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SIMULTANEOUS ESTIMATION OF CEFEPIME HCl AND TAZOBACTAM SODIUM IN INJECTION DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

*A Simple, rapid, specific and sensitive High PerformanceLiqidChromatographic method was developed for the "SIMULTANEOUS ESTIMATION Of CEFEPIMEHClAND TAZOBACTAM SODIUM IN INJECTION DOSAGE FORM BY USING RP-HPLC". Chromatographic was achieved on Inertsil-ODS C_{18} column (250mm*4.6mm, i.d.5 Microns) at ambient temperature using a binary mixture of ACN and Potassium Dihydrogen Orthophosphate as a mobile phase adjusted to pH 3.0 with Orthophoshoric acid in the ratio 40: 60 v/v was delivered through a column at a flow rate of 1ml/min. Measurement was performed at a wavelength of 226nm. The developed method was linear over the concentration range of 60-140µg/ml (r^2= 0.998) forCefepimeHCland 7.5-17.5 µg/ml (r^2= 0.991) for Tazobactam Sodium. Thepercentage recovery found to be 99.93% for Cefepime and 101.28% for Tazobactam, The method was validated for linearity, precision, accuracy, sensitivity andrhobustness as per ICH Q_2R_1guidelines.*

KEY WORDS

CefepimeHCl,Tazobactam Sodium, Validation, HPLC.

INTRODUCTION

Cefepime hydrochloride (CEFE) isfourth-generation, semisynthetic, broad spectrum, cephalosporin antibiotic for parenteral administration. Chemically, it is 1-[[(6R, 7R)-7-[2-(2-Amino-4-thiazolyl)-glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo oct-2-en-3-yl] methyl]-1-methylpyrrolidinium chloride, 72-(Z)-(O-methyl oxime), mono hydrochloride, monohydrate (**Figure 1**). CEFE is azwitterionicoxymino β-lactam with an amino-thiazole side chain, which enhances the ability of CEFE to penetrate rapidly the outer cell membrane of gram-negative bacteria. CEFE used in the treatment of moderate-to-severe infections such as pneumonia, uncomplicated urinary tract infections, skin and soft tissue infections, intra-abdominal infections and febrile neutropenia[1-5].

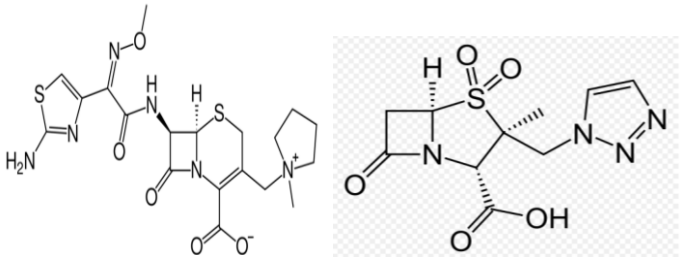

Figure 1: Chemical structure of (a) cefepime (b) tazobactam.

Tazobactam sodium (TAZO) is chemically known as (2S, 3S, 5R)-3-methyl-7-oxo-3-(1H-1, 2, 3-triazol-1-ylmethyl)-4-thia-1-azabicyclo heptane-2-carboxylic acid 4, 4-dioxide (Figure 1). TAZO is semi synthetic parenteral penicillin. TAZO is a β-lactamase inhibitor with a broad spectrum of antibacterial activity against most gram positive, gram negative aerobic bacteria and anaerobic bacteria.

ENDEAVOUR is a sterile combination of CEFE and TAZO available as a dry powder for injection. The fixed dose combination of CEFE and TAZO is used parenterallyfor the treatment of moderate to severe infection caused by or suspected of being caused by susceptible β-lactamases producing bacteria, while CEFE alone would be ineffective. CEFE and TAZO combination used for the treatment of uncomplicated and complicated urinary tract infection, uncomplicated skin and skin structure infection and complicated intra-abdominal infection.

A literature survey revealed that several liquid chromatography methods had been reported for the determination of CEFE alone as well as for stability and degradation study. CEFE in combination with other drugs had been estimated by numerous liquid chromatography methods.TAZO was also successfully determined by HPLC. Till date no chromatographic method is available for simultaneous estimation of CEFE and TAZO in combination. So, it was thought to develop simple RP-HPLC method for simultaneous estimation of CEFE and TAZO in bulk as well as in pharmaceutical formulation.

2. EXPERIMENTAL

Instrumentation: Chromatographic separation was performed using chromatography system equipped with the Shimadzu HPLC with UV/PDA detector. LC solution software was employed for data collecting and processing.

Reagents and Materials: CEFE and TAZO reference standard (RS) was obtained from a well reputed research laboratory. ENDEAVOUR, a fixed dose combination of CEFE and TAZO was purchased from the local pharmacy. Acetonitrile (HPLC grade) and potassium dihydrogen phosphate of AR grade were obtained from Merck, Mumbai.

Chromatography Condition: Chromatographic separation was performed onInertsil ODS C-18 stainless steel column with dimensions of 250*4.6mm i.d., 5 µm particle size. A binary mobile phase consisting of 25 mM potassium dihydrogen phosphate and dipotassium hydrogen phosphate buffer, pH 3.0 and acetonitrile (60: 40 v/v) was delivered through a column at a flow rate of 1 mL/min. The phosphate buffer, pH 3.0 and acetonitrile were filtered separately through a 0.45 µm membrane filter paper, mixed. The mobile phase was degassed before use. HPLC analysis was performed at ambient temperature with detection at 226 nm. The injection volume was 10 µL.

Preparation of Standard Solution

Standard Solution of CEFE (100 µg/mL): An accurately weighed quantity of powder equivalent to 10.0 mg of CEFE was transferred to 10 mL volumetric flask. The drug was dissolved and diluted to the mark with distilled water. An aliquot of about 1 mL was transferred to 10 mL volumetric flask and diluted with distilled water.

Standard Solution of TAZO (12.5 µg/mL): An accurately weighed quantity of powder equivalent to 12.5 mg of TAZO was transferred to 100 mL volumetric flask. The drug was dissolved and diluted to the mark with distilled water. An aliquot of about 1 mL was transferred to 10 mL volumetric flask and diluted with distilled water.

Preparation of sample solution: A quantity of Endeavour vial powder 125mg (equivalent to 100mg CEFEPIME and 12.5mg TAZOBACTAM) was weighed and transferred into 100ml volumetric flask. The mixture was dissolved in water, sonicated for 10 min and diluted to the mark with water to obtain a concentration of 100 µg/mLof CEFE and 12.5 µg/mLof TAZO.

Method validation:

The method was validated for accuracy, precision, intermediate precision, linearity, limit of detection, limit of quantification and ruggedness by following procedures.

Accuracy: The accuracyof the method was established from recovery experiments. The recovery was performed by adding CefepimeandTazobactam to the placebo in the range of 100 % to 140% of test

concentration. The solutions were injected into HPLC. The individual recovery and mean recovery values were also calculated.

Linearity: The linearity and range of the method was established by measuring the responses of the standard preparations of five different concentrations of Cefepime i.e. 60%, 80%, 100%, 120% and 140% and five different concentrations of Tazobctam i.e. 7.5%, 10%, 12.5%, 15% and 17.5%.Graph was shown in fig-3.

Precision: Here the precision was established by using:

Method precision: It is the closeness of agreement between the values obtained in an assay. Itis expressed as the coefficient of variation (% CV). CV is the standard deviation of the assay values divided by the concentration of the analyte. Several types of precision can be measured Intra-assay precision (repeatability) is the % CV of multiple determinations of a single sample in a single test run; Inter-assay precision (also called intermediate precision) measures the % CV for multiple determinations of a single sample, controls and reagents analysed in several assay runs in the same laboratory. The preposed method's reproducibility was checked by six samples of 100 mcg Cefepime and 12.5 mcg Tazobactam. The percentage RSD of two drugs CefepimeHCl & Tazobactam Sodium were found to be 0.65 and 1.74 respectively.

RESULTS AND DISCUSSION:

From the typical chromatogram of Cefepime and Tazobactam as shown in the **Fig 2**, it was found that the retention time of Cefepime was 3.140 min and of Tazobactam was 4.980 min, In the present developed HPLC method, the standard and sample preparations required less time and no tedious extraction were involved. A good linear relationship 0.998 was observed between a concentration range of 60-140µg/mL for Cefepime and 0.991 was observed between a concentration ranges of 7.5-17.5 µg/mL, low values of the standard deviation are indicative of high precision method. The assay of Cefepime and Tazobactam in injection dosage form were found to be 100.91% and 100.35% respectively, This indicates by high accuracy of the method. For the method, flow rate was 1.0 ml/min, retention time was found to be 3.140 min and 4.980, accuracy % mean recovery was found to be 99.93%and 101.28%, and precision % RSD was found to be 0.65% and 1.75% for Cefepime and Tazobactam respectively. Ruggedness and system suitability results were summarised in table-2&3.

CONCLUSION

The proposed method was found to be simple, sensitive, rapid and economical for the estimation of Cefepimeand Tazobactam in injection dosage form, The developed method was also checked for the performance characteristics and has also been validated. The assay by HPLC method adopted for Cefepime and Tazobactam was found to be precise, linear and accurate. It was also proved to be robust. Therefore, the proposed method can be used for routine analysis of estimation of Cefepime and Tazobactam in its injection formulation.

DRUG	CEFEPIME HCL	TAZOBACTAM SODIUM
Concentration range(µg/mL)	60-140	7.5-17.5
Slope (m)	807.55	171.73
Intercept (b)	2147.4	382.82
Correlation coefficient	0.998	0.991

Table 1: linear regression data for calibration curves

DRUG	CEFEPIME HCL	TAZOBACTAM SODIUM
No. of samples	6	6
Mean assay (%)	100.91	100.35
Std. Dev.	28.924	15.567
%RSD	0.65	1.74

Table 2: Observations for method precision

PARAMETER	CEFEPIME HCL	TAZOBACTAM SODIUM
%RSD	0.514	1.459

Table 3: Observations for ruggedness

Systemsuitability parameters	Cefepime Hydrochloride	Tazobactam Sodium
Tailing factor (T)	1.618	1.200
Number of theoretical plate(n)	2824	4573
Retention time (R_t)	3.140	4.980
LOD	0.13	0.008
LOQ	0.39	0.023

Table 4: system suitability parameters

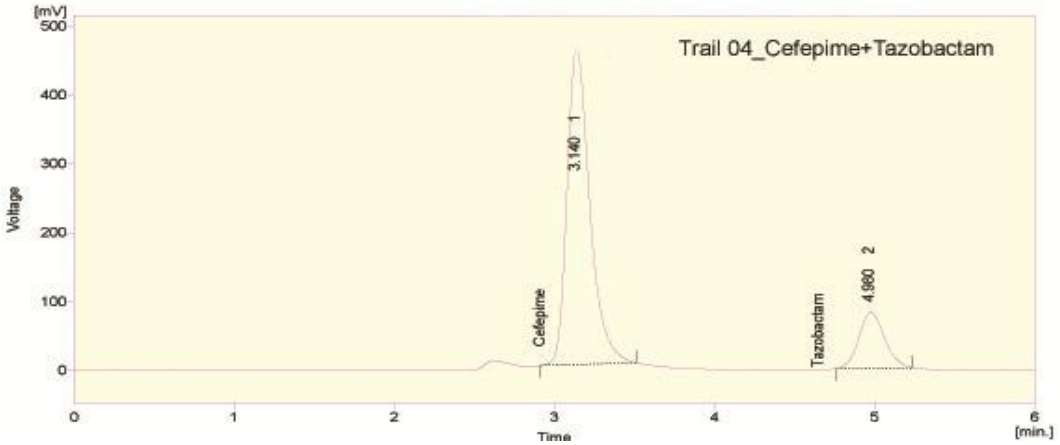

Fig 2: Optimized chromatographic conditions

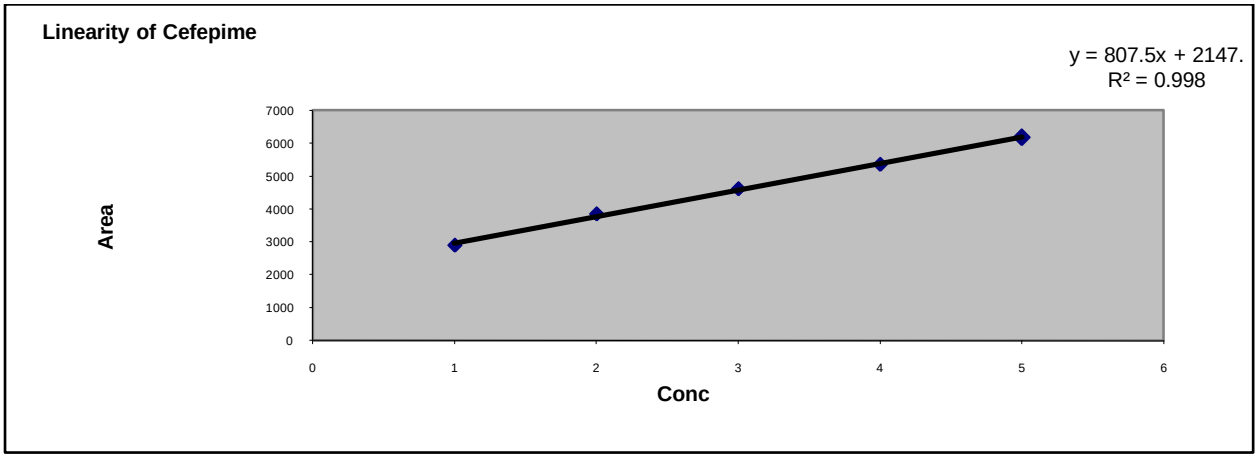

FIG 3: Chromatogram of Linearity Curve for CefepimeHCl

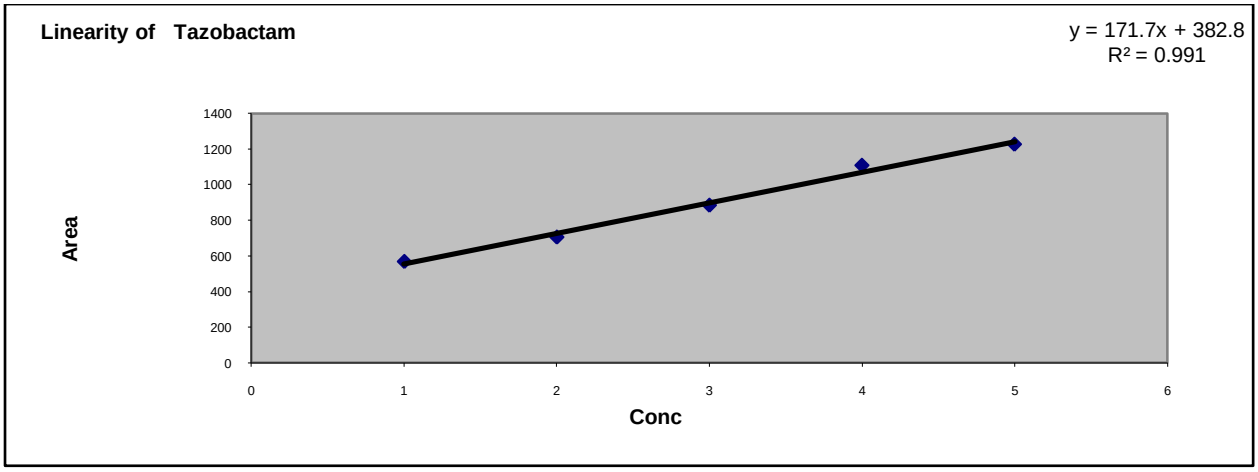

FIG 4: Chromatogram of Linearity Curve for Tazobactam Sodium

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