

Original Article

DEVELOPMENT AND METHOD VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFOPERAZONE AND TAZOBACTAM IN MARKETED FORMULATION

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ABSTRACT

Objective: A new precise, accurate, sensitive and robust RP-HPLC method was developed for third generation cephalosporin and β lactamase inhibitor i.e. cefoperazone and tazobactam in marketed preparation.

Methods: The Chromatographic separation was achieved on Thermo BDS Hypersil C18 column (250 × 4.6 mm i.d.5 μm) at ambient temperature. A Binary mobile phase consisting of 0.02 mM potassium dihydrogen phosphate buffer, pH 4.0 and Methanol (80:20, v/v) was delivered through a column at a flow rate of 1 ml/min. Measurement was performed at a desired wavelength of 250 nm.

Result: The method was linear over the concentration range of 20-60 μg/mL (r² = 0.9987) for cefoperazone (CEFO) and 2.5-7.5 μg/mL (r² = 0.9998) for tazobactam (TAZO). The percentage content for cefoperazone 97.64±1.0 and of tazobactam was 97.13±0.96 in the marketed formulation. The low value of % Relative Standard Deviation (%RSD) was 0.78 and 0.43 for CEFO and TAZO, respectively, indicates the reproducibility of this method. Tailing Factors for CEFO and TAZO were less than 2. A LOD and LOQ low value suggests the sensitiveness of method. The method was validated for linearity, precision, accuracy, and robustness as per ICH guideline.

Conclusion: It can be concluded from the results that the proposed RP-HPLC method was found to be simple, accurate, robust and precise for the analysis of cefoperazone and tazobactam in bulk and sterile dried injection dosage forms. This method was validated as per ICH guidelines. Thus, it can be used for routine quality control studies for assay of cefoperazone and tazobactam simultaneously.

Keywords: Cefoperazone, Tazobactam, Simultaneous estimation, Validation, RP- HPLC.

INTRODUCTION

Infectious diseases have always been created the threat to human being and animals[1]. Therefore, the treatment is necessary using suitable antimicrobial agents. Although various antibiotics have been developed but cephalosporin group of antibiotics are widely used[2]. Cefoperazone is a third generation cephalosporin antibiotic indicated for the treatment of patients infected with susceptible strains of microorganisms like respiratory tract, skin and soft tissue, bone and joint infections, septicemia, meningitis[3]. Cefoperazone (CEFO) is chemically [6R-[6α,7β(R)]]-7-[[[(4-Ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino](4-hydroxy phenyl) acetyl]amino]-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid. The chemical structure of CEFO is presented in figure 1. It is official drug in Indian Pharmacopoeia [4], British Pharmacopoeia [5].

Tazobactam (TAZO) is a compound that inhibits the action of bacterial β-lactamase. TAZO is semi-synthetic parenteral penicillin with a broad spectrum of antibacterial activity [6-7]. Tazobactam (TAZO) chemically known as (2S,3S,5R)-3-Methyl-7-oxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide (Figure 2). TAZO is not official drug in any Pharmacopoeia.

The combination of CEFO and TAZO is more effective for the treatment of moderate to severe infections than single drug therapy alone. CEFO and TAZO combination are used for the treatment of urinary tract infections and skin infections. A literature survey revealed that a liquid chromatography method has been reported for determination of CEFO in formulation [8]. CEFO in combination with other drugs also have been estimated by various liquid chromatography methods [9-11]. TAZO was also successfully determined by HPLC, HPTLC [12, 13]. Till date no HPLC method is available for simultaneous estimation of CEFO and TAZO in combined dosage form. Therefore present work involves the development of a simple RP-HPLC method for simultaneous

estimation of CEFO and TAZO in bulk as well as in pharmaceutical formulation.

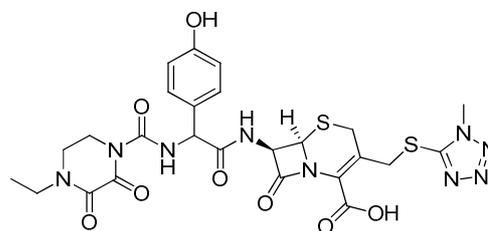


Fig. 1: Chemical structure of Cefoperazone

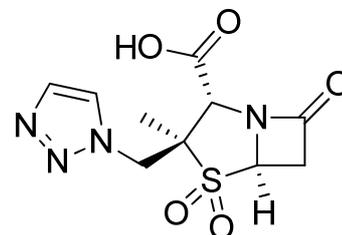


Fig. 2: Chemical structure of Tazobactam

MATERIALS AND METHODS

The reference standard (RS) of CEFO and TAZO were obtained from Molecule Laboratory Pvt. Ltd, Ahmedabad, India. The fixed dose combination of CEFO and TAZO (Lupitam) was purchased from the local pharmacy. Each unit of Lupitam contains 1000 mg of Cefoperazone and 125 mg of Tazobactam. The Methanol (HPLC

grade) and potassium dihydrogen phosphate (of AR grade) were obtained from Merck, Mumbai, India.

Instrumentation and Chromatographic Conditions:

Chromatographic separation was performed using chromatography (LC2010C_{HT}, Shimadzu, Japan) system equipped with the Agilent SPD-20AT prominence diode array detector. Spin Chrome software was employed for data collecting and processing Chromatographic separation was performed on BDS Hypersil C-18 stainless steel column with dimensions of 250×4.6 mm, 5 µm particle size.

A binary mobile phase consisting 0.02M potassium dihydrogen phosphate buffer, pH 4.0 and Methanol (80:20 v/v) was delivered through a column at a flow rate of 1 mL/min. The phosphate buffer, pH 4.0 and Methanol was filtered separately through a 0.45 µm membrane filter paper. The mobile phase was degassed before use. HPLC analysis[14,15] was performed at ambient temperature with detection at 250 nm. The injection volume was 20 µL.

Preparation of standard stock solution

Standard stock solution of CEFO (400µg/mL): An accurately weighed quantity of powder equivalent to 40.0 mg of CEFO was transferred to 100 mL volumetric flask. The drug was dissolved and diluted up to the mark with methanol.

Standard stock solution of TAZO (50µg/mL): An accurately weighed quantity of powder equivalent to 5.0 mg of TAZO was transferred to 100 mL volumetric flask. The drug was dissolved and diluted up to the mark with methanol.

Assay of marketed formulation:

To determine the content, 10 vial units (Lupitam) were individually weighed and average weight was recorded. Dry powder from all vials was mixed together to make a pooled sample. A quantity of vial powder (≅ 40.0 mg of CEFO and 5.0 mg of TAZO) was weighed and transferred into 100 mL of volumetric flask.

The mixture was dissolved in methanol, sonicated for 10 min and diluted to the up to mark with methanol to obtain a concentration of 400 µg/mL of CEFO and 50 µg/mL of TAZO. The solution was filtered using Whatmann filter paper No. 41. This solution was further diluted with mobile phase to obtain final concentration of CEFO (40 µg/ml) and TAZO (5µg/ml). The final sample solution was filtered (Whatmann filter paper No. 41) and injected. Run time of analysis was kept 15 min and detection is carried out at 250 nm. All the determinations were carried out in triplicate.

METHOD VALIDATION

Validation of an analytical procedure is the process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirements for the intended analytical application. The developed chromatographic method was validated for system suitability, linearity and range, accuracy, precision, and robustness, as per ICH guidelines [16].

System suitability test

The system suitability test was performed by five replicate analyses of working standard solution. Results of %RSD, retention time, theoretical plates and tailing factor (peak symmetry) are presented in Table 1.

Analysis of marketed formulation

Results of assay were in good agreement with the label claim. The drug content in the unit was found to be 97.64±1.00 % and 97.13±0.963% for Cefoperazone and Tazobactam, respectively, are shown in Table 2.

Linearity and range

Working solutions were injected under the operating chromatographic conditions and peak areas for each drug were calculated at 250 nm. The calibration curve was plotted between areas against corresponding concentrations of each drug. Linear regression data for calibration curves were shown in Table 3. The

range of solution has been decided according to correlation coefficient of regression equation.

Accuracy (% recovery)

The accuracy of the method was determined by calculating % recovery of each drug by standard addition method. Percent recovery of CEFO and TAZO was determined at three different level 80%, 100% and 120% of the target concentration in triplicate, the results obtained were compared with expected results and statistically validated, are shown in Table 4.

Precision

Inter-day and Intraday precision were carried out in following concentration range 20, 40, 60µg/ml of CEFO and 2.5, 5, 7.5µg/ml of TAZO, respectively. At different time intervals in the same day and at the same time on different days. The variation in the results was analysed and statistically validated. The % relative standard deviation (%RSD) values for inter-day and intra-day precision for CEFO and TAZO are shown in Table 5.

Robustness

Robustness evaluation showed the reliability of analysis respect to deliberate variations in method parameters. The method was studied by changing flow rate (±0.2 ml/min), change in pH (±0.2), and change in mobile phase (±2%v/v) during analysis. Sample solution of 100% concentration is prepared and injected in triplicate for every condition and % Assay was calculated for each condition are shown in Table 6.

LOD and LOQ

The standard deviation of the Y-intercept and average slope of the calibration curve was used to calculate LOD and LOQ using following formulae.

$$LOD = 3.3 \frac{SD}{S} \quad LOQ = 10 \frac{SD}{S}$$

LOD - Limit of detection,

LOQ - Limit of quantitation

Where, S is average value of slopes of calibration plots and SD is calculated using values of y intercepts of regression equations are shown in Table 7.

RESULTS AND DISCUSSION

Initially various mobile phases were tried in attempt to obtain the better separation and good resolution between CEFO and TAZO combined dosage form. Finally potassium dihydrogen phosphate (0.02M KH₂PO₄) Buffer: Methanol (80:20) at pH 4.0) was found to be an appropriate mobile phase allowing good separation of both the compounds using, Hypersil BDS C18 column at 40±5°C at 1 mL/min. flow rate. As the CEFO and TAZO exhibit significant absorbance at wavelength 250 nm, therefore it was selected as detection wavelength for simultaneous estimation of CEFO and TAZO in marketed formulation. These optimized conditions had acceptable system suitability parameters indicate good resolution for both the peaks (Table 1). The value of % Relative Standard Deviation (%RSD) was 0.78 and 0.43 for CEFO and TAZO, respectively, indicates reproducibility of the method. The Number of theoretical plates for CEFO and TAZO were 3523 and 4253, respectively, in acceptance limit. Tailing Factors for CEFO and TAZO were 1.54 and 1.56, respectively, not more than 2. LOD and LOQ for CEFO was 5.22, 16.02 that for TAZO was 0.4245, 14.15, respectively (Table 7) and these values were acceptable and therefore the optimized conditions were used for further analysis. The retention time for CEFO and TAZO were 3.86 and 7.57 min., respectively. The values of correlation coefficient for CEFO and TAZO (Table 3) demonstrated the good relationship between peak area and concentration. Therefore, the developed method was linear in concentration range of 20-60 µg/mL for CEFO and 2.5 -7.5µg/mL for TAZO. The percentage assay of CEFO and TAZO in sterile dried powder samples were 97.64% and 97.13%, respectively (Table 2). Percent recovery was 99.51±0.70% for CEFO and 98.42±0.51% for TAZO

demonstrated accuracy. The high recovery obtained indicates that the proposed method is highly accurate. The low value of % RSD in intra-day and inter-day precision (Table 5) indicated reproducibility of this method.

Finally, deliberate variations were made to check the significant variations in experimental conditions (Table 6) suggested robustness of developed method.

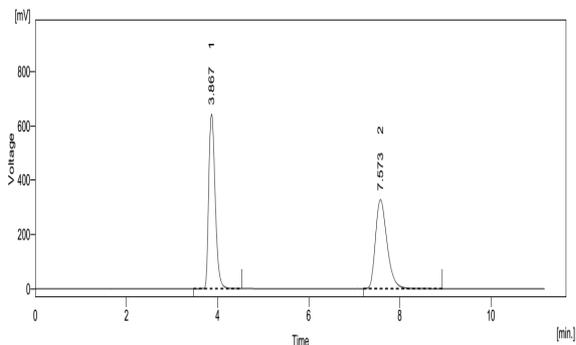


Fig. 3: Standard Solution of CEFO and TAZO

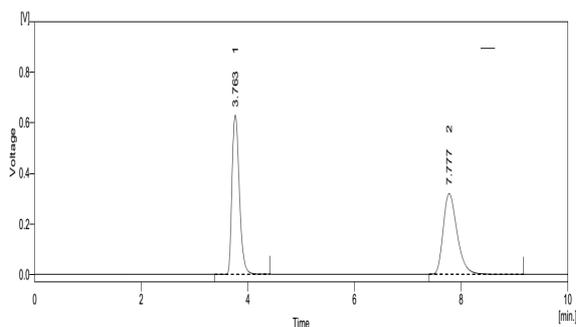


Fig. 4: Sample solution of CEFO and TAZO

Table 1: Results of system suitability test

Parameter	Cefoperazone	Tazobactam
SD*	2490.83	2071.57
%RSD*	0.783	0.435
RT(min)	3.86	7.57
Theoretical Plate	3523	4253
Tailing Factor	1.54	1.56

*n=5

Table 4: Accuracy data of CEFO and TAZO

Drug	Amount Added (µg/ml)	Amount recovered (µg/ml)	Mean % Recovery ± SD*	% RSD*
CEFO	80% (16 µg/ml)	15.90	99.84% ± 0.5575	0.558
	100% (20 µg/ml)	19.90	99.51% ± 0.7084	0.711
	150% (24 µg/ml)	23.87	99.49% ± 0.1756	0.176
TAZO	80% (2 µg/ml)	1.96	99.17% ± 0.8605	0.867
	100% (2.5 µg/ml)	2.48	98.92% ± 0.5112	0.516
	120% (3.5 µg/ml)	2.95	98.94% ± 0.4056	0.409

*n=3

Table 5: Precision data of Cefoperazone and Tazobactam

Parameter	Cefoperazone	Tazobactam
Inter-day(%RSD)*	0.4606	0.6179
Intra-day(%RSD)*	0.7839	0.4352

(*n=6)

Table 2: Results of Assay of Marketed formulation

Parameters	Cefoperazone	Tazobactam
Label Claim (mg)	1000	125
Actual content found (mg)	974.68	122.73
% Avg Assay(n=3)	97.64	97.13

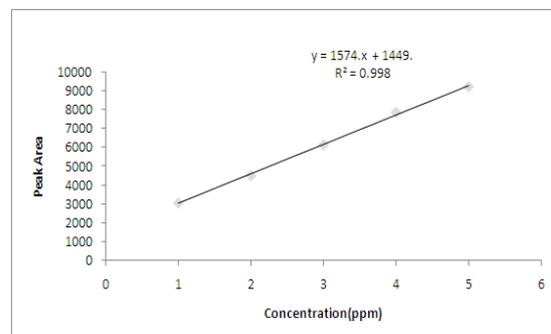


Fig. 5: Calibration curve of Cefoperazone

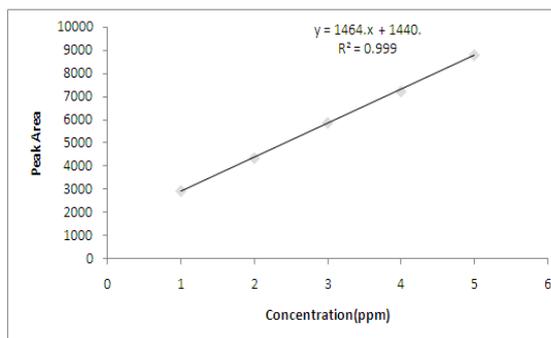


Fig. 6: Calibration curve of Tazobactam

Table 3: Linear regression data for calibration curves of Cefoperazone and Tazobactam

Parameters	Cefoperazone	Tazobactam
Linearity range (µg/ml)	20 – 60	2.5 – 7.5
Coefficient of correlation	0.9987	0.9998
Slope	1574.00	1464.00
Intercept	1449.00	1440.00

Table 6: Robustness studies of CEFO and TAZO

Change in flow rate(1 ml/min)	Flow rate(ml/min)	Average*	Std Dev	%RSD
Cefoperazone	1.2 ml/min	6011.004	69.80938	1.16136
	0.8 ml/min	6385.375	38.39933	0.60136
Tazobactam	1.2 ml/min	5707.367	62.59649	1.09676
	0.8 ml/min	6066.191	63.03829	1.03917
Change in mobile phase composition Buffer:Methanol(80:20)v/v	Mobile phase(80:20)v/v	Average*	Std Dev	%RSD
Cefoperazone	(78:22)v/v	6003.18	68.59503	1.142645
	(82:18)v/v	6307.907	58.89389	0.933652
Tazobactam	(78:22)v/v	5703.431	57.92355	1.015591
	(82:18)v/v	5998.32	52.38804	0.873379
Change in pH(4.0) ±0.2	Change in pH	Average*	Std Dev	%RSD
Cefoperazone	4.2	5890.12	64.815	65.997
	3.8	6322.73	1.1004	1.0438
Tazobactam	4.2	5598.82	54.336	50.325
	3.8	6013.94	0.9704	0.8368

*n=3

Table 7: LOD and LOQ

Parameter	Cefoperazone	Tazobactam
LOD	5.22	0.4245
LOQ	16.02	14.15

CONCLUSION

The present work represents the first report that deals with simultaneous analysis of cefoperazone and tazobactam in bulk and sterile dried injection dosage forms using RP-HPLC. It can be concluded from the results that the proposed method is simple, accurate, robust and precise. This method was validated as per ICH guidelines. Thus, it can be used for routine quality control studies for assay of cefoperazone and tazobactam.

CONFLICT OF INTERESTS

Declared None

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