Vara Prasad A. et al. / Vol 3 / Issue 2 / 2013 / 61-66.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR CEFTIBUTEN IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

***[1]Vara Prasad A, [2]K.E.V.Nagoji, [3]V.Girija Sastry, [4]Sree Rama Murthy Pyla, [5]N.Sriram**

[*1]Jyothishmathi Institute of Pharmaceutical Sciences, Thimmapur, Karimnagar, Andhra Pradesh, India.
[2] Sri Venkateswara college of Pharmacy, Etcherla, Srikakulam, Andhra Pradesh, India.
[3]University College of Pharmaceutical Sciences, Andhra University Visakhapatnam, Andhra Pradesh, India.
[4] Raghu College of Pharmacy, Dakamarri (V), Bheemili (M), Visakhapatnam Dist. Andhra Pradesh, India.
[5]Smt.Sarojini Rammulamma college of Pharmacy, Mahabubnagar, Andhra Pradesh, India.

ABSTRACT

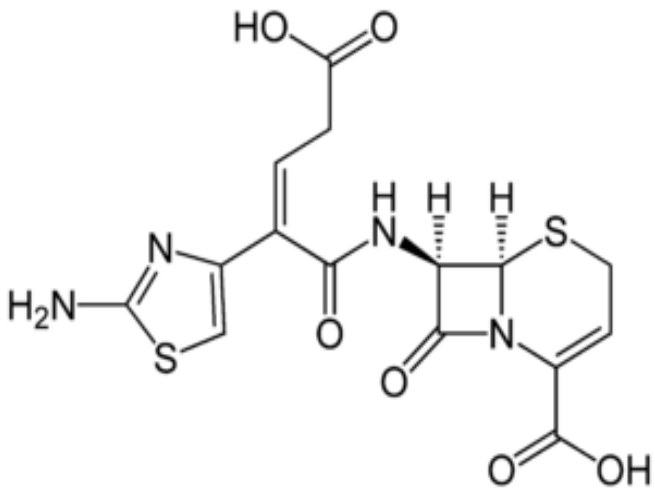
A simple, precise, rapid and accurate reverse phase HPLC method has been developed for the determination of Ceftibuten in bulk and its pharmaceutical dosage form. An enable C18G, 250mmX4.6mm i.d, 5µm particle size column was used with photo diode array UV-Visible detector. The mobile phase consisting of a mixture of phosphate buffer pH3.0 and acetonitrile HPLC grade (35:65) as the mobile phase at a flow rate 1.0 mL/min, the detection was carried out at 228nm. The retention time of the drug was 2.435 minutes. The method was linear over the concentration range of 10-80µg/ml. the limit of detection and limit of quantification were 0.11 and 0.34 respectively. The percentage recovery of Ceftibuten was 99.41 – 100.83%. The validation of method was carried out utilizing ICH guidelines.

Keywords: Ceftibuten, RP- HPLC, Development, Validation.

INTRODUCTION

Ceftibuten is a third generation cephal osporin antibiotic. It is an orally administered agent, with 2 dosage forms, capsule or oral suspension. It is marketed by Pernix Therapeutics under the trade name Cedax. It is active against Haemophilus influenzae, Moraxella catarrhalis, Escherichia coli (K. pneumoniae, K. oxytoca), Proteus vulgaris, P. mirabils, P. providence, Salmonella sp., Shigella sp., Enterobacter sp. and Streptococcus sp. Ceftibuten exerts its bactericidal action by binding to essential target proteins of the bacterial cell wall. This binding leads to inhibition of cell-wall synthesis. Ceftibuten is used to treat acute bacterial exacerbations of chronic bronchitis (ABECB), acute bacterial otitis media, pharyngitis, and tonsillitis. It is also indicated for pneumonia, infections of the urinary tract, enteritis and gastroenteritis. The chemical name of Ceftibuten is (6R, 7R)-7-([(Z)-2-(2-amino-1,3-thiazol-4-yl)-5-hydroxy-5-oxopent-2-enoyl]amino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [1-4].

Ceftibuten



Corresponding Author:- Vara Prasad. Adepu Email:- varaprasadadepu@gmail.com

EXPERIMENTAL

Instrumentation

Quantitative HPLC was performed on a binary Shimadzu prominence HPLC in Gradient mode with a 20µl sample injection loop (manual), and SPD 20A Photo diode array UV-Visible detector. The output signal was monitored and integrated using LC solutions software. An enable C-18 (250 x 4.6 mm, packed with 5 µm) column was used for the separation.

Reagents used

a. HPLC water (QUALIGENS LTD)
b. Acetonitrile (HPLC grade, Finar Chemicals)
c. Disodium hydrogen phosphate (Na_2HPO_4) (AR grade)
d. Citric acid monohydrate (AR grade)

OPTIMIZATION

Optimization of the method

To develop a suitable and robust HPLC method for the determination of Ceftibuten. different mobile phases methanol: water, Acetonitrile: water, Acetoniotrile: buffer, methanol: buffer were used in different compositions of mobile phases (80:20, 40:60, 55:45, 35:65, 80:20) at different flow rates (0.5,1.0, 1.2, 1.5, 1.8 ml/min). Then the composition of the mobile phase Phosphate Buffer : acetonitrile in the ratio of 35:65 at flow rate of 1.0 ml/ min gave sharp peaks with minimum tailing and good resolution for Ceftibuten. Whereas with other compositions of mobile phases at other flow rates broad peaks and pronounced tailing was observed. Then, Ceftibuten was eluted at retention times around 2.435 min with symmetric peak shape. Optimized chromatographic conditions were shown in Table 1 [5-7].

METHOD

Preparation of the phosphate buffer (P^H3.0)

0.9 gms of anhydrous disodium hydrogen phosphate and 1.298 gms of citric acid monohydrate were dissolved in sufficient water to produce 1000 ml an adjust the P^H to 3.0 [8-12].

Preparation of the Mobile phase

650 ml of acetonitrile and 350 ml of prepared buffer solution were properly mixed in the ratio of 55:45.

Preparation of standard drug and solutions

Stock solution of the drug (pure) was prepared by dissolving 100 mg of Ceftibuten in 50 ml of mobile phase in 100 ml volumetric flask and the final volume was made up to 100 ml using mobile phase. Daily working standard solutions of Ceftibuten were prepared by taking suitable aliquots of drug solution from the standard solutions 100 µg/ml [13-16].

Preparation of sample drug solution for pharmaceutical formulations

Twenty Capsules containing Ceftibuten of marketed formulation (CEDAX 400mg) were taken and the powder equivalent to 100 mg of Ceftibuten was dissolved in 100 ml of mobile phase to get a stock solution of 1 mg/ml and then sonicated for 15 min. This solution was filtered through a 0.45 micron membrane filter. The solution was further diluted stepwise with mobile phase and spiked with required amount of standard drug and diluted with mobile phase to get concentrations with in the linearity range [17-19].

Procedure for calibration curve

The contents of the mobile phase were filtered before use through a 0.45µm membrane filter and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. Then, twenty micro liters of each of standard and sample solutions were injected into the HPLC system for six times and the retention time, average peak areas of component area of were recorded. The linearity range was found to be in between 20-100 µg/ml for Ceftibuten. The linearity range was shown in Table 2 and Calibration curve in figure 2. A typical chromatogram of Ceftibuten was shown in Fig 1.

Analysis of formulation

The amount of drug present in each pharmaceutical formulation was calculated through peak area drug by using the standard calibration curve (concentration in µg/ml was taken on x-axis and peak area on y-axis) [20].

Method Validation

Linearity

The linear fit of the system was illustrated graphically. Least square regression analysis was carried out for the slope, intercept and correlation coefficient. The results are presented in Table 2 [21].

Precision

The precision of each method was ascertained separately from the peak areas obtained by actual determination of eight replicates of a fixed amount of drug. The percent relative standard deviation and percentage range of errors (at 0.05 and 0.01 confidence limits) were calculated for Ceftibuten and presented in the table. The precision of the assay was also determined in terms of intra-and inter-day variation in the peak areas for a set of drug solutions on three different days. The intra-and inter-day variation in the peak area of the drug solution was calculated in terms of % RSD and the results are presented in the Table 3 [20].

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, 120%) of bulk samples of Ceftibuten along with within the linearity range were taken and added to the pre-analyzed formulation of concentration 20 µg/ml. From that percentage recovery values were calculated. The results were shown in Table 4 [21].

System suitability parameters

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed. (or) The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Resolution (R), Tailing factor (T), LOD (µg/ml) and LOQ (µg/ml) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of Ceftibuten in pharmaceutical formulations was validated or not. The results were shown in Table 5.

Fig 1. Typical chromatogram of Ceftibuten (Standard drug)

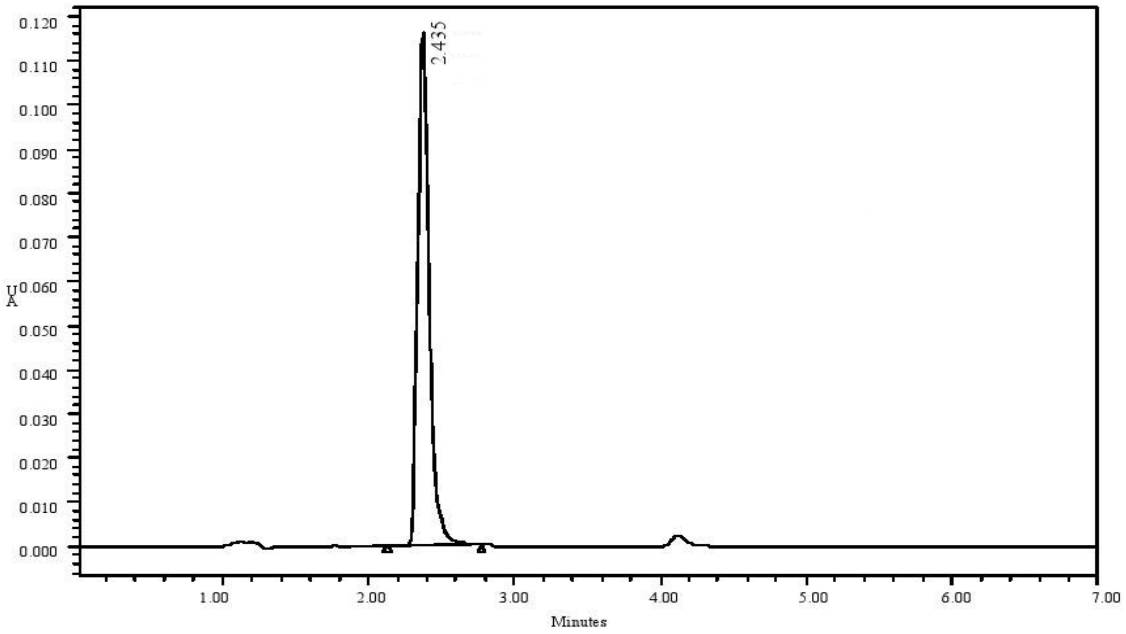


Fig 2. Calibration curve of Ceftibuten

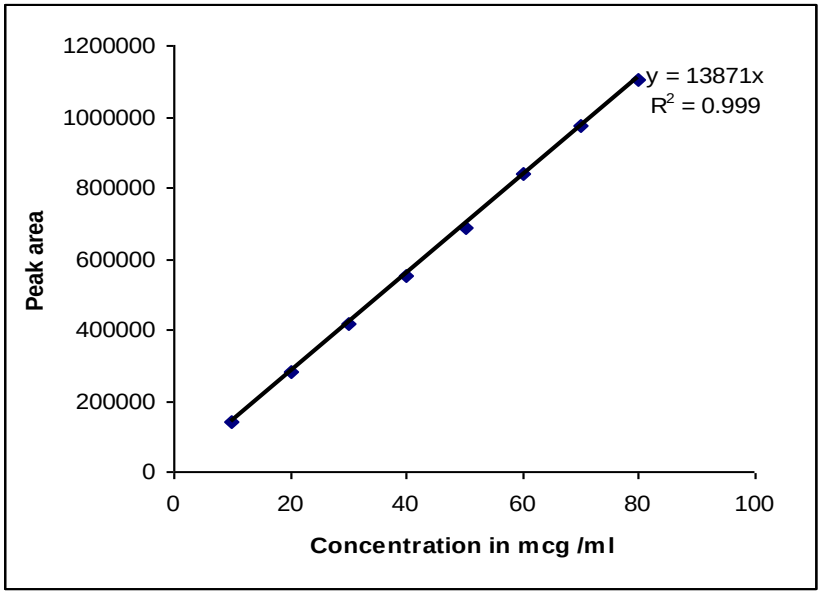


Table 1. Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	An enable C-18G
Mobile Phase	Buffer: acetonitrile: (35:65)
Flow rate (ml/min)	1.0 ml
Column back Pressure (kgf/cm2)	165
Run time (minutes)	07
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	228
Drug RT (min)	2.435

Table 2. Linearity

Concentration(µg/ml)	**Area**	**Statistical Analysis**
10	139549.3	Y=13871x
20	279086	
30	418649	
40	550903	Correlation coefficient=0.999
50	689758	
60	837291	
70	976842	
80	1103623	

Table 3. Precision results for Ceftibuten:

S.No.	**Concentration(µg/ml)**	**area**	**Statistical analysis**	
1	50	689758	**Mean**	689071.
2.	50	688739		
3.	50	689636		
4.	50	688649	**SD**	680.7
5.	50	688798		
6.	50	687699		
7.	50	689701	**% RSD**	0.11
8.	50	689594		

Table 4. Accuracy results for Ceftibuten

Sample ID	**Concentration (µg/ml)**		**%Recovery of pure drug**	**Statistical Analysis**	
	Pure drug	**Formulation**			
S_1 : 80 %	16	20	99.83	Mean	99.97%
S_2 : 80 %	16	20	99.86	SD	0.2227
S_3 : 80 %	16	20	100.23	% RSD	0.22
S_4 : 100 %	20	20	99.78	Mean	99.44%
S_5 : 100 %	20	20	99.78	SD	0.480
S_6 : 100 %	20	20	99.10	% RSD	0.48
S_7 : 120 %	24	20	98.99.	Mean	99.52%
S_8 : 120 %	24	20	100.01	SD	0. 692
S_9 : 120 %	24	20	99.03	% RSD	0. 70

Table 5. System suitability parameters

S.No	Parameters	Obtained Values
1.	Theoretical plates (N)	4031.2
3.	Tailing factor (T)	1.3
4.	LOD (µg/ml)	0.11
5.	LOQ (µg/ml)	0.34

RESULTS AND DISCUSSION

From the linearity Table 2, it was found that the drug obeys linearity within the concentration range of 10-80 µg/ml for Ceftibuten. From the results shown in precision Table 3, it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility. From the results shown in accuracy Table 4, it was found that the percentage recovery values of pure drug from the pre-analyzed solutions of formulations were in between 99.44 – 99.97%, which indicates that the method was accurate and also reveals that the commonly used recipients and additives present in the pharmaceutical formulations were not interfering the proposed method. The system suitability parameters also reveal that the values were within the specified limits for the proposed method.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Ceftibuten from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation recipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Ceftibuten in pure form and its dosage forms and can also be used for dissolution or similar studies.

REFERENCES:

1. Bhudavari S. The Merck Index, Merck Research Laboratories, New Jersey, Edn, 1953, 13.
2. Sweetman CS. Martindale:The complete Drug Reference, Edn, 34, 2005, 179.
3. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically – Third Edition. Approved Standard NCCLS Document M7-A3, Vol. 13, No. 25, NCCLS, Villanova, PA. December, 1993.
4. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests – Fifth Edition. Approved Standard NCCLS Document M2-A5, Vol. 13, No. 24, NCCLS, Villanova, PA. December, 1993.
5. Bauernfeind A. Ceftibuten and bactericidal kinetics. Comparative in vitro activity against enterobacteriaceae producing extended spectrum b-lactamases. *Diagn Microbiol Infect Dis,* 14, 1990, 89 –92
6. Jones RN. Ceftibuten: a review of antimicrobial activity, spectrum and other microbiologic features. *Pediatr Infect Dis J*, 12(suppl), 1993, S37–S44.
7. Jones RN, Barry AL. Antimicrobial activity, spectrum and recommendations for disk diffusion susceptibility testing of ceftibuten (7432-S: SCH 39720), a new orally administered cephalosporin. *Antimicrob Agents Chemother*, 32, 1988, 1576 –1582.
8. Barr WH, Lin CC, Radwanski E, et al. The pharmacokinetics of ceftibuten. *Diagn Microbiol Infect Dis,* 14, 1991, 89 –92.
9. Lin C, Radwanski E, Affrime M, et al. Multiple-dose phar-macokinetics of ceftibuten in healthy volunteers. *Antimicrob Agents Chemother*, 39, 1995, 356 –358
10. Chirurgi VA, Edelstein H, Oster SE, et al. Ceftibuten versus cefaclor for the treatment of bronchitis. *J Antimicrob Chemother*, 28, 1991, 577–580.
11. Kammer RB, Ress R. Randomized comparative study of ceftibuten versus cefaclor in the treatment of acute lower respiratory tract infections. *Diagn Microbiol Infect Dis,* 14, 1991, 101–105.
12. Pan HT, Kumari P, Lim J, et al. Determination of a cepha-losporin antibiotic, ceftibuten, in human plasma with column-switching high-performance liquid chromatography with ultraviolet detection. *J Pharm Sci*, 81, 1992, 663– 666.
13. Marlin GE, Nicholls AJ, Funnell GR, et al. Penetration of cefaclor into the bronchial mucosa. *Thorax*, 39, 1984, 813– 817.
14. Valcke Y, Pauwels R, Van Der Straeten M. Pharmacokinetics of antibiotics in the lung. *Eur Respir J*, 3, 1990, 715–722.
15. Nix DE, Goodwin SD, Peloquin CA, et al. Antibiotic tissue penetration and its relevance: models of tissue penetration and their meaning. *Antimicrob Agents Chemother,* 35, 1991, 1947–1952.
16. Carbon C. Significance of tissue levels for prediction of antibiotic efficacy and determination of dosage. *Eur J Clin Microbiol Infect Dis*, 9, 1990, 510 –516.
17. Lin C, Lim J, Radwanski E, et al. Pharmacokinetics and dose proportionality of ceftibuten in men. *Antimicrob Agents Chemother*, 39, 1995, 359 –361.

18. Andrews JM, Wise R, Baldwin DR, et al. Concentrations of ceftibuten in plasma and the respiratory tract following a single 400 mg dose. *Int J Antimicrob Agents*, 5, 1995, 141–144.
19. Wise R. The pharmacokinetics of the oral cephalosporins: a review. *J Antimicrob Chemother*, 26(suppl E), 1990, 13–20.
20. Baldwin DR, Andrews JM, Wise R, et al. Bronchoalveolar distribution of cefuroxime axetil and in vitro efficacy of observed concentrations against respiratory tract pathogens. *J Antimicrob Chemother*, 30, 1992, 377–385.
21. Baldwin DR, Wise R, Andrews JM, et al. Concentrations of cefpodoxime in serum and bronchial mucosal biopsies. *J Antimicrob Chemother*, 30, 1992, 67–71.