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Development and Validation of New RP-HPLC Method for the Determination of Cefaclor in Pharmaceutical Dosage forms and in Human Plasma

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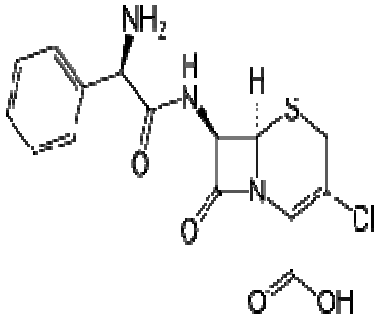
Abstract

A simple, rapid and precise reverse phase high performance liquid chromatography method has been developed and validated for the determination of Cefaclor in pharmaceutical dosage forms and in serum. Chromatography was performed by Shimadzu model LC-20 AT_{VP} with Kromasil C-18 column using a mobile phase comprising of acetonitrile: orthophosphoric acid (1%) : 0.01M ammonium dihydrogen phosphate (50:45:5 v/v), at a flow rate 1.0ml/min. The analyte was monitored using PDA detector at 270nm and the Run time was 10min. for Cefaclor. The proposed method was found to have linearity in the concentration range of 2-10 µg/ml.

Keywords: Cefaclor, accurate, precise, recovery, linearity, orthophosphoric acid.

Introduction

Cefaclor[1] having molecular formulae $C_{15}H_{14}ClN_3O_4S$ and molecular weight 367.808 belongs[2,3] to the family of second-generation cephalosporin antibiotic known as the cephalosporins and are used to treat[4] certain infections caused by bacteria such as pneumonia, ear, lung, skin, throat and urinary tract infections. Chemically cefaclor is (6*R*,7R)-7-[[(2*R*)-amino-phenyl acetyl]-amino] - 3-chloro-8-oxo-5-thia-1- Azabicyclo [4.2.0] oct-2-ene- 2-carboxylic acid monohydrate, which is soluble in water and insoluble in methanol, chloroform and benzene.



Cefaclor: Cobalt(II) and nickel(II) complexes of the Cefaclor have been synthesized[5]. X Chen etal[6], reported sensitive and specific liquid chromatographic-tandem mass spectrometric method for the determination of cefaclor in human plasma. Masaaki Kai etal[7], reported another method for the determination of cefaclor based on the chemical derivatization of the drug with 4-(2′ cyanoisoindolyl) phenylisothiocynate (CIPIC) under the reaction conditions with heating at 80°C for 7min in the presence of pyridine. Trajče Stafilov, etal, presented simple high-performance liquid chromatographic (HPLC) method to measure simultaneously the blood plasma concentration of cefaclor and cephalexine[8].

Material and Methods

Shimadzu model LC-20 AT_{VP}, using PDA detector was equipped with Empower software. Column make: Kromasil C-18 column, Particle Size: 5µ, Column length: 250 x 4.6mm ID, Injector Type: Rheodyne type injector, Injection Volume: 20 µl, Detection wavelength: 270nm, Flow rate: 1.0ml/min, Pump Pressure: 25.8 Mpa, Run time: 10 min, Mobile Phase Used: Acetonitrile: Orthophosphoric acid (1%):0.01M Ammonium dihydrogen phosphate (50:45:5 v/v).

Chemicals and Reagents: Cefaclor was obtained as a gift sample; all the chemicals were procured from E-Merck, India, Limited. Water (HPLC grade), acetonitrile (HPLC grade), orthophosphoric acid and ammonium dihydrogen phosphate (AR grade) were used.

Chromatographic Conditions: The mobile phase consisted of acetonitrile, 1%Orthophosphoric acid and 0.01M ammonium dihydrogen phosphate (50:45:5 v/v). Prepared mobile phase was filtered through 0.45µ membrane filter and sonicated. Sample solution was prepared by dissolving the drug in mobile phase and sonicated for 30 minutes. The mobile phase was delivered isocratic ally at a flow rate of 1 ml/min. All solutions were filtered through a 0.45 µ membrane filter before use. Kromasil C_{18} column 250 x 4.6 mm ID with 5µ particle size and the column were maintained at ambient temperature. The injection volume was 20µl and the total run time was 10min. The detection was carried out at 270nm. The chemicals were procured from E-Merck, India, Limited.

Preparation of Mobile Phase Solution: The mobile phase was prepared by mixing acetonitrile,1% orthophosphoric

acid and 0.01M ammonium dihydrogen phosphate (50:45:5 v/v) by ultra bath sonicated for 30 min.

Preparation of Standard: Stock solution of Cefaclor was prepared by dissolving accurately weighed 10mg of drugs in 10ml methanol (final concentration, 1000µg/ml). The prepared stock solutions were stored away from light. From the stock, standard solutions was freshly prepared during the day of analysis.

Preparation of Working Standard Solution (a.p.i): From the stock solution 1mmg/ml solution was prepared.

Preparation of Working Standards for Linearity: Solutions in the concentration range of 0.1-0.5mg/ml were prepared from the standard working solution.

Linearity and Calibration: Linearity was assessed by performing single measurement at several analyte concentration varying quantities of stock standard solution diluted with the mobile phase to give a concentration of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml. Injection was made at intervals of 6min. The linearity was tested for the concentration ranging from 0.1-0.5mg/ml. The peak area ratio of the drug was plotted against concentration. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision: Reproducibility was performed by injecting three replicates concentrations of standard and sample solutions which were prepared and analyzed by same analyst on same day. Inter-day variations in the peak area of drug solutions and the amount of drug were calculated in terms of Percentage Relative Standard Deviation. Sample concentration is 1mg/1ml.

Accuracy: Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 80%, 100% and 120% to the pre analyzed sample formulation.

Assay: The estimation of drug in pharmaceutical dosage forms. Cefalor tablets of 0.1mg strength were evaluated for the amount of Cefaclor present in the formulation. Each sample was analyzed in triplicate after extracting the drug. The amount of drug present in formulation was calculated by comparing the mean peak area from standard.

Intermediate Precision or Ruggedness: Inter-day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation.

Robustness: Robustness was carried out by varying two parameters from the optimized chromatographic conditions**.**

Specificity: The method was determined as specific by comparing test results obtained from analyses of sample solution containing excuse ingredients with that of test results those obtained from standard drug.

System Suitability Parameter: System suitability tests were carried out on freshly prepared standard stock solutions of Cefaclor and it was calculated by determining the standard deviation of Cefaclor standards by injecting standards in five replicates at 10 minutes interval and the values were recorded.

Preparation of Formulation Sample Solution: 20mg Ceflor Dps powder (50mg formulation) was weighed and dissolved in 10ml mobile phase. The resultant sample solution concentration is 2mg/ml then sonicated by ultra bath sonicated for 30 minutes and filtered through 0.45µm membrane filter. The amount of drug present in the 100mg formulation was calculated from linearity graph.

Preparation of Serum Sample Solution: 0.5ml of this serum was taken in a test tube and added 100µl of diltizem hydrochloride (1µg/ml) and 0.1ml of 1M NaOH and 5ml of dichloromethane and mixed about 20min in vortex mixer and centrifuged at 3000 rpm for 10min. From this centrifuged solution 4ml of organic layer was separated and evaporated to dryness to get residue. To this residue 100µl of 1M acetic acid and 3ml of n-hexane and mixed for 5 min by vortex mixer and evaporated the organic layer and finally the remaining sample was injected into HPLC and chromatogram was recorded. The amount of drug present in the blood sample was calculated from linearity graph.

Results and Discussion

The Reverse Phase high performance liquid Chromatography method was developed by a stability indicating assay method. Pure drugs chromatogram was run in different mobile phases containing methanol, acetonitrile, THF, and different buffers like potassium dihydrogen phosphate, sodium dihydrogen phosphate, Ortho phosphoric acid in different volumes ratios. Different columns like C_8, C_{18}, phenyl, cyano with different dimensions were used. Then retention time and tailing factor were calculated. Finally acetonitrile and 1% orthophosphoric acid and 0.01M ammonium dihydrogen phosphate in the ratio of 50:45:5 v/v (P^H:3.4) and Kromasil C_{18} analytical column was selected which gave a sharp and symmetrical peak with 1.82 tailing. Calibration graph was found to be linear at range 0.1mg/ml to 0.5mg/ml. Five different concentrations of Cefaclor in range given above were prepared and 20µl of each concentration injected in HPLC as shown in the table 1, figure 1. The slope (m) and intercept (c) obtained were found to be 170955.95 and -0.03.The correlation of coefficient (r^2) obtained was found to be 0.9626 as shown in the table 1. It was observed that the concentration range showed a good relationship. The limit of detection for Cefaclor was found to be 40µg/ml and the limit of quantification was found to be 75µg/ml. It

proves the sensitivity of method. The Percentage assay of Cefaclor in formulation was found to be 67.02%. as shown in the table 1 and figure 3. The relative standard deviation value obtained was below 1 which indicates the precession of the method. The validation of the proposed method was further verified by recovery studies. The data was presented by in the table 2 and figure 2. The percentage recovery was found to be 104.35% which shows a good index of accuracy of the developed method. The amount of drug present in the human serum sample was calculated from the linearity graph was found to be 1.079 mg/ 0.5ml as shown in table 3 and figure 4.

Table -1
Optical Chracterisation of Cefaclor

Parameters	Cefaclor
Linearity range(mg/ml)	0.1 – 0.5
Correlation coefficient(r)	0.9626
Slope(m)	170955.95
Intercept(c)	-0.03
Limit of detection (LOD; µg/ml)	40
Limit of Quantification (LOQ; µg/ml)	75
Tailing factor	1.82
Retention time (min)	6.053
Theoretical plates	3927.42
(%) R.S.D	0.148
(%)Accuracy	104.35
(%)Formulation	67.02
Serum (mg/0.5ml)	1.079

Table-2
Recovery Data of Cefaclor

Pharmaceutical formulation (brand name)	Labeled amount (mg)	Percentage Assay	Percentage recovery
KEFLOR	50 mg	67.02	104.35

*Average value of three different levels in triplicate

Conclusion

The RP-high performance liquid chromatographic method for the analysis of Cefaclor from their formulations was found to be accurate and precise. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of Cefaclor formulations. This method could be a simple for the practical applications and comparatively better method than the reported ones in the literature.

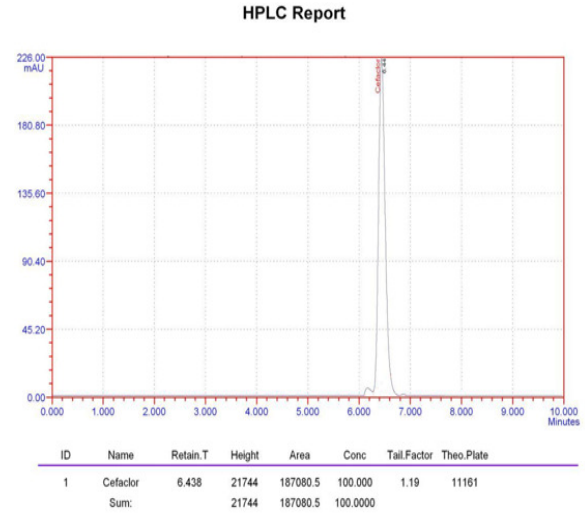


Figure-1
Chromatogram of cefaclor (standard)

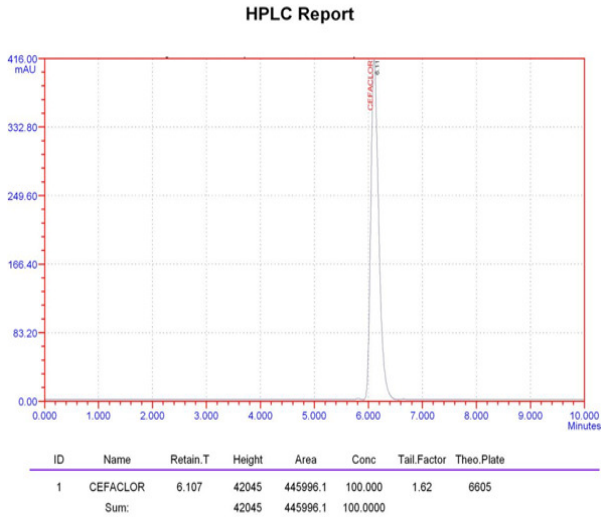


Figure-2
Chromatogram of Cefaclor (Accuracy)

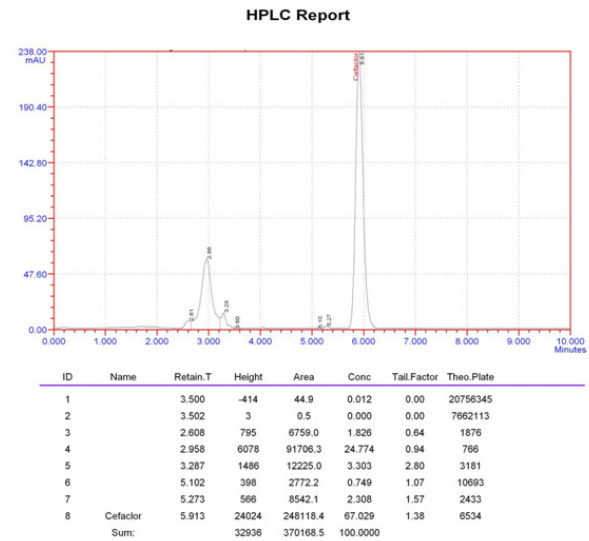
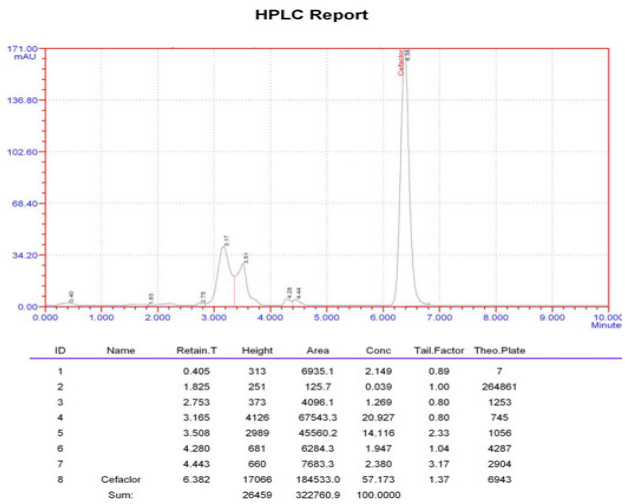


Figure-3
Chromatogram of cefaclor (formulation assay)



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		0.405	313	6935.1	2.149	0.89	7
2		1.825	251	125.7	0.039	1.00	264861
3		2.753	373	4096.1	1.269	0.80	1253
4		3.165	4126	67543.3	20.927	0.80	745
5		3.508	2989	45560.2	14.116	2.33	1056
6		4.280	681	6284.3	1.947	1.04	4287
7		4.443	660	7683.3	2.380	3.17	2904
8	Cefaclor	6.382	17066	184533.0	57.173	1.37	6943
	Sum:		26459	322760.9	100.0000		

Figure-4
Chromatogram of cefaclor (serum)

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