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Validated RP-HPLC Method for the Simultaneous Determination of Tazobactam and Cefepime in Injectable Generic Combination Formulation

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ABSTRACT

In the present communication here we reported the development and validation of a new isocratic RP-HPLC method for the assay of tazobactam and cefepime in injectable generic combination form. The experimental operating factors influencing the maximum elution of these drugs were exclusively studied and optimized {Hypersil C_{18} column (250×4.6 mm, 5µ) using the mobile phase [KH_2PO_4 buffer (pH-3.5) and acetonitrile in the ratio of 45:55%v/v] with a flow rate of 1.0 mL min^{-1} and UV detection at of 230 nm in ambient column temperature}. The retention times for tazobactam and cefepime were found to be 2.329 min and 4.252 min respectively. Linearity was observed over the concentration range of 10-30 µg mL^{-1} for tazobactam and 50-150 µg mL^{-1} for cefepime respectively. The limits of detection and quantitation of the proposed method were 0.00717 and 0.0239 µg mL^{-1}, for tazobactam and 0.0147 and 0.049µg mL^{-1} for cefepime respectively. The values of other parameters precision, accuracy, sensitivity and robustness etc., are within the acceptance limits of ICH Q2 (R1) guidelines. The student's t and F-values at 95% confidence level did not exceed the tabulated t- and F-values, showing excellent agreement with those achieved by the reported methods. The validation results of the proposed method offered preferential advantages over most of the reported methods in terms of easy, precise, reliable, and economical.

Graphical Abstract

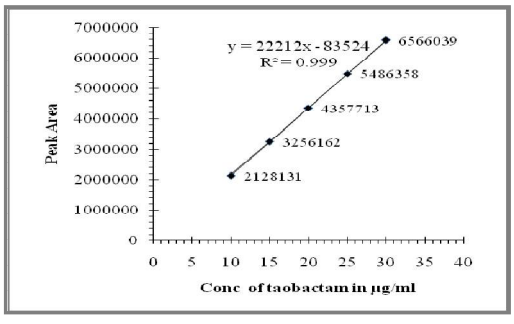

Figure 3. Calibration curve of tazobactam.

Keywords: RP-HPLC, Tazobactam, Cefepime, Validation, Injectable generic combination form, ICH Guidelines.

INTRODUCTION

Tazobactam [**1**], [2*S*-(2α, 3β, 5α)]-3-Methyl-7-oxo-3-(1*H*,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo [3.2.0] heptan-2-carbonsäure-4,4-dioxid is an penicillanic acid sulfone derivative (Figure 1a) used to treat infections caused by gram negative aerobic bacteria and anaerobic bacteria. And where as cefepime hydrochloride [**2-4**], 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-methyl pyrrolidinium chloride,72-(Z)-(O-methyl oxime), mono hydrochloride, monohydrate (Figure 1b) is fourth-generation, semisynthetic, cephalosporin antibiotic 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.

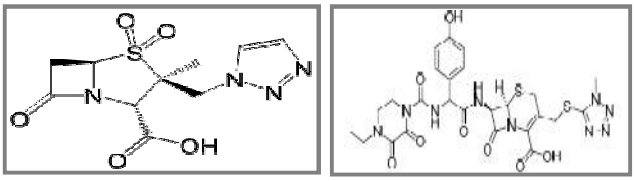

Figure 1a. Chemical structure of tazobactam, **b**, Chemical structure of cefepime

Fixed dose combination of tazobactam and cefepime (1–Vial Injection Salt containing Cefepime-1000mg;Tazobactam-125mg) is available under 17 brands for parenteral administration, at local pharmacies (FORPAR XP by Cipla, RESIPIME-T by Rescuers Life science, DRAPIME-TZ By Rapid Life Drugs and Healthcare etc) and is used for the treatment of uncomplicated and complicated urinary tract infection, uncomplicated skin and skin structure infection and complicated intra-abdominal infection [**5**].

However, there are few HPLC methods [**6-11**] were reported for the simultaneous estimation of these drugs. Hence, in the present study an attempt has been made to develop simple, and accurate, sensitive, precise and repeatable RP-HPLC method, for the simultaneous estimation of both drugs in injection dosage form.

MATERIALS AND METHODS

Instrumentation: The present analysis was carried on Water's 2695 HPLC system provided with Hamilton Syringe, Hypersil C_{18} column (250×4.6 mm, 5µ), auto sampler and 2996 UV-Photodiode array detector. Data was acquired and processed with Empower 2 software. Shimazdu (Tokyo, Japan) electronic weighing balance (Model BL 220 H) was used for weighing the samples. Elico pH meter (Hyderabad, India) LI 120 model was used for pH measurements. All dilutions were performed in standard class-A, volumetric glassware (Borosil make).

Chemicals and reagents: Pharmaceutical grade pure samples of tazobactam and cefepime (99.9%) were obtained from Cipla Ltd, Hyderabad as gifted samples and its commercial formulation (Generic form) in the brand name of DRAPIME-TZ injectable vial (Cefepime-1000mg; Tazobactam-125 mg) were procured from the local pharmacy**.** Milli-Q water, Methanol (HPLC Grade), Acetonitrile (HPLC Grade), Orthophosphoric acid (GR Grade), and potassium dihydrogen orthophosphate monohydrate (GR Grade) were purchased from Qualigens Ltd., Mumbai.

Preparation of phosphate buffer: The buffer was prepared by dissolving 2.72g of Potassium dihydrogen ortho phosphate in 1000 mL of milli-Q water. The pH of the buffer solution was adjusted to 3.5 ± 0.05 with ortho phosphoric acid.

Mobile phase preparation: Prepare a filtered and degassed mixture of acetonitrile and phosphate buffer (pH-3.5) in the ratio of 55:45 %v/v respectively.

Diluent preparation: Methanol is used as diluent in the present assay.

Preparation of stock and working standard solutions: Standard stock solutions of the present studied drugs was prepared by weighing accurately 10 mg of tazobactam and 100 mg cefepime were transferred into a clean and dry 100 mL volumetric flask. To this flask about 50 mL of diluent was added and sonicated for five minutes. Later, the volume of the flask was made upto the mark with the mobile phase (Concentrations 100 µg mL^{-1} for tazobactam and for 1000 µg mL^{-1} cefepime). From the above prepared stock solution pipette out suitable aliquots and transferred into a clean and dry 10 mL volumetric flask, mobile phase was added up to the mark to get final concentration of 10-30 µg mL^{-1} for tazobactam and 50-150 µg mL^{-1} for cefepime respectively.

Preparation of sample solution: 10 vial units (DRAPIME-TZ injectable vial (Cefepime-1000 mg, Tazobactam-125 mg) 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 (10 mg of tazobactam and 1000 mg cefepime) was weighed and transferred into 100ml 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 100 µg mL^{-1} for tazobactam and for 1000 µg mL^{-1} cefepime respectively. The solution was filtered using Whatmann filter paper No.41. From above prepared sample stock solution pipette out aliquots of the above solution and transferred into a clean and different dry 10 mL volumetric flasks. Mobile phase was added up to the mark 10ml to get final concentration of 10-30 µg mL^{-1} for tazobactam and 50-150 µg mL^{-1} for cefepime, respectively. 20 µL volumes of these standard and sample solutions were injected five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

RESULTS AND DISCUSSION

HPLC method development: In the development of the present method for the selected combination a number of experimental trials were made by changing the columns and mobile phase by varying its composition as well as by changing the solvents. All these trials have resulted either in low resolution or asymmetric peaks or peaks with more tailing factors or longer time of elution.

Varying compositions of acetonitrile and KH_2PO_4 buffer (pH 3.5 adjusted with phosphoric acid) 60 : 40, 50 : 50, 55 : 45, and 40 : 60 % v/v were evaluated as mobile phase in order to achieve good peak shape and short run time. Using the above mobile phase and column the effect effluent flow rate (0.5-1.5 mL min^{-1}) on resolution was monitored. From these studies flow rate of 1.0 mL min^{-1}, was found to be satisfactory to obtain good peak symmetry, resolution of the two drugs respectively. As tazobactam and cefepime exhibited significant absorbance at wavelength 230 nm, and this was selected as detection wavelength in the current study.

However, finally the Hypersil C_{18} column (250×4.6 mm, 5 µ) with a flow rate of 1.0 mL min^{-1} of mobile phase and UV detection at a wavelength of 230 nm and ambient column temperature with mobile phase of and phosphate buffer (pH-3.5) in the ratio of 55:45 %v/v resulted in excellent elution of the two drugs with low retention and run times. With the above optimized conditions the chromatogram (Figure 2) of the cited drugs (tazobactam and cefepime) were resolved with retention times (2.329 min and 4.252 min for tazobactam and cefepime respectively) and theoretical plates and good resolution respectively.

Method validation: The developed RP-HPLC method was validated in accordance with ICH guidelines [**12**] using the following parameters.

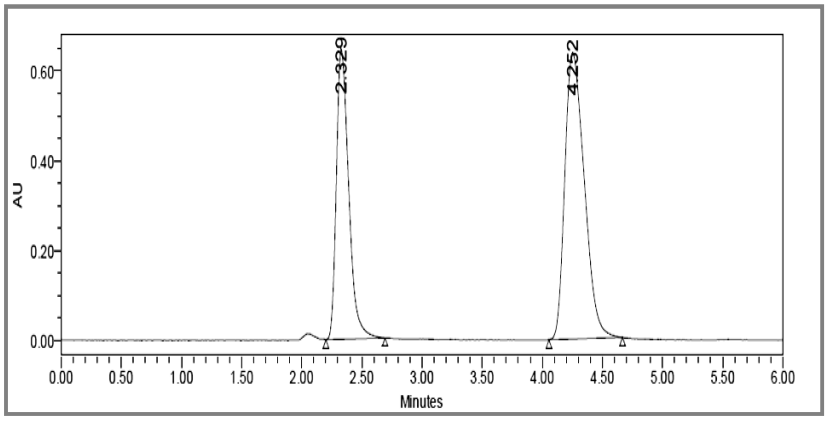

Figure 2. Typical chromatogram of tazobactam and cefepime.

System Suitability: System suitability parameters like number of theoretical plates, HETP and peak tailing were determined for both the drugs with the proposed method and their values were presented in table 1 and were that all the system suitability parameters for developed method for tazobactam and cefepime were within the acceptance criteria.

Table 1. System suitability of tazobactam and cefepime

Parameters	Tazobactam	Cefepime
No. of theoretical plates	2517	3072
Tailing factor	1.45	1.37
Area	4612362	7178596
Retention Time	2.329	4.252

Specificity:

Blank and Placebo Interference: The specificity of the proposed RP-HPLC method was established by injecting blank and placebo using the above chromatographic conditions. The chromatograms of blank and placebo solution showed no peaks at the retention time of tazobactam and cefepime peak revealing that the diluent and placebo solution used in sample preparation did not interfered in assay of tazobactam and cefepime in their formulations.

Linearity and Detector Response: The linearity was performed by plotting, and calculating linear regression analysis for the standard curves obtained for tazobactam and cefepime (Figures 3 and 4) respectively. Two standard curves were obtained in the concentration range of 10-30 µg mL^{-1} for a tazobactam and 50-150 µg mL^{-1} for cefepime respectively. The slope and intercept value were **y = 222120x-83524(r^2 = 0.9997)** for tazobactam and **y= 69463x-33736 (r^2 = 0.9998)** for cefepime respectively (Table 2). From the data obtained it is revealed that an excellent correlation exists between response factor and concentration of cited drugs within the concentration range indicated as above respectively.

Table 2. Results of linearity of tazobactam and cefepime

Tazobactam		Cefepime	
µg mL^{-1}	Peak Area Ratio	µg mL^{-1}	Peak Area Ratio
10	2128131	50	3404977
15	3256162	75	5177577
20	4357713	100	6974281
25	5486358	125	8658599
30	6566039	150	10347326
Slope, b	222120	**Slope, b**	69462.9
Intercept, a	-83524	**Intercept, a**	-33736
Correlation, r^2	0.9997	**Correlation, r^2**	0.9998

The LOD values for tazobactam and cefepime were found to be 0.00717µg mL^{-1} and 0.0147 µg mL^{-1} respectively and the LOQ values for tazobactam and cefepime were found to be 0.0239 µg mL^{-1} and 0.049 µg mL^{-1} respectively revealing good sensitivity of the proposed method (Table 3).

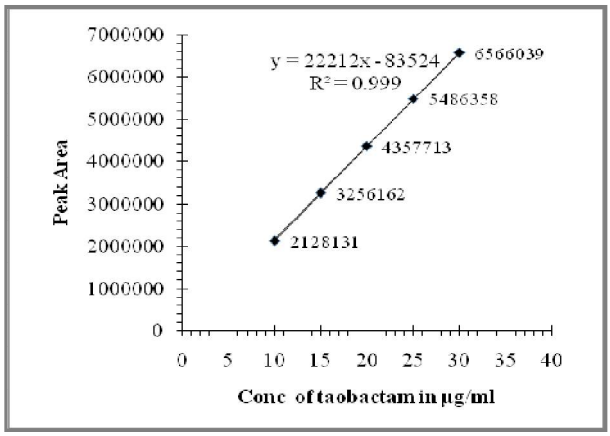

Figure 3. Calibration curve of tazobactam.

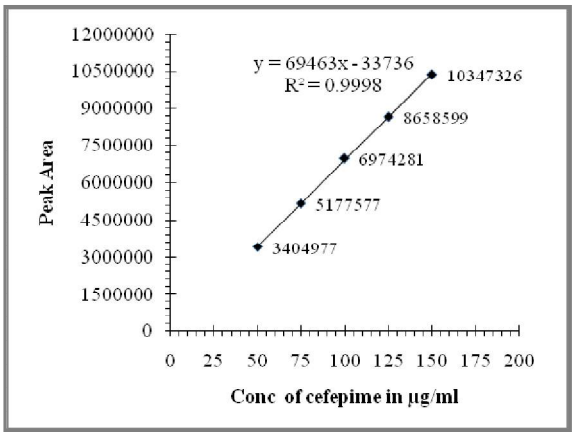

Figure 4. Calibration curve of cefepime.

Table 2. Results of linearity of tazobactam and cefepime

Tazobactam		Cefepime	
µg mL^{-1}	**Peak Area Ratio**	**µg mL^{-1}**	**Peak Area Ratio**
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30	6566039	150	10347326
Slope, b	222120	Slope, b	69462.9
Intercept, a	-83524	Intercept, a	-33736
Correlation, r^2	0.9997	Correlation, r^2	0.9912

Table 3. LOD and LOQ values of tazobactam and cefepime

Parameter	Tazobactam	Cefepime
LOD(µg mL^{-1})	0.0071	0.014
LOQ(µg mL^{-1})	0.0239	0.049

Precision: The precision of the developed RP-HPLC method was evaluated by carrying out intra-day analysis by injecting six replicate injections of 100% test concentration of the above mentioned drugs and the results were expressed in terms of standard deviation and %RSD. The results were summarized in table 4. From the results (%RSD of 0.208 for tazobactam and 0.191 for tazobactam) it was revealed that the developed RP-HPLC method was found to be precise, respectively.

Table 4. Results of precision of tazobactam and cefepime

Tazobactam			Cefepime		
S.No	**RT**	**Area**	**S.No**	**RT**	**Area**
Injection1	2.323	4376443	Injection1	4.200	6938292
Injection2	2.325	4380958	Injection2	4.198	6915281
Injection3	2.317	4392409	Injection3	4.184	6951575
Injection4	2.319	4365637	Injection4	4.182	6949221
Injection5	2.339	4377514	Injection5	4.214	6946878
Injection6	2.317	4385992	Injection6	4.172	6943228
*Mean		4379826	*Mean		6940746
*Std. Dev.		9118.669	*Std. Dev.		13321.73
*% RSD		0.208	*% RSD		0.191

*Average of six determinations

Accuracy: The accuracy of the proposed RP-HPLC method was determined at three concentration levels (50, 100 and 150%) by recovery experiments that were carried out in triplicate preparations on composite blend collected from 10 injection vials of prescribed generic formulation. The percentage recoveries were ranged from 99.91-100.12% for tazobactam and 99.96-100.07% for cefepime respectively. From the data reported in table 5, revealed that the developed RP-HPLC method was found to be accurate for tazobactam and cefepime assay.

Table 5. Results of accuracy of tazobactam and cefepime

Recovery Level	Tazobactam				Cefepime				
	Amount Added		Amount Found	% Recovery	Recovery Level	Amount Added		Amount Found	% Recovery
	Standard	Test				Standard	Test		
50%	10	5.0	14.99	99.93	50%	50	5.0	54.98	99.96
100%	20	5.0	25.03	100.12	100%	100	5.0	105.08	100.07
150%	30	5.0	34.97	99.91	150%	150	5.0	154.99	99.99
Mean Recovery* and %RSD	99.98% with %RSD- 0.11%				Mean Recovery* and %RSD	100.0% with %RSD-0.0056%			

*Average of three determinations

Robustness Studies: The robustness study of the developed RP-HPLC method assay method for tazobactam and cefepime was established in the mentioned variance conditions (± 2 units change in flow rate and detection wavelength). From these studies it was found that the assay values of the test preparation solution were not affected and were in accordance with that of actual. More over the system suitability parameters were also found satisfactory concluding the robustness of the proposed method Table 6.

Table 6. Results of robustness studies of tazobactam and cefepime

Chromatographic parameters	Changed value	Retention time		Tailing factor	
		Tazobactam	Cefepime	Tazobactam	Cefepime
Flow Rate	1.0 mL min^{-1}	2.930	4.907	1.444	1.308
±0.2 mL min^{-1}	1.4mL min^{-1}	1.780	2.980	1.368	1.185
Wavelength	225 nm	2.223	3.710	1.36	1.219
±5 nm	235 nm	2.203	3.207	1.409	1.219

Ruggedness: Under the prescribed experimental conditions the ruggedness studies are carried out on different days for tazobactam and cefepime respectively. The results are showed the ruggedness values the %RSD is less than 2 for tazobactam and cefepime, concluding the developed RP-HPLC method is rugged.

Solution stability study: The stability studies at 100% test concentration of the above mentioned drugs in mobile phase were carried out for 24 h at 25ºC. The solution stability and mobile phase stability experimental data confirmed that sample solutions and mobile phase used were stable up to 24 h (Table 7) there by reducing the analysis time and number of samples to be analyzed respectively.

Table 7. Stability data of tazobactam and cefepime

Drug	% Assay at 0 Hr	% Assay at 24 Hr	% Deviation
Tazobactam	99.40	99.94	0.99
Cefepime	99.91	99.98	0.99

*Average of six determinations

Analysis of marketed formulation: Analysis of generic combination form (DRAPIME-TZ injectable vial containing Cefepime-1000 mg and Tazobactam-125 mg) was carried out using the above said optimized mobile phase and optimized HPLC conditions. The % drug content of tablets obtained by

the proposed method for tazobactam and cefepime were found to be 99.71 and 99.92 %, respectively, table 9. In addition the statistical comparison (Students-t and F-tests) of the proposed method with the reference method [**11**] in formulations for the prescribed combination established no significant difference revealing 95% confidence limit respectively.

Table 8. Results for analysis in formulations

Parameters	Drapime-TZ-1.125 mg Injectable vial	
	Tazobactam Found (125 mg)	Cefepime Found (1000 mg)
Sample	124.98	999.87
	124.36	999.90
	124.58	999.08
AVG*	124.64	999.25
*% Recovery	99.71	99.92
SD*	0.314	1.09
†t-Test	1.04	0.278
†F-Test	3.65	1.02

* Average ± standard deviation of six determinations, †Students t-and F-test values referto comparison of the proposed method with the reference method [**11**].
Theoretical values at 95% confidence limit, F = 5.05, t = 2.262

APPLICATION

The proposed RP-HPLC method has a relatively short run time (< 5min) that allows quantifying large number of pharmaceutical preparations and plasma of the patients using these combination drugs. In addition to this the developed HPLC method seemed to be simple, selective, cost-effective, and reproducible and can be reliably used by almost every pharmaceutical laboratory.

CONCLUSION

The paper presented in this communiqué describes the development of a selective, accurate and sensitive isocratic RP-HPLC method for the assay of tazobactam and cefepime in generic fixed combination dose formulation. The results of validation studies (percentage, mean, R.S.D., percentage difference and recovery %) ensured good compliance in accordance with ICH guidelines [**12**]. The proposed method deduced high recoveries with good linearity and precision. From the above studies it can be concluded that the developed method can be easily used for the routine quality control of tazobactam and cefepime in fixed combined formulations within a short analysis time by quality control labs.

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