DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF FOURTH GENERATION CEPHALOSPORINS (CEFEPIME) IN PURE AND DOSAGE FORM

SYAMA SUNDAR B, GURUCHARANA DAS V*
T R R Government Degree College, Kandukur, A.P.
***Corresponding author:vavilaladas@gmail.com**

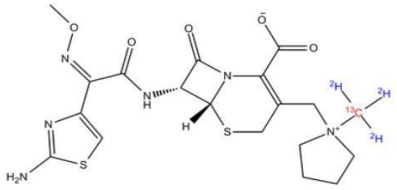
ABSTRACT

The Present research work deals with RP-HPLC method for the development and validation of cefepime which can be extended to the remaining fourth generation Cephalosporins. In this method mobile phase is a mixture of o-phosphoric acid, OPA, (1%) and methyl alcohol 80:20 (v/v). A non polar C18 column was chosen as the stationary phase for this study. A flow rate of 1.0 mL/min mobile phase was found to be suitable in the studied range of 0.5—1.5 mL/min. The retention time obtained for cefepime was 2.79 min.

Keywords: Cefepime,o-phosphoric acid, methyl alcohol, UV detection, Validation

INTRODUCTION

The work has been taken up in view of developing High Performance Liquid Chromatographic method for the determination of fourth generation cephalosporins at micro gram level, which are pharmaceutically and biologically important components. Cefclidine, Cefepime (Magapime), Cefluprenam, Cefoselis, Cefozopran, Cefpirome (Cefrom), Cefquinome are the fourth generation cephalosporins which are used for the treatment of surgical infections which include a variety of entities such as secondary peritonitis, intra-abdominal abscesses, obstetric and gynecological infections as well as bone-joint and soft-tissue infections.



Structure of Cefepime

Literature survey revealed that a few analytical methods were reported for the determination of cefepime in pure drug and dosage forms and in biological samples using high performance liquid chromatography,LC-Mass Spectrometry, Gas Chromotography, Chemi luminescence, Ion chromatography,[13]either in sample form or combined forms.

High-performance liquid-chromatographic methods have been developed and validated for analysis of cefepime in several biological matrices20 using dichloromethane, 94.5:5.5 (v/v) water and acetonitrile containing 0.015 M pentane sulfonic acid sodium salt, acetonitrile and tetra butyl ammonium hydroxide adjusted to pH 5.0 with ortho- phosphoric acid in ratio 20:80 (v/v) ratio, aqueous mobile phase of dibasic potassium hydrogen phosphate (pH 7.0) and methanol as a mobile phase.

MATERIALS AND METHODS

Chemicals and Reagents: Methanol – HPLC grade (Merck Industries, Worli, Mumbai) ortho phosphoric acid- HPLC grade (SD Fine chemicals, Mumbai) were used. The reference samples of cefepime was supplied by Venus Remedies Limited, India and the branded formulations of cefepime,(Magapime, Novapime and cepime) were used.

Instrumentation and chromatographic conditions: A Shimadzu HPLC equipped with a Luna C_{18}column (250 mm x4.6mm, 5µ) an LC 20 AD pump and a SPD 20 AD UV- Visible detector was employed in this study. Chromatographic analysis and data acquisition was monitored by using spinchrome software. A 20 µL Hamilton syringe was used for sample injection. Degassing of the mobile phase was done by using a spectra lab.DGA 20A3 ultra sonic bath sonicator. A Shimadzu electronic balance was used for weighing the materials.

Column: A non polar C_{18} column was chosen as the stationary phase for this study.

Mobile Phase: A mixture of 1% ortho phosphoric acid and methanol in 80:20 (v/v) was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were well defined, resolved and free from tailing. A flow rate of 1.0 mL/min mobile phase was found to be suitable in the studied range of 0.5—1.5 mL/min.

Preparation of Solutions

Standard Solution of cefepime (in dosage form): Standard solution of cefepime was prepared by dissolving 100 mg of drug sample (Novapime – 100 mg – Lupin laboratories, Mumbai) in 100 mL of distilled water. Working solutions of drug sample (100µg / mL) were prepared by diluting aliquots of the stock solutions with distilled water.

Wave length: The spectra of diluted solutions of cefepime in methanol were recorded on UV spectrophotometer in order to elicit λ_{max} (maximum absorbance). The spectra of cefepime showed a balanced wavelength at 253 nm.

Retention Time: Under the above optimized conditions retention of 2.79 min was obtained for cefepime.

Method validation: The developed method of analysis was validated as per the ICH Q2 (R1) guidelines for the parameters like specificity, linearity, precision, accuracy, robustness and system suitability, limit of detection and limit of quantification.

Specificity: The blank solution was prepared by mixing the excipients in the mobile phase without the drug. The drug to excipient ratio used was similar to that in the commercial formulations. The commonly used excipients in formulations like lactose, microcrystalline cellulose, ethyl cellulose, hydroxyl propyl methyl cellulose, magnesium stearate and colloidal silicon di oxide were used for the study. The mixtures were filtered through 0.45µ membrane filter before injection. An observation of chromatograms indicates absence of excipients peaks near the drug peak in the study runtime. This indicates that the method is specific.

Precision: The precision of the method was studied in terms of repeatability in intra-day assay and inter-day assay (intermediate precision). The intra day and inter day variation for determination of cefepime was carried out at four different concentrations. %RSD values are presented in the Table.2. shows that the method provides acceptable (<2) intra day and inter day variation.

Linearity: The mean peak areas were noted from the chromatograms and a plot of concentrations over the peak areas was constructed at 253 nm. The regression of the plot was computed by least square method. The linearity was found to be in the range of 20—100 µg/mL between the concentration of cefepime and peak area response .This regression equation was later used to estimate the amount of cefepime in pharmaceutical dosage forms.

Robustness: A study was conducted to determine the effect of deliberate variations in the optimized chromatographic condition of the mobile phase, flow rate, and the pH of the mobile phase. A single condition was carried at a time keeping all other parameters constant. The results were found to be within the allowed limits which indicate that the method is robust.

Variation in composition of mobile phase: The tailing factor and the number of theoretical plates showed a little change with change in mobile phase composition.

Variations in flow rates: A study was conducted to determine the effect of variation in flow rate. The system suitability parameters were evaluated at 0.9 mL/min and 1.1 mL/min. The results were within the acceptable criteria. Hence the allowable variation in flow rate is 0.9 mL/min. to 1.1mL/min.

System Suitability*:* System precision and system suitability studies were carried out by injecting six replicates of the working standard solution. The % RSD for the peak areas obtained was calculated. The data presented in Table.3. reveals that %RSD is <1 and establishes reproducible performance of the instrument.

Limit of Detection and Limit of Quantification: In this study the analyte response is 10 times greater than the noise response. For this study six replicates of the analyte at lowest concentration in the calibration range were measured and quantified. The LOD and LOQ of Cefepime obtained by the proposed method were 20 and 60µg/mL respectively.

Estimation of the drug from dosage forms: Ten tablets of cefepime were weighed and powered into uniform size in a mortar. An average weight of a tablet was calculated from this powder. An accurately weighed portion from this powder equivalent to 100 mg of cefepime was transferred to 100mL volumetric flask containing 20 mL of mobile phase. The contents of the flask were sonicated for about 20 min. for complete solubility of the drug and the volume was made up to 100 mL with water. Then the mixture was filtered through 0.45µ membrane filter.4mL of above solution was taken into a separate 100mL volumetric flask and made upto the volume with mobile phase and mixed well. The above solution (20µL) was then injected six times into the column and the mean peak area of the drug was calculated and the drug content in the formulation was calculated by the regression equation.

RESULTS AND DISCUSSION

A mixture 1% ortho-phosphoric acid and methanol in a proportion of 80:20(v/v) was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention time obtained for cefepime was 2.79 min.

A good linear relationship (r=0.9982) was observed between the concentration of cefepime and the respective peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was **Y=388732.5X-0.0259** where Y gives peak area and X is the concentration of the drug. When cefepime solutions containing 20, 40, 60, 80µg/mL were analyzed by the proposed method for finding out intra and inter day variations, low % RSD was observed. High recovery values obtained from the dosage form by the proposed method (99.4%) indicates the method is accurate. The absence of additional peaks indicates non interference of common excipients used in the tablets.

The deliberate changes in the method have no effect on the tailing of the peak, theoretical plates and percent assay. This indicates that the present method is robust. The lowest values of LOD and LOQ for the proposed method indicate that the method is sensitive. The standard solution of the drug was stable upto 24 hours as the difference in percent assay is within acceptable limit.

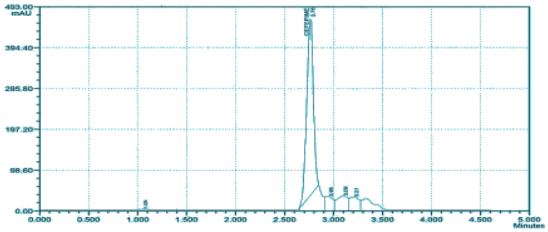


Figure.1.Chromatogram of Cefepime

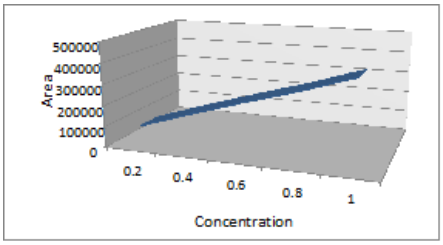


Figure.2.Linearity Curve

Table.1.Linearity and other parameters of Cefepime by the proposed HPLC method

Concentration	Area	Parameter	Value
0.2 mg/mL	86352	Linearity Range(µg/mL)	20-100
0.4 mg/mL	167809	Slope(a)	388732.5
0.6 mg/mL	245006	Intercept(b)	-0.0259
0.8 mg/mL	317974	Correlation Coefficient	0.99982
1.0 mg/mL	400002	Regression Equation	Y=388732x-0.0259

Table.2.Intra & Inter-day precision

Concentration of Cefepime µg/mL	Intra-Day Precision			Inter-Day Precision		
	Mean amount	% Amount	%RSD n=3	Mean amount	% Amount	%RSD n=3
20	19.78	98.9	0.91	20.02	100.1	0.18
40	40.5	100.25	0.44	39.92	99.8	0.18
60	59.95	99.91	0.32	59.52	99.2	0.18
80	80.55	100.68	0.22	79.65	99.56	0.18

Table.3.Accuracy Data

Amount taken µg	Amount found µg	Percent %Recovery	Mean Recovery	%RSD
0.2	0.196	98	99.66	1.7
0.2	0.198	99		
0.2	0.204	102		
0.4	0.395	98.75	98.5	0.54
0.4	0.391	97.75		
0.4	0.396	99		
0.6	0.602	100.33	100.05	0.64
0.6	0.604	100.66		
0.6	0.595	99.16		

Table.4.Results of Robustness Study

Variation of Mobile Phase		Chromatographic Parameters		
		Tailing factor	Theoretical plates	% Assay
MeOH	OPA (0.1%)			
15	85	0.56	6170	99.76
20	80	0.15	6138	99.94
25	75	0.32	6151	99.83

Table.5.LOD and LOQ of Cefepime

Parameter	Value (µg/mL)
LOD	20
LOQ	60

Table.6.System Precision

Injection Number	Peak Area	Theoretical Plates
1	609507.9	6266
2	601945.3	6240
3	605718.5	6343
4	609815.2	6274
5	606922	6319
6	607234.5	6298
Mean	**606781.78**	–
SD	**3208.3925**	–
%RSD	**0.528**	–

Table.7.Analysis of formulations & Recovery experiments

Sample	Labeled Amount	Amount found	%Recovery
CEPIME	500mg	498.7 mg	99.74
MEGAPIME	500mg	499.3 mg	99.86

CONCLUSION

Hence it can be assessed that the proposed RP- HPLC method for the determination of Cefepime is sensitive, precise and accurate and reproducible for the routine analysis of Cefepime in bulk and pharmaceutical dosage forms.

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