ISSN: 2395-0536

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

SIMULTANIOUS ESTIMATION OF CEFEPIME AND TAZOBACTAM IN PHARMACEUTICAL FORMULATIONS BY RP-HPLC

M. Suresh Babu[*1] and A. Sowmya[2]

[*1]Department of Pharmaceutical Analysis, JITS College of Pharmacy, Kalagampudi, Andhra Pradesh, India.
[2]Department of Pharmaceutical Chemistry, Sri Siddartha College of Pharmacy, Nuzvid, Andhra Pradesh, India

ABSTRACT

The main aim of the present work is to develop a simple, precise, rapid and accurate RP- HPLC method for the estimation of Cefepime and Tazobactam in Injectable Solution, and it should be validated according to the ICH recommended guidelines. A wavelength 248nm was selected and the mobile phase consists of Heptane-1 sulphonic acid and Adjust pH to (+/-0.05) with dilute glacial acetic acid and Acetonitrile in the ratio of 85:15 v/v at a flow rate of 1 ml/min were found to be optimum conditions for analysis. Cefepime and Tazobactam show linearity in the range of 50-150µg/ml. The accuracy of Cefepime and Tazobactam studies showed % recovery of the 98.0% to 102.0%. Robustness studies reveal that the method was reliable.

Key words: Cefepime, Tazobactam, RP- HPLC, validation

Author for correspondence:
M. Suresh Babu,
Department of Pharmaceutical Analysis, JITS College of Pharmacy, Kalagampudi,
Andhra Pradesh, India.
Email id: sureshbabu3377@gmail.com.

INTRODUCTION

Analytical chemistry may be defined as the "Science and art of determining the composition of materials in terms of the elements or compounds contained". Pharmaceutical analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principles from various branches of science like Chemistry, Physics, Microbiology, Nuclear Science, Electronics, etc.

Analytical method is a specific application of a Technique to solve an analytical problem. Analytical Instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air, generally used for drug analysis are spectral methods, chromatographic methods, electro analytical techniques, and miscellaneous techniques like conventional titrimetric, gravimetric and Polari metric methods (1-10).

Cefepime hydrochloride (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) is a white to pale yellow powder. Cephalosporins are bactericidal and have the same mode of action as other beta-lactam antibiotics (such as penicillins). Cephalosporins 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 (PBPs).

Tazobactam ((2*S*,3*S*,5*R*)-3-methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide) increases efficacy of βlactam antibiotics by inactivating β lactamase enzymes of βlactam resistant microbes.

So, far there is no methods reported for the estimation of Cefepime and Tazobactam in combinatined Injectable Solution using RP-HPLC but available in combination with the Tazobactam. There are UV, Spectrophotometric methods available there for determination of Cefepime and Tazobactam and also LC/MS methods are there for the estimation of Cefepime and Tazobactam in Biological fluids and Ocular tissues. Therefore there it is need to develop a Specific, precise, accurate and validated HPLC method for the estimation of in ophthalmic solutions.

Therefore there is need to develop a method for the determination of Cefepime and Tazobactam in combined Cefepime and Tazobactam injectable solution.

EXPERIMENTALS

Chemicals and Reagents

Cefepime and Tazobactam from Aurbindo, Potassium Dihydrogen orthophosphate
(KH2PO4) from Rankem, Ortho phosphoric acid, Acetonitrile, Methanol and Triethylamine from Merck.

Methods

Method development involves evaluation and Optimization of the various stages of sample Preparation, chromatographic separation, detection and quantification. Optimization of various parameters was performed in order to develop a selective and sensitive method for analysis on HPLC using detection.

Standard solution

Accurately weigh and transfer 250 mg of Cefepime and 31.25mg of Tazobctam working standards into a 100ml clean, dry volumetric flask, add 70ml of diluent and sonicate to dissolve and make up to volume with water. Pipette 2ml of this solution and transfer into a 10ml volumetric flask and dilute to the volume with diluent.

Sample Preparation (100ppm)

Weigh accurately about 5.0g of test sample into a 100 ml volumetric flask, add to it 50 m of diluents and shake well, dilute to the volume with diluents and mix well.

Chromatographic condition

Optimized Chromatographic conditions are Column-Phenomex Luna, 150*4.6mm, 5um; Flow rate: 1.0 ml/min; Detector: 250nm; Injection volume : 10uL ; Mobile phase: Buffer, and Acetonitrile in the ratio of 95:5 v/v; Run time : 15min; Column oven temp: 30 °C; Pump mode: Isocratic; Diluent : Water: Acetonitrile(50:50).The following parameters are validated for the stability indicating method of Cefepime and tazobactam specificity, linearity, precision, ruggedeness (intermediate precision), accuracy (recovery), stability of solution, robustness and system suitability (11-13).

RESULTS AND DISCUSSION

Cefepime and tazobactam both were eluted, and the peak shape was proper the tailing of the peak and .plate count was good using above optimized method.

Linearity results of Cefepime and Tazobactam

Linearity results of Cefepime and Tazobactam are given in table-1 and 2 and fig-1 and 2

Table-1 Linearity results of Cefepime

Conc in ppm	Area
0	0
250	3645928
375	5394743
500	7077915
625	8672776
750	10813787
Correlation coefficient	0.999

Table-2 Linearity results of Tazobactam

11	Area
0	0
31.25	621764
46.87	932041
62.5	1289303
78.125	1598881
93.75	1959428
Correlation coefficient	0.999

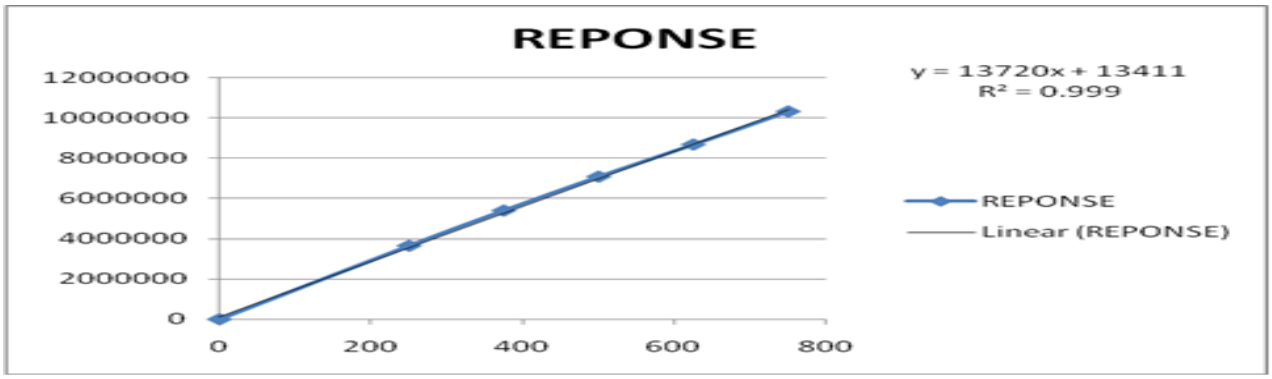

Fig-1 Linearity Graph for Cefepime

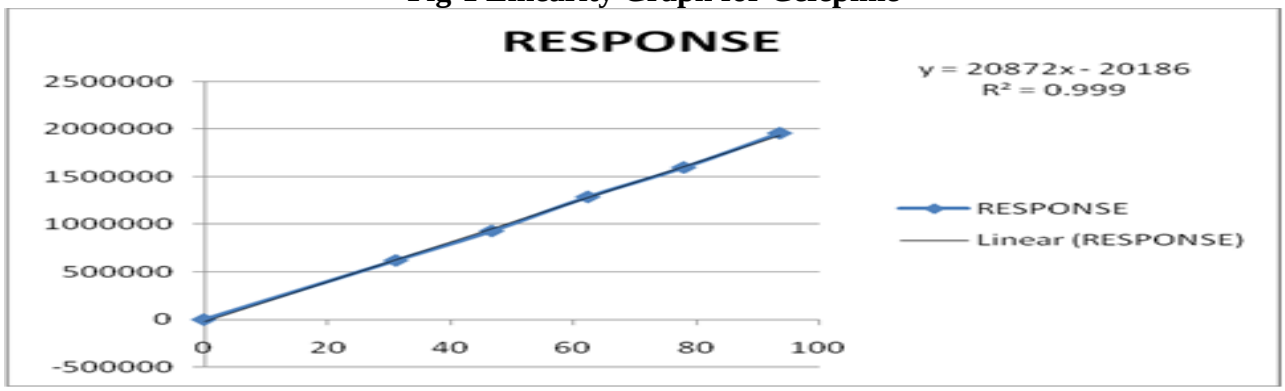

Fig-2 Linearity Graph for Tazobactam

Response is Linear over the concentration range from 50 % to 150 % of test concentration.

Six Sample solutions were prepared individually using single unit batch of Cefepime and Tazobactam , solution as per methodology and injected each solution into HPLC. From the results it is concluded that the method is precise.

The results (Method Precision & Intermediate Precision) are indicating that the test method is rugged for analyst to analyst, system to system, column to column and day to day variation.

Solutions were prepared in triplicate at levels from 50% to 150% of test concentration using Cefepime and tazobactam solution as per the methodology and injected each solution into HPLC.

The recovery results indicating that the test method has an acceptable level of accuracy for the assay of Cefepime and Tazobactam, Solution from 50 % to 150% of test concentration (Table-3).

Table-3 Results of accuracy

Concentration/Sample ID	% Recovery	Statistical Analysis	
50% Level Sample – 1	99.76	Mean	100.24
50% Level Sample – 2	99.95	SD	0.720
50% Level Sample – 3	101.09	% RSD	0.718
100% Level Sample – 1	99.066	Mean	99.80
100% Level Sample -2	99.255	SD	1.116
100% Level Sample -3	101.08	% RSD	1.118
150% Level Sample -1	99.56	Mean	101.10
150% Level Sample -2	101.86	SD	1.34
150% Level Sample -3	101.90	% RSD	1.32

The results (table-4) indicating that the test method is robust for all the variable conditions outlined in the table.

Table-4 Results of Robustness

Parameter	Variation	System Suitability		
		Tailing factor	**Theoretical Plates**	**%RSD**
Mobile phase	-10%	1.25	8729	0.1
	+10%	1.30	9528	0.1
Flow	-10%	1.60	6794	0.12
	+10%	1.31	6040	0.11
Column oven temperature	**-5%**	1.36	8155	0.12
	+5%	1.23	4466	0.11

Simple, precise, rapid and accurate RP-HPLC method was developed for the estimation of Cefepime and Tazobactam in Injectable Solution.. In RP-HPLC method, optimization of chromatographic parameters was done. Parameters optimized were, selection of wavelength, effect of nature of mobile phase, ratio of mobile phase, P^H of the Buffer and effect of flow rate. A wavelength 248nm was selected and the mobile phase consists of Heptane-1 sulphonic acid and Adjust pH to (+/-0.05) with dilute glacial acetic acid and Acetonitrile in the ratio of 85:15 v/v at a flow rate of 1 ml/min were found to be optimum conditions for analysis. System suitability studies were also carried out which includes theoretical plates, resolution and tailing factors etc.The accuracy studies were shown as % recovery for Cefepime and Tazobactam Tartrate at 50%, 100% and 150% the limits of % recovered were found to be within the limits. Hence the method was found to be accurate. The accuracy of Cefepime and Tazobactam studies showed % recovery of the 98.0% to 102.0%. In the System precision study, %RSD was found to be less than 2%, For Cefepime and Tazobactam, which indicates that the system has good reproducibility.

For ID precision studies 6 replicate injection of Cefepime and Tazobactam was performed. %RSD was determined for peak areas of Cefepime and Tazobactam .The acceptance limit was should be not more than 2% and the results were found to be with in the acceptance limits. Using the optimized chromatographic conditions, chromatograms of standard solutions of Cefepime and Tazobactam were recorded. Retention time was found to be 10.42 min. Calibration curve were obtained by using peak area vs. concentration. Cefepime and Tazobactam show linearity in the range of 50-150µg/ml. Calibration curve was plotted and correlation co-efficient found to be 0.999. Precision of the method was studied by making the replicate injections of the standard solutions and standard deviation was determined. The reliability and sensitivity of the method could be seen from recovery studies. There is no interference due to excipients. The proposed method is simple, rapid and accurate. For robustness studies the chromatograms were recorded for standard solutions of Cefepime and Tazobactam by changing flow rate ±10 and column Temperature $\pm 10^0$ C . Robustness studies reveal that the method was reliable. Finally the HPLC developed method could be used for estimation Cefepime and Tazobactam in Cefepime and Tazobactam injectable Solution.

CONCLUSION

A simple, rapid & precise RP-HPLC method was developed for the determination of in Cefepime and Tazobactam injectable, formulation. The developed method was free from interferences due to placebo and Cefepime and Tazobactam peak is homogeneous and has no co eluting peaks. Hence the developed method was simple, rapid precise & specific, and stability indicating and it can be used for routine quality control analysis of Cefepime and Tazobactam in injectable solutions. The test method is validated for Specificity, Linearity, Precision, Accuracy, Range, and Stability of Solution, Ruggedness and robustness and found to be meeting the predetermined acceptance criteria the validated method is specific, Precise, Accurate, Stable, Robust and Rugged for the determination of assay of Cefepime and Tazobactam injectable Formulation, Solution. Hence, this method can be introduced into the routine

use for determination of assay of Cefepime and Tazobactam injectable, Solution.

REFERENCES

1. Vogel's, Textbook of quantitative inorganic analysis, 4th ed. p. 1-12, page 1.
2. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry, CBS Publishers and Distributors; Delhi, 4th Ed, volume 2: p.157-174, 199, page 2.
3. Sharma BK. Instrumental methods of chemical analysis,Goel Publishing House; Meerut, 19th Ed, 2000, page 2.
4. Snyder LR, Kirkland JJ, Joseph LG. Practical HPLC Method Development, Wiley Inter Science; New York, 2nd Ed,p.1-56, 234-289,685-712, 1997, page 3.
5. Willard HH, Merrit LL, Dean JA, Settle FA. Instrumental methods of analysis. 6th Ed. New Delhi: CBS Publishers and Distributors; p.1-15, 1986, page 6.
6. Douglas A. Skoog, F. James Holler, Timothy A. Nieman, Principles of instrumental analysis p.725-760, page 7.
7. David G.Watson. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed., p.221-232, p.267-311, page 8.
8. Remingtonn's The Science and Practise of Pharmacy,20th Edition 2000., page 9.
9. Connors KA. A Textbook of Pharmaceutical Analysis, Wiley intersciences Inc; Delhi, 3r^{d} Ed, p.373-421, 1994, page 10.
10. Gurdeep R.chatwal, Sham K.Anand, Instrumental methods of chemical analysis, p. 2.566-2.638, 2007, page 18.
11. ICH: Q2B, Analytical Validation – Methodology (November 1996), page 24.
12. ICH: Q2A, Text on validation of analytical procedure (October 1994), page 22.
13. ICH Q2 (R1), Validation of Analytical Procedures Text and Methodology November 2005, page 23.