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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF CEFTALAZONE AND TAZOBACTUM IN BULK AND IN ITS PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A validated RP-HPLC method was developed for simultaneous estimation of Ceftalazone and Tazobactam in bulk and in its pharmaceutical dosage form. The current method is simple, precise, and accurate and can be used for the quantification in the regular quality control tests and in industries. The optimization of the method was done by using several combinations of mobile phases and different columns and finally the chromatograms showed good resolution, retention time, peak response and lowest noise base line ratio by using Acetonitrile and phosphate buffer of pH 4 at a ratio of 70:30%v/v at a wavelength of 234nm using UV detector for detection. The retention time of Ceftalazone and Tazobactum was found to be 2.42 & 4.42 at a flow rate of 1ml/min. The current method was validated for accuracy, % assay, precision, Linearity, LOD and LOQ. The % assay of Ceftalazone and Tazobactum was found to be 101.3% and 101.8%. The linearity shown by the drugs at a concentration range of 50-150ppm of Ceftalazone and 25-75 ppm of Tazobactum showing regression co-efficient of 0.999, respectively. The LOD of Ceftalazone & Tazobactum was found to be 1.46 and 4.45 and LOQ was found to be 0.47 and 1.42, respectively. The current newly developed method was validated as per the ICH guidelines.

Keywords *– RP-HPLC, Quantification, Zerbaxa injection, Acetonitrile, Phosphate buffer pH 4, Ceftalazone, Tazobactum.*

1. INTRODUCTION

Chromatography is a separation technique in which individual components gets separated from a mixture using a combination of stationary phase and mobile phase through equilibrium distribution between two phases. There are two types of modes of separation [1-5].

a. Normal phase: Polar stationary phase and Non- Polar mobile phase

b. Reverse phase: Polar mobile phase and Non-Polar stationary phase.

HPLC is a separation technique that can be used for the analysis of organic molecules and ions. It involves a solid stationary phase, normally packed inside a stainless-steel column, and a liquid mobile phase. Separation of components based on the differences in the relative distribution ratios of the solute between two phases [6].

2. MATERIALS AND METHODS

Materials used like Potassium di-hydrogen phosphate (KH_2PO_4), Di-Potassium hydrogen phosphate (K_2HPO_4), Water, Methanol, Acetonitrile, Ortho-phosphoric acid provided by Merck, Ceftalazone and Tazobactum provided by In-house (KP Labs)

2.1 Instrumentation

HPLC Analysis Is carried on in Waters, Empower software version-2 and spectral and absorbance measured on an UV spectrophotometer - Lab India, UV win software UV- 3000+

2.2 Method Development

The objective is to develop a new method which is simple, precise, accurate and validated according to ICH guidelines.

2.2.1 Preparation of solutions

a. Preparation of phosphate buffer of pH 4

Weigh accurately 2.94 grams of KH_2PO_4, transfer into a 1000ml volumetric flask, add 100ml of HPLC grade water to dissolve it completely, diluted to 1000ml with the same and pH was adjusted to 4 with ortho-phosphoric acid. The resulting solution is filtered through 0.22µ filter paper and sonicated in a Sonicator for 15 min.

b. Preparation of Mobile phase

Mix 700ml (70%) of Acetonitrile & Phosphate buffer 300ml (30%) in a HPLC glass bottle degassed in ultrasonic water bath for 10min and filtered through 0.22µ filter under vacuum filtration.

c. Diluent preparation

The mobile phase is used as diluent.

d. Preparation of standard stock solutions (10 µg/ml and 5 µg/ml)

Weigh accurately 10 mg of Ceftalazone and 5 mg of Tazobactum, transfer into a 10ml clean dry volumetric flask, add 2ml of diluent, sonicate for 10min and make sure for complete dissolution. Make up to the mark with mobile phase / diluent. (Stock solution- 1000µg/ml). From this, 0.1ml was pipetted out into 10ml volumetric flask, add 2ml of diluent, and make up to the mark with diluent and finally sonicate the solution for 10 mins**.** (10 µg/ml of Ceftalazone and 5 µg/ml of Tazobactum)

e. Sample preparation

The vial powder is taken and weight equivalent to 10 ng of Ceftalazone and Tazobactum are taken in a 10ml clean dry volumetric flask, add 7ml of diluent/ mobile phase, sonicate to dissolve it completely and made up to the mark with diluent/ mobile phase. Further 2ml from the above solution was pipetted out into 10ml volumetric flask and diluted to the mark with diluent and then the solution is sonicated for 10 min.

2.2.2 Optimization of Chromatographic parameters

The method was optimized by performing several trials and the final method was optimized and selected based on the good retention time, resolution, and theoretical plate count and peak shape.

Trial-5: The separation of peaks is good and peak shapes obtained were good. The retention time for Ceftalazone and Tazobactum was found to be 2.42 and 4.42, respectively.

The final optimized condition for the method development and validation of Ceftalazone and Tazobactum was selected based on the trials, and the satisfactory results were obtained in the following conditions: (Trial -5.)

3 RESULTS AND DISCUSSION

The standard solutions of both the drugs was prepared at a concentration of 10 µ/ml and scanned under the UV spectrophotometer with a wavelength range of 200-400nm. The overlay spectrum of both the spectra is considered as isosbestic point and absorbance maximum shown at 234nm considered for the development and validation.

3.1 Method development optimized conditions

Table 1: Optimized chromatographic conditions

S. No.	Parameters	Description
1	Column (stationary phase)	Agilent RPC18 column (4.6x150mn) 5µ
2	Mobile phase A	Phosphate buffer of pH 4
3	Mobile phase B	Acetonitrile
4	Detector Wavelength	234nm
5	Flow rate	1ml/min
6	Mobile phase ratio	30%:70% v/v
7	Detector used	UV detector
8	Retention time	Ceftalazone – 4.42 Tazobactum – 2.24
9	Run time	10 min
10	Injection volume	10µl

3.2 Validation Results

a. Assay:

The assay calculated for Ceftalazone and Tazobactum individually by injecting the samples and standards as per the given protocol and estimated by using formulae:

$$\%\ ASSAY = \frac{\text{sample area} \times \text{dilluition of sample} \times P \times \text{average weight}}{\text{standard area} \times \text{dilution of standard} \times 100 \times \text{Label claim}} \times 100$$

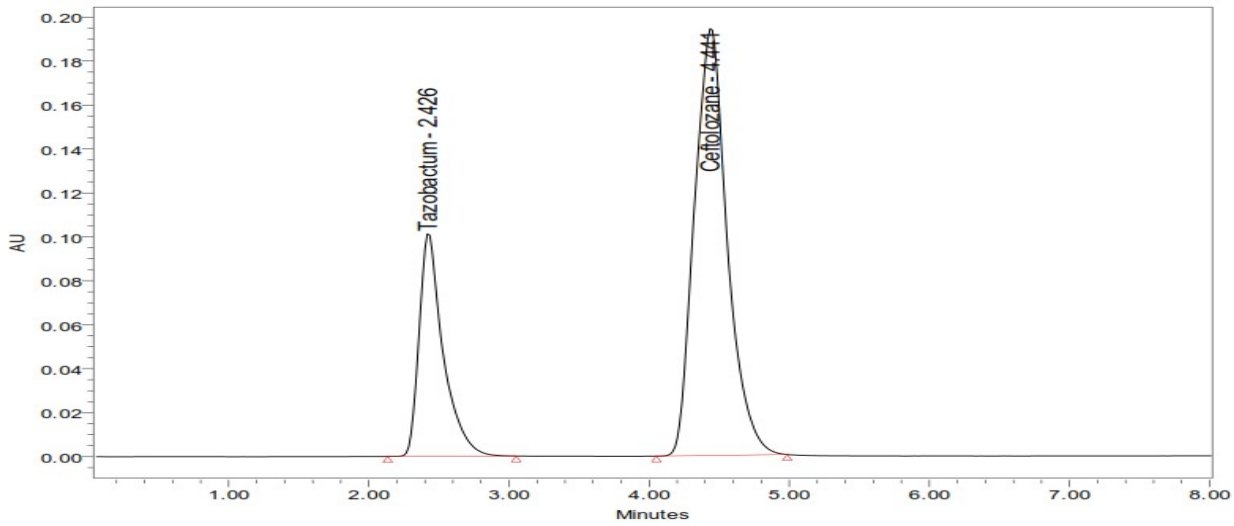

Fig. 1: Chromatogram showing assay of sample

Table 2: Results of Assay of Ceftalazone and Tazobactum

S. No.	Name of the compound	Label claim (mg)	Amount taken (mg)	% purity
1	Ceftalazone	1000	1012	101.3%
2	Tazobactum	500	510.6	101.8%

b. Accuracy

The accuracy was performed by injecting 50%, 100% and 150% of spiked samples in triplicate into chromatographic system and the peak areas, RSD and % RSD was noted, and results shown in the below tabular form:

Table 3: Results of Accuracy of Ceftalazone

% concentration at specification level	Average area	% Recovery	Mean recovery
50%	1596980	100.2%	100.34%
100%	3185292	100.1%	
150%	4810556	100.6%	

Table 4: Results of Accuracy of Tazobactum

% concentration at specification level	Average area	% Recovery	Mean recovery
50%	611071	100.2%	100.36%
100%	1221848	100.1%	
150%	1849373	100.8%	

c. Precision

The precision is carried for repeatability and intermediate precision. The samples are injected 6 times in repeatability and intermediate precision and % RSD calculated and found to be within the limits:

The intermediate precision was carried by two different analysts and % RSD calculated and found to be within the limits.

Table 5: Showing % RSD results of Repeatability Precision of Ceftalazone and Tazobactum

S. No	Sample name	Peak area	Mean	Standard deviation (SD)	% RSD
1	Ceftalazone	3170928	3175743	3311.6	0.10
2	Ceftalazone	3177606			
3	Ceftalazone	3172805			
4	Ceftalazone	3175392			
5	Ceftalazone	3181200			
6	Ceftalazone	3176524			
1	Tazobactum	1211677	1214507	1807.088	0.148
2	Tazobactum	1215077			
3	Tazobactum	1212640			
4	Tazobactum	1215705			
5	Tazobactum	1216988			
6	Tazobactum	1214954			

Table 6: Showing % RSD results of Intermediate Precision of Ceftalazone and Tazobactum

S. No	Sample name	Peak area	Mean	Standard deviation (SD)	% RSD
1	Ceftalazone (Analyst –1)	3178180	3174899	2424.5	0.076
2	Ceftalazone	3174077			
3	Ceftalazone	3172410			
4	Tazobactum (Analyst –1)	1214728	1215705	724.05	0.059
5	Tazobactum	1216495			
6	Tazobactum	1215928			
1	Ceftalazone (Analyst –2)	3176344	3176706	2796.808	0.08
2	Ceftalazone	3173476			
3	Ceftalazone	3180298			
4	Tazobactum (Analyst –2)	1216522	1218425	1795.91	0.14
5	Tazobactum	1217919			
6	Tazobactum	1220833			

d. Linearity

The linearity was determined by injecting 5 different concentrations of Ceftalazone and Tazobactum and area of each level was used for calculating correlation coefficient by plotting a graph between concentration and peak area on x-axis and y-axis respectively.

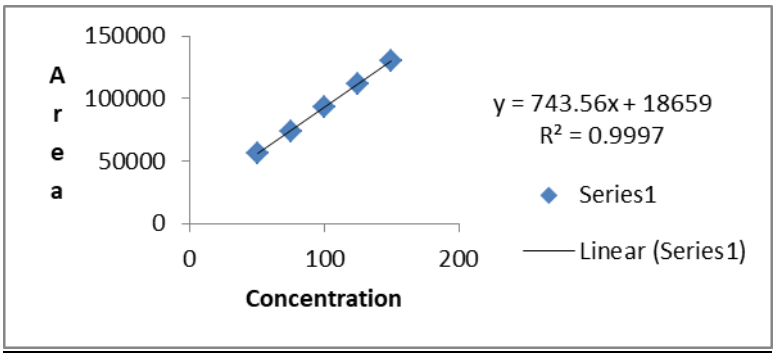

Fig. 2: Calibration curve of Ceftalazone

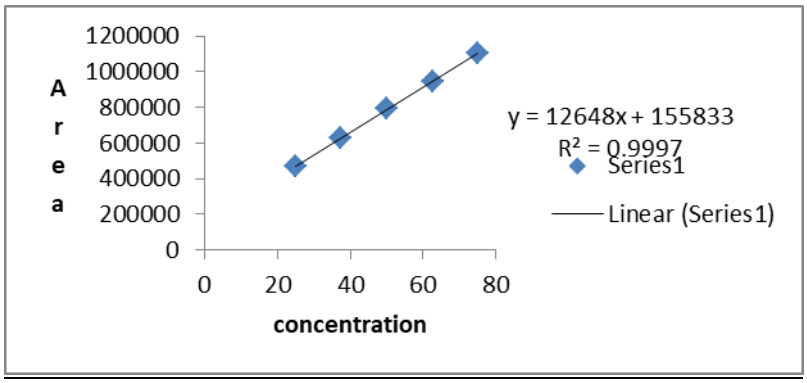

Fig. 3: Calibration curve of Ceftalazone

e. Range

Based on the results of Accuracy, Precision and Linearity data, the method was accurate, simple, precise, linear within a range of 50-150 µg/ml concentration of Ceftalazone and 25-75 µg/ml concentrations of Tazobactum respectively.

f. Limit of detection and Limit of quantification

The limit of detection and quantification was calculated based on standard deviation and slope obtained from the linearity graph.

Table 7: Results of Limit of detection

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Tazobactam	1828.2	12648	0.47
Ceftalazone	3627.6	18659	0.64

Table 8: Results of Limit of quantification

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Tazobactam	1828.2	12648	1.44
Ceftalazone	3627.6	18659	1.94

The LOD was found to be as follows: Ceftalazone – 2.34 and for Tazobactum is - 2.174.

The LOQ found to be as follows: Ceftalazone – 9.2 and for Tazobactum is - 6.7.

g. System suitability

The system suitability was done by changing the flow rate of mobile phase, organic composition of mobile phase and note down the tailing factor and plate count.

Table 9: System suitability results for flow rate

S. No.	Flow rate (ml/min)	Plate count		Tailing factor	
		Ceftalazone	Tazobactum	Ceftalazone	Tazobactum
1.	0.8	998	1517	1.5	1.6
2.	1.0	1505	1045	1.2	1.5
3.	1.2	2382	1195	1.1	1.3

4. CONCLUSION

A new method was established for simultaneous estimation of Tazobactum and Ceftalazone by RP-HPLC method. The chromatographic conditions was successfully developed for the separation of Tazobactum and Ceftalazone by using Agilent column (4.6×150mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) ACN: phosphate buffer ($KH_2PO_4$and K_2HPO_4) phosphate pH 4 (pH was adjusted with orthophosphoric acid, detection wavelength was 230 nm. The instrument used was WATERS HPLC, separation module 2695, photo diode array detector 996, Empower-software version-2. The retention time was found to be 2.437 mins and 4.466 mins. The percent purity of Tazobactum and Ceftalazone was found to be 99.87% and 100.27% respectively. The system suitability parameters for Tazobactum and Ceftalazone such as theoretical plates and tailing factor was found to be 1045, 1.6 and 1505 and 1.2. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Tazobactum and Ceftalazone was found in concentration range of 25µg-75µg and 50µg-150µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, percent recovery was found to be 100.1% and 102.3%, %RSD for repeatability was 0.2 and 0.1, % RSD for intermediate precision was 0.2 and 0.1 respectively. LOD value was 2.174 and 2.34 and LOQ value was 6.7 and 9.2, respectively.

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