Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(5):23-27
(http://www.derpharmachemica.com/archive.html)

Method Development and Validation for the Simultaneous Determination of Cefepime and Tazobactam in Injectable Dosage form by RP-HPLC

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ABSTRACT

A Simple, Accurate and Precise method was developed and validated for the simultaneous determination of cefepime and tazobactam in injectable dosage form. Isocratic separation was achieved on YMC C18 column (250 × 4.6 mm, particle size 5 µm) using a mobile phase consisting of methanol-phosphate buffer (60:40 v/v) at a rate of 1 ml per minute and using ultra violet detector (230 nm). Linearity was observed over the concentration range of 12.5 to 37.5 µg/ ml (r^2-0.99) for tazobactam and 100 to 300 µg/ml (r^2-0.999) for cefepime. The % mean recovery of the method was 99% for tazobactam and 100% for cefepime. The limits of detection (LODs) were 0.149 and 1.2024 for tazobactam and cefepime and limits of quantification (LOQs) were 0.495 and 4.0080, respectively. The method was validated according to International Conference on Harmonization (ICH) Guidelines. The results of this method are proved that the method would have great value when used for the analysis of dosage form.

Keywords: Cefepime, Tazobactam, International Conference on Harmonization (ICH), RP-HPLC, Validation

INTRODUCTION

Cefepime is used to treat moderate to severe nosocomial pneumonia, infections caused by multiple drug-resistant microorganisms (e.g. *Pseudomonas aeruginosa*) and empirical treatment of febrile neutropenia. Multiple drug-resistant *Streptococcus pneumonia* (MRSA). Chemically, cefepime is 1-{[(6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxylato-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl}-1 methylpyrrolidin-1-ium (Figure 1a) cefepime is soluble in methanol, water and acetonitrile. Cephalosporins are bactericidal and have the same mode of action as other beta-lactam antibiotics (such as penicillins). These disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity, especially in Gram positive organisms. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidases known as penicillin binding proteins.

Tazobactam administered an effective treatment for patients with lower respiratory tract, intra-abdominal, urinary tract, gynecological and skin/soft tissue infections and for fever in patients with neutropenia. Chemically, Tazobactam is (2S,3S,5R)-3-methyl-4,4,7-trioxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4$l^{6}-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (Figure 1b). tazobactam is soluble in water, ethanol, methanol and acetonitrile. Tazobactam broadens the spectrum of piperacillin by making it effective against organisms that express beta-lactamase and would normally degrade piperacillin [1-10].

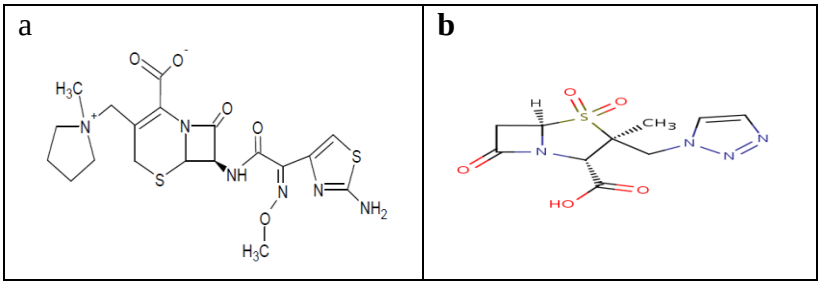

Figure 1: Chemical structures of cefepime (a) and tazobactam (b)

This method was very economic, simple precise and accurate for the determination of cefepime and tazobactam in bulk drugs as well as formulations. Some other methods for the determination of cefepime HCl and tazobactam sodium in pharmaceutical formulations including High Performance Liquid Chromatography (HPLC), gas chromatography (GC), ultra violet (UV). It was an attempt to develop economic RP-HPLC Method for simultaneous determination of cefepime and tazobactam in injectable dosage form. In the present study, a simple, economic and precise reverse phase HPLC method for simultaneous determination of cefepime and tazobactam in injectable dosage form was developed and validated successfully according to the International Conference on Harmonisation (ICH) guidelines.

EXPERIMENTAL

Cefepime and tazobactam (purity >99.5%) were produced from the Sura Laboratories Ltd., Hyderabad, India. Methanol (Merck Ltd, Mumbai) was of HPLC grade. Analytical grade di potassium hydrogen phosphate, triethylamine, phosphoric acid and sodium hydroxide were produced from S.D. Fine chemicals Ltd, Mumbai. The HPLC water was purchased from local chemical store. Injectable dosage form was purchased from local medical store. All the other chemicals used were of analytical grade.

HPLC instrumentation and analytical conditions

HPLC system (Schimadzu LC-20AD System) equipped with a pump and a UV detector was used in this study. For data acquisition and processing EMPOWER software was employed. The analysis was performed on YMC C18 column (250 × 4.6 mm, particle size 5 µm). The temperature of column was maintained at 30°C. Isocratic elution was performed using a mobile phase of methanol/phosphate buffer pH 3.0 (60:40) at a flow rate of 1 ml/min. The volume of the injection was 10 µl and the detector wavelength was 230 nm.

Standard and sample solutions

Phosphate buffer was prepared by dissolving 4.35 g of di potassium hydrogen phosphate in 250 ml of water and filtered through 0.45 µm membrane filter and adjusting the pH with dilute phosphoric acid solution. Standard solutions were prepared by dissolving 1000 and 125 mg of cefepime and tazobactam in 10 ml of mobile phase and 0.1 ml of each solution was further diluted to 50 ml using mobile phase in order to obtain a concentration of 0.2 and 0.025 mg/ml of cefepime and tazobactam, respectively. Sample solution was prepared by commercially available injection powdered equivalent to the 1000 mg of cefepime HCl and 125 mg of tazobactam sodium was weighed and transferred into a volumetric flask of 10 ml and add 10 ml of mobile phase, transfer 0.1 ml of above sample solution into a 50 ml of volumetric flask, dilute to volume with mobile phase and mix [11-21].

RESULTS AND DISCUSSION

Development of method

Preliminary studies were carried out in order to optimize a suitable method for simultaneous determination of cefepime and tazobactam in injectable dosage form. Trail runs were performed by using C8 and C18 reversed-phase columns, several compositions of solvents (mobile phase) and different flow rates for separation of both drugs with good chromatographic conditions (resolution, symmetry, tailing factor etc.). Stationary phase of A C18 YMC column (25 × 4.6 mm, particle size 5 µm) with mobile phase of methanol/phosphate buffer (60:40 v/v) at a flow rate of 1 ml/min and a detection wavelength of 230 nm afforded with the better separation with well-determined and sharp peaks of both the drugs. The separation was performed on an isocratic mode and the volume of injection was 10 µl.

Method validation

Development of optimized method was completed then this method was validated in terms of following parameters: linearity and range, accuracy and percentage recovery, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness.

Linearity and range

The linearity of this method was evaluated by analyzing five working solutions (calibration standards) of cefepime containing 100, 150, 200, 250, 300 µg/ml and of tazobactam 12.5, 18.75, 25, 31.25, 37.5 µg/ml. The plots of peak areas versus concentrations were linear in the range from 100 to 300 µg/ml and 12.5 to 37.5 µg/ml of cefepime and tazobactam respectively. The regression equations were obtained as follows: y=43363x (r^2-0.99) for tazobactam and y=34582x (r^2-0.99) for cefepime, where y=peak area and x=concentration of solution; r=the square of determined correlation coefficient. These results are proved that the developed method was linear over the specified range.

Accuracy and percentage recovery

Recovery and Accuracy studies were carried out at three different levels of concentration that are 50, 100 and 150%. The study was performed three times (n=3). The average recovery of cefepime was found 100.24% and of tazobactam was found 99.15% (Table 1), which indicates the accuracy of the method.

Table 1: Accuracy results of cefepime and tazobactam

Drug	Spiked level (µg/ml)	Spiked amount* (µg/ml)	Measured amount* (µg/ml)	Recovery (%)
Cefepime	50	100	99	99
	100	200	199.7	99.85
	150	300	305.6	101.87
Mean (%)	100.24			
Tazobactam	50	12.25	11.8	96.97
	100	24.5	24.6	100.4
	150	36.75	36.8	100.1
Mean (%)	99.15			

*Mean of three determinations (n=3) for each concentration

Precision

The relative standard deviation for precision was observed that not more than 2%. The percentage assay for precision study was observed between 100 to 101% for cefepime and 97 to 98% for tazobactam. The Precision results are presented in Tables 2 and 3.

Table 2: Precision data for cefepime

S. No.	RT	Area	%Assay
Injection 1	4.2	6998292	101
injection 2	4.198	6995281	101
injection 3	4.184	6951575	100
injection 4	4.182	6949221	100
injection 5	4.214	6946878	100
injection 6	4.172	6943228	100
Mean	-	-	100
Std. Dev.	-	-	0.37
%RSD	-	-	0.37

Table 3: Precision data for tazobactam

S. No.	RT	Area	%Assay
Injection 1	2.323	4306443	97
Injection 2	2.325	4320958	97
Injection 3	2.317	4342409	98
Injection 4	2.319	4325637	98
Injection 5	2.339	4367514	98
Injection 6	2.317	4345992	98
Mean	-	-	98
Std. Dev.	-	-	0.49
% RSD	-	-	0.5

LOD and LOQ

The LODs for cefepime and tazobactam corresponding to a signal-to-noise ratio of were 1.2024 and 0.149 respectively. The LOQs corresponding to a signal-to-noise ratio of 10 were 4.0080 and 0.495 respectively.

Robustness

Robustness of the method was studied by applying minor variations in the chromatographic conditions like pH of the eluent, flow rate and column temperature. System suitability parameters such as number of theoretical plates, retention time, tailing factor were studied. The performance of the developed method was unaffected even after small deliberal changes made in the selected chromatographic conditions which proved that the method was robust. The results of system suitability parameters were found to be satisfactory. The results of robustness and system suitability parameters are given in Tables 4-6.

Table 4: Robustness data for tazobactam

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate (0.8 ml/min)	2.322	3182	1.45
Increased flow rate (1.2 ml/min)	2.314	3268	1.46
Decreased temperature (25°C)	2.890	3594	1.43
Increased temperature (35°C)	1.954	2948	1.44

Table 5: Robustness data for cefepime

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate (0.8 ml/min)	4.191	4015	1.46
Increased flow rate (1.2 ml/min)	4.109	4197	1.39
Decreased temperature (25°C)	5.033	4769	1.37
Increased temperature (35°C)	3.469	3672	1.39

Table 6: System suitability data of tazobactam and cefepime

Parameter	Tazobactum	Cefepime	Acceptance criteria
Retention time	2.3	4.2	
Theoretical plates	2520	3051	>2500
Tailing factor	1.39	1.33	<2.00
% RSD	0.7	0.6	<2.00

Assay of cefepime and tazobactam in injectable dosage form

Once development and validation of the method successful then this method was applied for analysis of cefepime and tazobactam in injectable dosage form Figure 2. The method shows excellent separation with good resolution between the two analytes. The higher percentage recovery and non-interference of the formulation excipients in retention time of the drugs show the selectivity of this method for the determination of both the analytes in dosage form. Satisfactory results were obtained that the mean found for cefepime is 101% and tazobactam is 101% were good agreement with the label claim (Table 7).

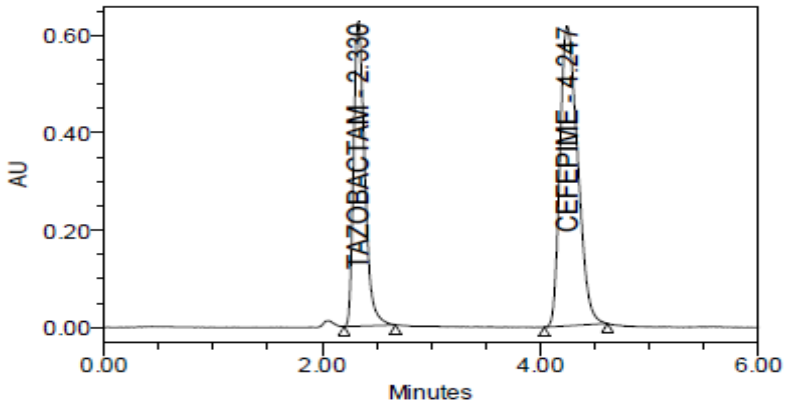

Figure 2: Chromatogram of cefepime and tazobactam. Peak asymmetry and theoretical plates of cefepime are 1.33 and 3051 and of tazobactam are 1.39 and 2520, respectively

Table 7: Assay results of cefepime and tazobctam in injection dosage form

Drug	Label claim (mg)	Amount found* (mg)	%estimated
Cefepime	1000	999.0 ± 1.1	99.9
Tazobactam	125	124.37 ± 0.6	99.5

*Mean of three determinations (n=3)

CONCLUSION

A simple, economic, accurate and precise method was developed and validated as per ICH guidelines in terms of accuracy, precision, linearity, LOD, LOQ, robustness, for the determination of cefepime and tazobactam in injectable dosage form. The developed method was free from interferences due to excipients present in the formulation. Therefore, this method may be useful for the analysis of commercial formulations economically [22-36].

ACKNOWLDGEMENTS

The authors are thankful to jyothishmathi institute of pharmaceutical sciences, Karimnagar, Telangana for providing necessary facilities to carry out this work.

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