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Reverse Phase High Performance Liquid Chromatographic Estimation of Ceftazidime in Pharmaceutical Formulations

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An accurate, precise and reproducible high performance liquid chromatographic method was developed for the estimation of ceftazidime (CFZ) in bulk drug and pharmaceutical formulations. In this method, Luna C_{18} column (250 mm × 4.6 mm; 5 µ) with mobile phase consisting of buffer (0.01M disodium hydrogen phosphate, pH was adjusted to 5.0):acetonitrile:water (50:25:25 v/v/v). The flow rate was 1.5 mL/min and the detection wavelength was 254 nm. The linearity was observed in the range of 50-150 µg/mL with a correlation coefficient of 0.9995. The proposed method was validated for its linearity, accuracy, precision and robustness. The proposed method is simple, rapid, accurate, precise and reproducible hence can be applied for routine quality control analysis of ceftazidime (CFZ) in capsule dosage forms.

Key Words: Ceftazidime, HPLC, Estimation, Validation.

INTRODUCTION

Ceftazidime (CFZ)[1] is a third generation cephalosporin antibacterial with enhanced activity against pseudomonas aeruginosa. It is used in the treatment of susceptible infections especially those due to pseudomonas species. They include biliary tract infections, bone and joint infections, cystic fibrosis (respiratory tract infections) endophthalmitis, infections in immuno compromised patients (nutrophenic patients) meliodosis, meningitis, peritonitis, pneumonia, upper respiratory tract infections, septicaemia, skin infections (including burns, ecthymagangrenosum and ulceration) and urinary tract infections. It is also used for surgical infection prophylaxis (Fig. 1). Ceftazidime is chemically (Z)-(7R)-7-[2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(1-pyridiniomethyl)-3-cephem-4-carboxylate pentahydrate.

Literature survey revealed that a several analytical methods have been reported for the determination of ceftazidime in pure drug, pharmaceutical dosage forms and in biological samples using liquid chromatography[2-15], spectrophotometry[16-22], high performance thin layer chromatography[23] and electrokinetic chromatography[24] either in single or in combined forms. The aim of the present work is to develop and validate a simple, fast and reliable isocratic RP-HPLC method with UV detection for the determination of ceftazidime in bulk and in tablet dosage forms. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH)[25] for the determination of ceftazidime in bulk and in tablet dosage form.

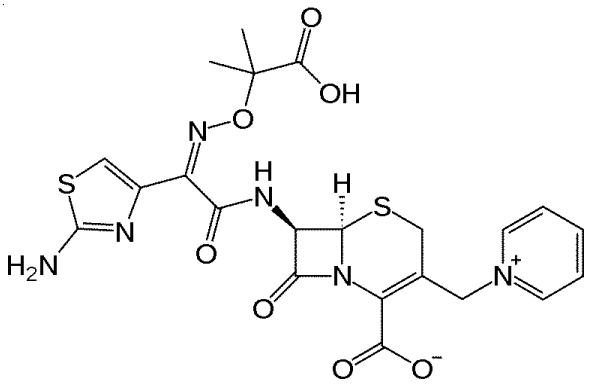

Fig. 1. Chemical structure of ceftazidime

EXPERIMENTAL

The analysis of the drug was carried out on a waters HPLC system equipped with a reverse phase Luna C_{18} column (250 mm × 4.6 mm; 5 µm), a 2695 Quaternary pump, a 20 µL injection loop and a 2487 dual absorbance detector and running on Waters Empower 2.0 software.

The gift sample of ceftazidime hydrochloride was supplied by Sun Pharmaceutical Industries Ltd., Baroda. Commercial tablets of CFZ were procured from local market. HPLC grade Acetonitrile and water were purchased from E. Merck (India) Ltd., Mumbai. Disodium hydrogen phosphate and orthophosphoric acid of AR Grade were obtained from SD Fine Chemicals Ltd., Mumbai.

Preparation of the 0.01 M buffer solution (pH 5.0): About 1.42 g of disodium hydrogen phosphate was transferred into a 1 L volumetric flask containing 200 mL of water. The contents were sonicated for 10 min and volume was made up to 1 L with water. The solution was filtered through 0.22 µ membrane filter and pH of the solution was adjusted to 5.0 with orthophosphoric acid.

Preparation of the mobile phase: A mobile phase mixture of acetonitrile, water and disodium hydrogen phosphate buffer (pH 5.0) in a ratio of 25:25:50 v/v/v was prepared by diluting 250 mL acetonitrile, 250 mL of water and 500 mL of buffer in a 1 L flask. The mixture was also used as diluent for preparing working standard solutions of the drug.

Procedure: A mixture of buffer (0.01M disodium hydrogen phosphate, pH was adjusted to 5):acetonitrile:water (50:25:25 v/v/v) was found to be the most suitable mobile phase for ideal separation of CFZ. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.5 mL/min. The column was maintained at ambient temperature. The pump pressure was set at 2200 psi. The column was equilibrated by pumping the mobile phase through the column for at least 0.5 h prior to the injection of the drug solution. The detection of the drug was monitored at 254 nm. The run time was set at 10 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 6.447 min. A typical chromatogram showing the separation of the drug is given in Fig. 2.

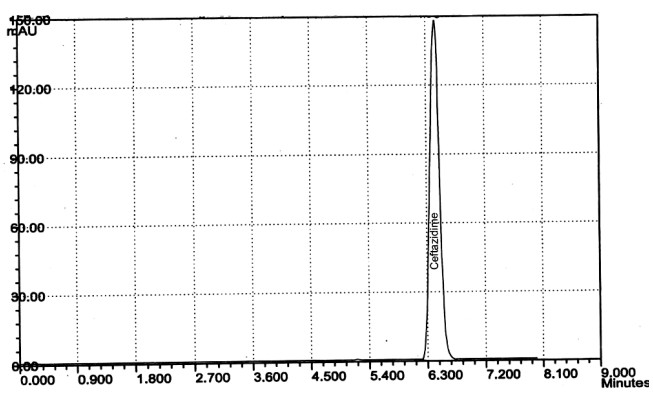

Fig. 2. Typical chromatogram of ceftazidime

Calibration plot: The quantitative determination of the drug was accomplished by the external standard method. The mobile phase was filtered through a 0.45 µ membrane filter before use. The flow rate of the mobile phase was adjusted to 1.5 mL/min. The column was equilibrated with the mobile phase for at least 0.5 h prior to the injection of the drug solution. The column temperature is maintained at 25 ± 1 ºC throughout the study. Linearity of the peak area response was determined by taking six replicate measurements at seven concentration points. Working dilutions of ceftazidime in the range of 50-150 µg/mL were prepared by taking suitable dilutions of the standard solutions in different 10 mL volumetric flasks and diluted up to the mark with the mobile phase. Twenty µL of the dilution were injected six times into the column. The drug in the eluents was monitored at 254 nm and the corresponding chromatograms were obtained. From the chromatograms, the mean peak areas were noted and a plot of concentrations over the peak areas was constructed. The regression of the plot was computed by least squares method. The linear relationship was found to be in the range of 50-150 µg/mL between the concentration of ceftazidime and peak area response. This regression equation was later used to estimate the amount of ceftazidime in pharmaceutical dosage forms.

TABLE-1
CALIBRATION DATA OF THE METHOD

Concentration of ceftazidime (µg/mL)	Mean peak area (n = 6)
50	77062
80	123301
90	136811
100	154123
110	169533
120	184954
150	231186

Validation of the proposed method: The method was validated according to ICH guidelines. The linearity, precision, accuracy, limit of detection, limit of quantification, robustness and other system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of CFZ. Solutions containing 25, 50 and 75 µg/mL of CFZ were subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table-2.

The accuracy of the HPLC method was assessed by analyzing solutions of CFZ at 50, 100 and 150 % concentrated levels by the proposed method. The results are furnished in Table-3. The system suitability parameters are given in Table-4.

Estimation of ceftazidime in pharmaceutical dosage forms: Twenty tablets of ceftazidime were weighed and

TABLE-2
PRECISION OF THE PROPOSED HPLC METHOD

Concentration of ceftazidime (µg/mL)	Intra-day precision			Inter-day precision		
	Mean amount found (n = 3)	Amount found (%)	RSD (%)	Mean amount found (n = 9)	Amount found (%)	RSD (%)
25	25.06	100.24	0.36	24.91	99.64	0.66
50	49.92	99.84	0.41	50.81	101.62	0.43
75	74.93	99.90	0.33	75.01	100.01	0.31

TABLE-3
ACCURACY STUDIES

Amount taken (µg)	Amount found (µg)	Recovery (%)	Mean percentage recovery	RSD (%)
25 + 20 = 45	44.91	99.8		
25 + 20 = 45	45.01	100.02	99.85	0.25
25 + 20 = 45	44.89	99.75		
25 + 25 = 50	50.06	100.12		
25 + 25 = 50	49.98	99.96	99.73	0.12
25 + 25 = 50	50.10	100.2		
25 + 30 = 55	54.68	99.42		
25 + 30 = 55	54.93	99.90	99.78	0.32
25 + 30 = 55	55.01	100.02		

TABLE-4
SYSTEM SUITABILITY PARAMETERS

Parameter	Result
Linearity (µg/mL)	50-150
Correlation coefficient	0.9995
Theoretical plates (N)	7692
Retention time (min)	6.447
Tailing factor	1.08
LOD (µg/mL)	0.12
LOQ (µg/mL)	0.40

powdered into uniform size in a mortar. From this the average weight of a tablet was calculated. An accurately weighed portion from this powder equivalent to 100 mg of ceftazidime was transferred to a 100 mL volumetric flask containing 20 mL of the methanol. The contents of the flask were sonicated for *ca.* 20 min for complete solubility of the drug and the volume was made up to 100 mL with mobile phase. Then the mixture was filtered through 0.45 µ membrane filter. From the above solution a 5 mL of aliquot was taken into a separate 50 mL volumetric flask and made up to the volume with mobile phase and mixed well. The above solution (20 µL) was then injected eight times into the column. The mean peak area of the drug was calculated and the drug content in the formulation was calculated by the regression equation of the method. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the pharmaceutical dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-5.

TABLE-5
ASSAY AND RECOVERY STUDIES

Sample	Labeled amount (mg)	Amount found* ± SD	Recovery* (%) ± RSD
Amceft tablets	250	249.6 ± 0.11	99.84 ± 1.2
Amceft tablets	1000	999.8 ± 0.23	99.98 ± 1.8

RESULTS AND DISCUSSION

In the proposed method, the retention time of CFZ was found to be 6.447 min. Quantification was linear in the concentration range of 50-150 µg/mL. The regression equation of the linearity plot of concentration of CFZ over its peak area was found to be Y = 1540X - 209.4 (r^2 = 0.9995), where X is the concentration of CFZ (µg/mL) and Y is the corresponding peak area. The number of theoretical plates calculated was 7692, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.12 and 0.40 µg/mL, respectively, which indicate the sensitivity of the method. The use of buffer (0.01M disodium hydrogen phosphate, pH was adjusted to 5):acetonitrile:water (50:25:25 v/v/v) resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in capsule formulations did not interfere with the estimation of the drug by the proposed HPLC method.

Conclusion

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of ceftazidime and can be reliably adopted for routine quality control analysis of ceftazidime in its pharmaceutical dosage forms.

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