RESEARCH ARTICLE ISSN: 2349-2678

Contents lists available at www.ijpba.in

International Journal of Pharmaceutical and Biological Science Archive

Index Copernicus Value: 65.90

Volume 6 Issue 4; July-August; 2018; Page No. 19-23

METHOD DEVELOPMENT AND VALIDATION OF CEFIXIM BY RP-HPLC METHOD IN BULK AND PHARMACEUTICAL DOSAGE FORM

A.Hanumappa Chowdary, R.Kiranjyothi and J.Priyanka

M.PHARM. (PHARMACEUTICAL ANALYSIS) JNTUA, OTPRI, Ananthapuramu

ABSTRACT

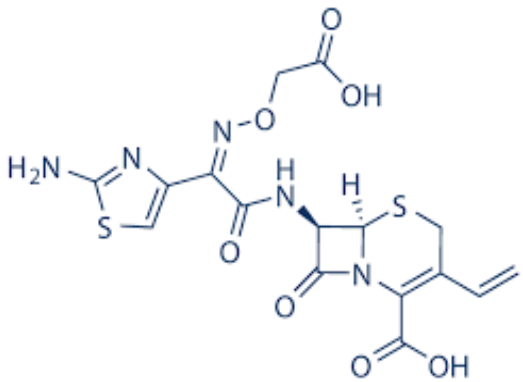
A simple, rapid and accurate RP-HPLC method was developed for the quantification of Cefixim in bulk and its formulation by RP-HPLC method using C18 ODS column (Phenomenex Luna, 250 x 4.6 mm, 5µm) in isocratic mode. The mobile phase consisted of methanol and 0.1% Orthophosphoric acid in the ratio of 70:30 (v/v) water: methanol was used. The flow rate was maintained at 1.0 mL/min and the injection volume was 20µL. Detection wavelength with UV detector at 289 nm and run time was kept 10 min. The retention time of Cefixim was 3.654 min. The method was linear over the concentration range50 -150µg/ml. The recovery was found to be 99.11 %. The validation of method was carried out utilizing ICH-guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations.

Key words: HPLC method, Cefixim, Method development, ICH-guidelines

INTRODUCTION

CEFIXIME:

Cefixime is an antibiotic useful to treat a number of bacterial infection gonorrhea [2]. This includes otitis media, strep throat, pneumonia, urinary tract infection, gonorrhea, and Lyme disease.[2]For gonorrhea typically only one dose is required.[3] In the United States it is a second line treatment to Ceftriaxone for gonorrhea[2] It is taken by mouth.[2]



Chemical Structure of Cefixime

Literature survey revealed that a quantification methods have been reported for the estimation in Cefixime pharmaceutical dosage forms. Bioanalytical methods(LC-MS/MS) have been reported for the quantification of Cefixime in biological fluids few chromatographic method have been reported for the analysis of Cefixim .The present work aims at developing simple, accurateand reproducible RP-HPLC method for the estimation of Cefixim in bulk and its formulation according to ICH guidelines.

MATERIALS AND METHODS

Instrumentation and Materials

The liquid chromatographic system consists of shimadzu LC Solutions- 20 AD UFLC with UV-VIS detector, binary pump and septum injector valve with 20 µl fixed loop. The analytes were monitored at 289 nm. Chromatographic analysis was performed on Phenomenex Luna C_{18} ODS column having 250 mm× 4.6 mm i.d. and 5 µm particle size.

**Corresponding author: A.Hanumappa Chowdary*

Materials used:

API of Cefixim was procured as a gift sample by , Orchid pharmaceutical pvt ltd, chennai , india Water was distilled and purified with Merck Millipore system. Formulation of Cefixim was purchased from local pharmacy.

Chromatographic Conditions

The Phenomnex C18 column ODS (250 x 4.6mm, 5µm) equilibrated with mobile phase methanol and 0.1% Orthophosphoric acid in the ratio of 70:30(v/v) was used and the flow rate was maintained at 1 mL/min. Detection wavelength with UV detector at 289 nm, and the injection volume was 20 µL and run time was kept 10 min.

Preparation of mobile phase

The mobile phase was prepared by taking 70% water and 30 % methanol adjusted using 0.1% orthophosphoric acid .It was filtered through 0.45µm membrane filter and degassed under ultrasonic bath prior use. The mobile phase was pumped through the column to stabilize the column.

Preparation of stock solution

10 mg of Cefixim drug was weighed accurately and it was dissolved in the mobile phase and after complete dissolution the volume was made up to 10ml. The stock solution was prepared.

VALIDATION OF METHOD DEVELOPED BY RP-HPLC

Specificity

The specificity of the proposed method was determined by comparing the results obtained by running the standard solution and with blank.

Linearity and Range

The linearity was determined for Cefixim. Solution of the drug at six different concentrations was analyzed and calibration curve was constructed by plotting mean response factor against the respective concentration. The method was evaluated by determination of the correlation coefficient and intercept value. Cefixim follows linearity in the concentration range of 50- 150 µg/mL respectively.

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100% and 150% to the pre analysed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate.

Robustness

The robustness was determined by injecting triplicate injections of standard and three-sample solutions in single at each different condition with respect to control condition. Robustness of the method was checked by varying the instrumental conditions; flow rate (±10%mL/min) and temperature (±5˚C). Sample solution was injected in each condition.

System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established.

Assay procedure

Marketed formulation of Cefixim is available in tablet dosage form. Drug equivalent to 50 mg/ml was transferred into 50ml volumetric flask and dissolved using mobile phase. The solution was sonicated for 15 mins. From the prepared solution optimized sample solutions were prepared and calculate the assay of pharmaceutical dosage form.

RESULTS AND DISCUSSION

Specificity

The comparison of the data of the drug solution before spiking and the spiked drug solution revealed that there was no significant interference of placebo with the recovery of

Cefixim inferring that the method was specific. The results were shown in table

Linearity and Range

The method was found to be linear. In the linearity study, regression equation and coefficient of correlation for isoproterenol was found to bey = 30386x + 5215.9, r2 =0.999. linearity data was shown in table no.2 and fig. 2.

Accuracy

The mean recovery was found to be 99.11%. The limit for mean recovery is 90-110%. Thus the method was found to be accurate. The results were shown in table no.

Robustness

This method is robust for the analysis of Cefixim within the specified range of deviations in the experimental conditions. The results were shown in table no.4.

Assay

The percent content of Cefixim formulation was found to be99.11 %.

Table 1: Specificity Data

S.No	Peak name	Cefixime peak area and Rt
1	Blank chromatogram	Nill
2	Placebo chromatogram	Nill
3	Standard chromatogram of cefixime	312524 and 3.65mints
4	Formulation chromatogram of cefixime	313652 and 3.64mits

Table 2: Linearity and range

Linearity data for cefixime		
% Level	**Concentration (µg/ml)**	**Peak area**
50%	0.5	135221
75%	0.75	343840
100%	1	312524
125%	1.25	354194
150%	1.50	564119
	Slope	42726
	Intercept	0.999

Fig. 2: Calibration curve of Cefixim

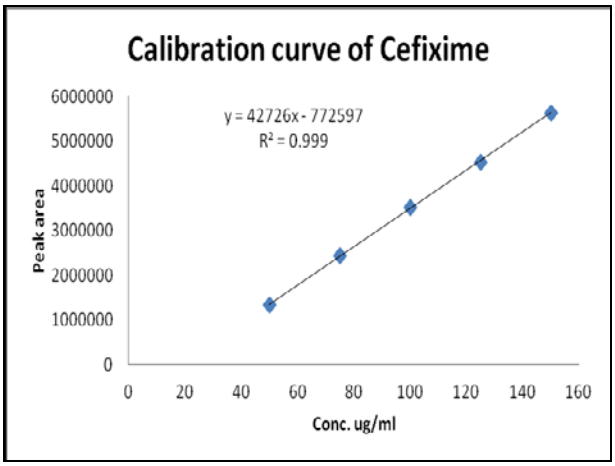


Fig. 3: Optimized chromatogram of Cefixim

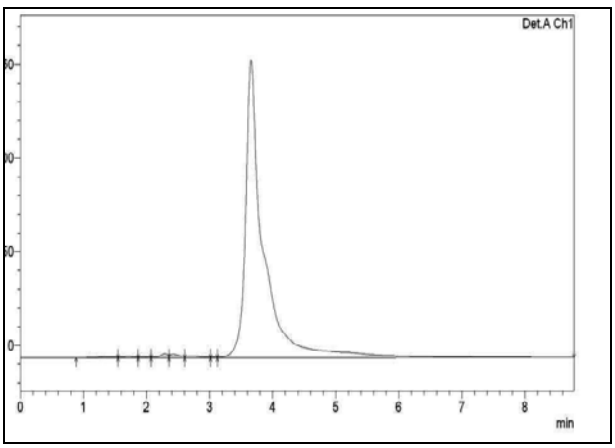


Table 3: Results for accuracy

Conc.	peak area	Amt. added	Amt. found	% recovery	%Mean recovery
	135224	7.45362	7.4453	98.425	
80%	1353212	7.45362	7.4453	99.152	98.42
	1362566	7.45362	7.4458	97.548	
	3526240	12.3458	12.258	98.897	
100%	3426251	12.3458	12.258	99.117	99.11
	3527250	12.3458	12.258	99.015	
	5641092	17.6258	17.645	99.021	
120%	5551192	17.6258	17.645	100.015	99.012
	5577825	17.6258	17.645	99.245	

Table 4: Results for robustness

S. No	Robust condition	Parameter change	Peak Area	Retention Time (min)
1	Wave length ± 2 nm	287 nm	3124256	3.598
2		289 nm	3125241	3.654
3		291 nm	3125261	3.684
4	Flow rate	0.9 mL/min	312625	3.587
5		1 mL/min	312524	3.654
6		1.1 mL/min	312621	3.641

Table 5: Results of System Suitability

S. No.	System suitability parameters	Value	Acceptance criteria
1	Retention Time (Rt)	3.645min	> 2 min
2	Theoretical plate (N)	3152	> 2000
3	Tailing factor (T)	1.6	Not more than 2
4	% RSD	0.92	< 2

CONCLUSION

The developed method was validated as per ICH guideline and was found to be within the prescribed limit. It concludes that the developed methods are simple, selective, sensitive and robust and suitable for both authentic and pharmaceutical dosage form.

REFERENCES

1. Ashutoshkar; Pharmaceutical Drug Analysis, *Published By New Age International* (P) Ltd,(2001).
2. P.Ravisankar, A Review On Analysis Method Development, *Indian Journal of Research In Pharmacy And Pharmaceutical Sciences*, 2(3), 2014, 1183-1195.
3. Ravi Shankar S. Text Book of Pharmaceutical Analysis. 3rd Ed. Tirunelveli: Rx Publications; 2001.
4. Kasture AV, Mahadik KR, Wadodker SG, More HN. Instrumental Methods of Pharmaceutical Analysis 2 (14),2006,1-30..
5. H.H. Williard, L.L. Merit, F.A.Dean And F.A. Settle, Instrument Methods of Analysis, Ed-

7th , CBS Publishers And Distributers, New Delhi, 1(156),1986,622-628.

6. Sharma .B.K, High Performance Liquid Chromatography, Instrumental Methods of Chemical Analysis. 13th Ed. Meerut: Geol. Publisher House; 2000,1, 7.
7. Chatwal, Anand, High Performance Liquid Chromatography, Instrument Methods Of Chemical Analysis. 1St Ed. Mumbai: Himalaya Publisher House; 2000,184.
8. International Conference On Harmonization (ICH) Guidelines Q2 (R1). Ich Guidelines: Validation Of Analytical Procedures: Text And Methodology Geneva, 2005.
9. International Conference On Harmonization (ICH) Guidelines Q2A, ICH Guidelines: Text On Validation Of Analytical Procedure, 1995.
10. Suddhasattya Dev, UV Spectrophotometric Determination Of Cefixime In Bulk And Its Dosage Form, *Journal Of Pharmacy Research* 2012,5(12),5419-5422.
11. Md. Ahasan Ullah Nayon, Development And Validation Of UV Spectrometric Method For The Determination Of Cefixime Trihydrate In Bulk And Pharmaceutical Formulation.
12. S.N.H. Azmi, B.Iqbal, Method Development And Validation For The Determination Of Cefixime In Pure And Commercial Dosage Forms By Spectrophotometry, *International Scholarly And Scientific Research & Innovation* 8(6) 2014.
13. Avanjia Dube; Spectrophotometric Estimation of cefixime tablet dosage form, International Journal of Pharmacy & Life Sciences 2(3):March, 2011.
14. Devkhile A.B: Method development and validation for third generation cephalosporin by UV-Vis Spectrophotometer, International Research Journal of Pharmacy, 2(1) 2011 222-229.
15. Heena J. Patel: Research article a new Spectrophotometric Method for the Simultaneous Multicomponent analysis Resolution of Cefixime and Cloxacillin Sodium in their tablet dosage form, Advance in Chemical Science
16. C.H Latha Saranya, Simultaneous Determination Of Cefotaxime Sodium And Paracetamol By LC-MS, *IOSR Journal Of Pharmacy*, Vol.4 ,2 (Feb-2014), Pp 12-18.
17. Ali Abdollahi, Determination Of Some Cephalosporins In Pharmaceutical Sciences, March 2016,22, 28-34.
18. Https://En.M.Wikipedia.Org/Wiki/Cefixime
19. Https://Www.Drugbank.Ca/Drug/DB00671