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New Validated RP-HPLC Method for the Estimation of Cefaclor in Pharmaceutical Formulation

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Cefaclor in Bulk and Pharmaceutical tablet Formulation. Isocratic elution at a flow rate of 1ml/min was employed on symmetry Shimadzu LC-20 AT_{VP} Kromasil C-18 column Column at ambient temperature. The mobile phase consisted of Acetonitrile : Orthophosphoric acid (1%) : 0.01M Ammonium Dihydrogen Phosphate (50:45:5 v/v). The UV detection wavelength was 270nm and 20 µl sample was injected. The run time for Cefaclor is 10 min. The flow rate was found to be 1ml/min. The percentage recovery of the method was found to be 104.35%. The LOD and LOQ for Cefaclor was found to be 40µg/ml and 75µg/ml respectively.The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of pharmaceutical formulation. The HPLC method can be successfully applied for the routine quality control analysis of Cefaclor formulations, which could be the better choices compared to the reported methods of literature

Key words: Cefaclor, Rp- HPLC, UV detection, Recovery, Precise.

INTRODUCTION

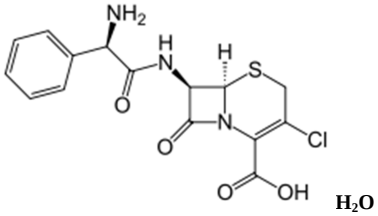


Fig: Structure of Cefaclor

Cefaclor belongs[1],[2] to the family of antibiotics known as the cephalosporins (cefalosporins). Cefaclor, also known as Cefachlor or cefaclorum (brandnames Ceclor, Distaclor, Keflor, Raniclor), is a second-generation cephalosporin antibiotic used to treat certain infections caused by bacteria such as pneumonia and ear, lung, skin, throat, and urinary tract infections. The Chemical name of 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. The frequency and severity of serum sickness-like reactions in children has led researchers to question its role in pediatric illness.[3] Cobalt(II) and nickel(II) complexes of the antibacterial drug Ceclor have been synthesized[4] and characterized on the basis of their elemental analysis, molar conductance, magnetic moment and electronic and infrared spectral data.

X Chen etal [5], A sensitive and specific liquid chromatographic-tandem mass spectrometric method is described for the determination of cefaclor in human plasma. The pretreated samples were analyzed on a C(18) HPLC column interfaced with a triple quadrupole tandem mass spectrometer. The lower limit of quantitation obtained as a result of the PPT procedure was 100 ng/ml. The intra- and inter-run precision, calculated from quality control (QC) samples was less than 12% for cefaclor. The accuracy as determined from QC samples was within +/-3% for the analyte. The SPE procedure could provide the lower limit of quantitation of 2 ng/ml. The precision and accuracy were measured to be below 7.1% and between -3.6% and 1.1%, respectively, for all QC samples. The method was applied for the evaluation of the pharmacokinetic profiles of cefaclor sustained-release formulation. Masaaki Kai etal[6], a sensitive method for the determination of cefaclor (CCL), a β-lactam antibiotic. It was based on the chemical derivatization of the drug with 4-(2′-cyanoisoindolyl)phenylisothiocynate (CIPIC) under the reaction conditions with heating at 80 °C for 7 min in the presence of pyridine. The derivatives emitted not only fluorescence (FL) at maximum emission wavelength of 410 nm with irradiation at 310 nm, but also chemiluminescence (CL) in the presence of H_2O_2, borate buffer (pH 9.6) and acetonitrile. The detection limit (S/N=3) in the chromatograph was 1 pmol by the CL detection and 10 pmol by the FL detection. The proposed CL method permitted the most sensitive determination of CCL in the human serum after its oral administration. Trajče Stafilov, etal [7], Developed a simple high-performance liquid chromatographic (HPLC) method to measure simultaneously the blood plasma concentration of cefaclor and cephalexine has been presented. The mobile phase was 0.025 mol l^{-1} KH_2PO_4 (pH 2.2) and methanol (75:25, V/V) using reversed phase C8 column. Analysis was run at a flow-rate of 1.2 ml min^{-1} and at a detection wavelength of 255 nm. The method was found to be reproducible with a RSD less than 6.0 % over the concentration range 0.2-30.0 μg ml^{-1} for cefaclor and 0.5-50.0 μg ml^{-1} for cephalexine in blood plasma samples. The limits of quantification were 0.2 and 0.5 μg ml^{-1}, respectively. A.V.D.Nagendrakumar et.al.,[8] proposed a Validated RP-HPLC Method for the Determination of Buspirone in Pharmaceutical Formulations. A simple, selective, linear, precise, and accurate RP-HPLC method was developed and validated for the rapid assay of the Buspirone in tablet dosage form. Isocratic elution at a flow rate of 1.0 mL/min was employed on a symmetry C18 (250 × 4.6 mm, 5 μm in particle size) at ambient temperature. The mobile phase consisted of water : acetonitrile :methanol 45 : 35 : 20(V/V). The UV detection wavelength was 210 nm, and 20 μL sample was injected. The retention time for the Buspirone was 7.057 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method can be successfully applied for the routine analysis of Buspirone in tablet dosage form.

MATERIALS AND METHODS

Instrumentation

Peak HPLC containing LC 20AT pump and variable wavelength programmable ***PDA detector*** and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a Kromasil C_{18} column 250 x 4.6 mm ID with 5 μ particle size and the column were maintained at ambient temperature. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar analytical balance was used for weighing the materials.

Chemicals and solvents

The reference sample of Cefaclor was obtained from Cipla, Mumbai. The Formulation was procured from the local market. Water, Methanol and potassium dihydrogen phosphate Acetonitrile Orthophosphoric acid and Ammonium dihydrogen Phosphate used were of HPLC grade and purchased from the chemicals were procured from E-Merck, India, Limited.

The mobile phase

The mobile phase was prepared by mixing Acetonitrile and 1% Orthophosphoric acid and0.01M 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.

Preparation of solutions
Stock Solution:
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:
From the stock solution 10mg/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.

Preparation of formulation sample solution
20mg KEFLOR 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.

Method Development
For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choosing stationary and mobile phases. The following studies were conducted for this purpose:

Detection of wavelength
The spectrum of 10ppm solution of Cefaclor was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength 270nm was observed.

Choice of stationary phase and mobile phase
Finally the expected separation and peak shapes were obtained on Kromasil C_{18} column 250 x 4.6 mm ID with 5 µ particle size.

Flow rate
Flow rates of the mobile phase were changed from 0.1-1.0 ml/min for optimum separation. It was found from experiments that 1.0 ml/min flow rate was ideal for elution of analyte.

Validation Procedure and Requirements
The analytical performance of the method of analysis was checked for specificity, System suitability, detection limit, and method precision.

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. KEFLOR 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.

$$\frac{\text{Sample peak area x Dilution factor of standard x Avg weight of tablets}}{\text{Standard peak area x Dilution factor of sample}}$$

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.

Cefaclor analysis in serum

Preparation of serum sample solution

From a local hospital blood was collected and serum was separated. 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.

Serum Data Of Cefaclor

From linearity graph we can estimate amount of drug present in the sample. Y=mx+c; Y =area

M=slope; X=concentration; C=intercept; Concentration $= \frac{\text{area - intercept}}{\text{Slope}}$; Amount of CEFACLOR present in serum $= \frac{184533 + 0.03}{170955.95}$; = 1.079mg/ 0.5ml

RESULT AND DISCUSSION

The Reverse Phase High Performance Liquid Chromatography method was developed 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 and0.01M 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 1.82tailing. 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 no: 3.4.01 Figure no: 3.4.1.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 no: 3.4.01. 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 no: 3.4.01 and figure no: 3.5.1. 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 no: 3.4.02 and figure no: 3.4.2. 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 no: 3.6.01 and Figure no: 3.6.1

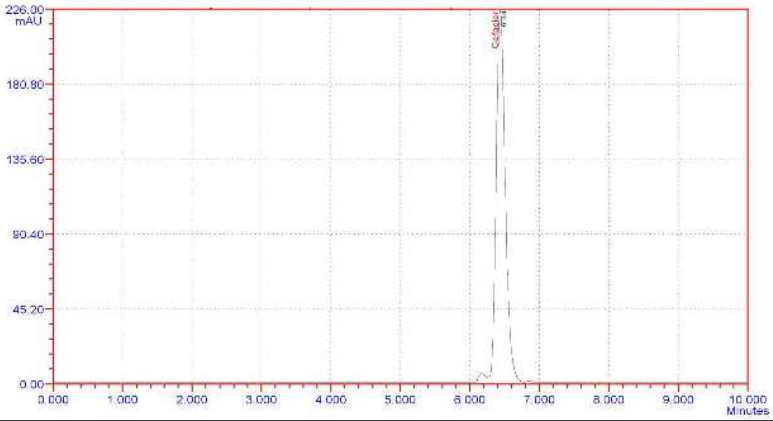
Table: 1 Optical chracterisation of cefaclor

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

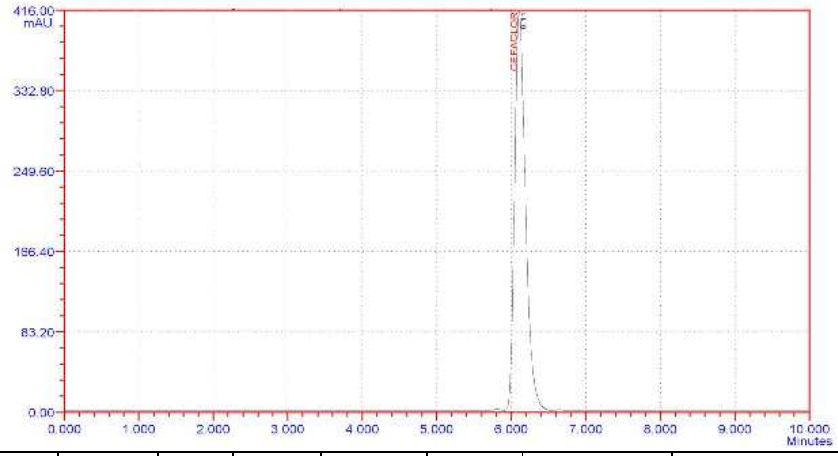
Pharmaceutical Formulationm (Brand Name)	Labeled Amount (mg)	Percentage Assay	Percentage Recovery
KEFLOR	50 mg	67.02	104.35

**Average value of three different levels in triplicate*



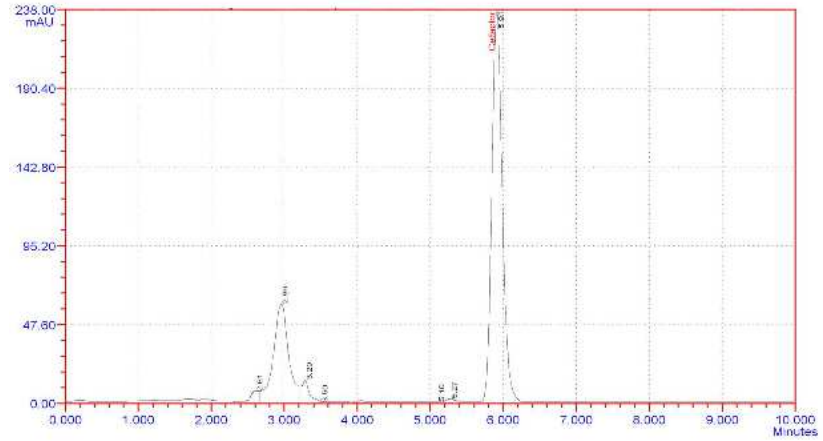
S.No	Name	Rt.	Height	Area	Conc.	Tailing factor	Theoretical plates
1	Cefaclor	6.438	21744	187080.5	100.000	1.19	11161
	SUM		21744	187080.5	100.000		

Fig: 2 Chromatogram of cefaclor and their values (Standard)



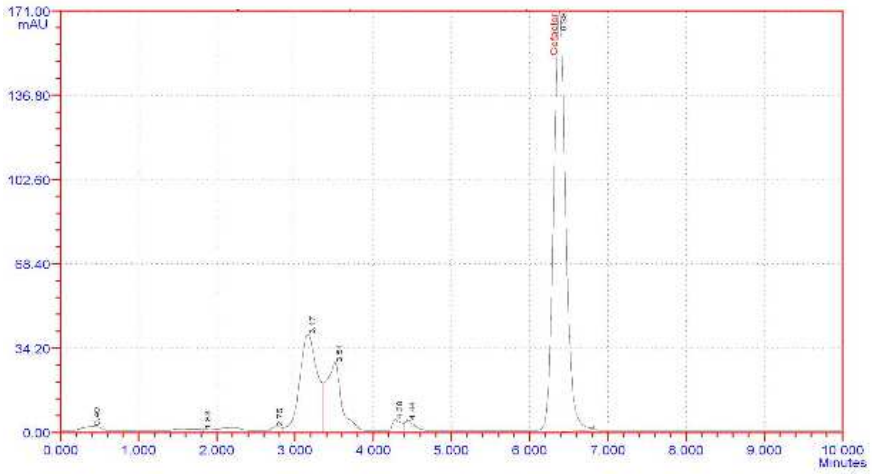
S.No	Name	Rt.	Height	Area	Conc.	Tailing factor	Theoretical plates
1	Cefaclor	6.107	42045	495966.1	100.000	1.82	6605
	SUM		42045	495966.1	100.000		

Fig.: 3 Chromatogram Of Cefaclor and their values (ACCURACY)



S.No	Name	Rt.	Height	Area	Conc.	Tailing factor	Theoretical plates
1	Cefaclor	3.500	414	44.9	0.012	0.00	20756345
		3.502	3	0.5	0.000	0.00	7682113
		2.608	795	6759.0	1.826	0.64	1876
		2.958	6078	91706.3	24.774	0.94	766
		3.287	1486	12225.0	3.303	2.80	3181
		5.102	398	2772.2	0.749	1.07	10693
		5.273	566	8542.1	2.308	1.57	2433
		5.913	24024	248118.4	67.029	1.38	6534
	Sum		32936	370168.5	100.000		

Fig: 4 Chromatogram Of Cefaclor and their values(Formulation Assay)



S.No	Name	Rt.	Height	Area	Conc.	Tailing factor	Theoretical plates
1	Cefaclor	0.405	313	6935.1	2.419	0.89	7
		1.825	251	125.7	0.039	1.00	264861
		2.753	373	4096.1	1.269	0.80	1253
		3.165	4126	67543.3	20.927	0.80	745
		3.508	2989	45560.2	14.116	2.33	1056
		4.280	681	6284.3	1.947	1.04	
		4.443	660	7683.3	2.380	3.17	1253
		6.382	17066	184533.0	57.173	1.37	745
	Sum		26459	322760.9	100.000		

Fig: 5 Chromatogram of Cefaclor and their values(SERUM)

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.

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REFERENCES

[1] A Hebert, E Sigman, M Levy *J Am Acad Dermatol*, **1991,** 25, 805–808.
[2] F Parra, J Igea, J Martín, M Alonso, A Lezaun, T Sainz, *Allergy*, **1992,** 47, 439–440.
[3] B A King, G C Geelhoed, *J Paediatr Child Health*, **2003,** 39, 677–681
[4] Z H Chohan, *Chem Pharm Bull*, **1991**, 39, 1578-1580.
[5] X Chen, D Zhong, B Huang, J Cui, *J Chromatogr B Analyt Technol Biomed Life Sci*, **2003**,784, 17-24.
[6] Masaaki Kai, Hiromi Kinoshita Kazuko Ohta, Shuuji Hara, Myung Koo Lee, Jianzhong Lu, *Journal of Pharmaceutical and Biomedical Analysis*, **2003**, 30, 1765-1771.
[7] Trajče Stafilov and Dragica Zendelovska, Determination of Cefaclor and cephalexine in blood Plasma by HPLC, Adnan Menderes University, 4th AACD Congress, 29 Sept-3 Oct.**2004**, Kuşadası-AYDIN/ TURKEY Proceedings Book 082.DOC
[8] M V Basaveswara Rao, A V D Nagendrakumar, Sushanta Maiti, G Raja, *Chromatography Research International* **2011**, Article ID 232505, 3 pages doi:10.4061/2011/232505