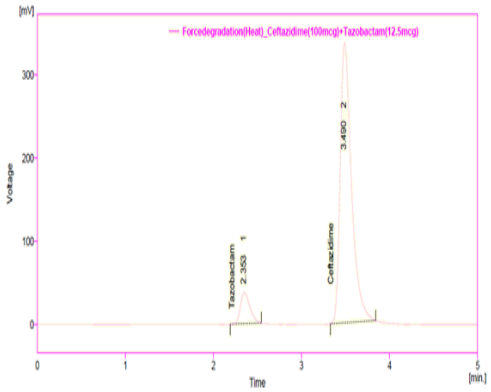

Fig: 8 Thermal Degradation

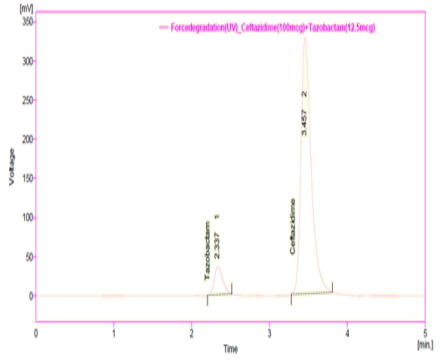

Fig: 9 Photolytic Degradation

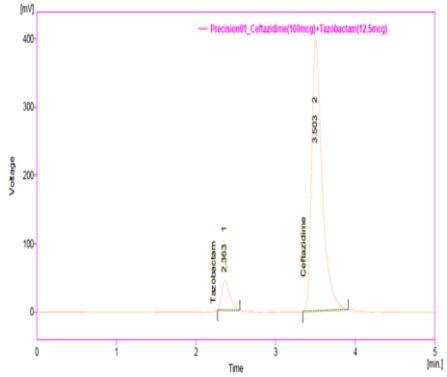

Fig: 10 Precision data

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CONCLUSION

Finally it concludes that all the parameters are within the limits and meet the acceptance criteria of ICH guidelines for method validation. The proposed method was simple, accurate, specific, precise, robust, rugged and economical. Hence this method is validated and can be used for routine and stability sample analysis.

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