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Analytical method development and Validation for the Quantitative estimation of Cefditoren Pivoxil in tablet formulation by RP-HPLC

S.Vidhya[1]*, B.Lakshmi Narayanan[2], P.Malairajan[3], R.Joseph Sahayaraja[4], E.P.Kumar[1], S.Mahibalan[1], M.Karpakavalli[1], C.Balakumar[1]

[1]Karpagam University, Coimbatore, Tamil Nadu, India
[2] Aditya Bangalore Institute of Pharmacy Education and research, Bangalore, Karnataka, India
[3]Rofel Shri G.M. Bilakhia College of Pharmacy, Vapi, Gujarat, India
[4]Dr.Miltons Laboratories, Puducherry, India

Abstract

A simple and accurate RP-HPLC method has been developed for the estimation of Cefditoren Pivoxil in tablet pharmaceutical dosage form using C_{18} Nucleosil column 150 x 4.6 mm i.d, 5 µm particle size in isocratic mode with mobile phase comprising of phosphate buffer (pH 3.0), acetonitrile and methanol in the ratio of 50:25:25 v/v. The flow rate was 1.0 ml/min and detection was carried out by UV-PDA detector at 230 nm. The retention time for Cefditoren Pivoxil was found to be 4.2 min. The linearity range, correlation co-efficient and accuracy of Cefditoren Pivoxil was found to be 40 -360 µg/ml, 0.9999 and 99.21% respectively. The developed method was found to be simple, precise and accurate for the estimation of Cefditoren Pivoxil in tablet formulations.

*Corresponding author, Mailing address:
Miss. S.Vidhya, M.Pharm
Karpagam University, Coimbatore,
Tamil Nadu, India.
Phone No: 09566629852
Email: vidhyasheerapathi@gmail.com

Key words:
Cefditoren Pivoxil, RP-HPLC, tablet pharmaceutical dosage form, method development, validation.

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1. INTRODUCTION

Cefditoren is a third-generation semi-synthetic cephalosporin antibiotic for oral administration. Cefditoren Pivoxil is chemically (-)

(6R,7R)-2,2-dimethylpropionyloxymethyl7-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(Z)-2-(4-methylthiazol-5-yl)ethenyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate[1]. It is a prodrug which can be hydrolyzed by esterase during absorption to the active drug, cefditoren, and the drug is distributed in the circulating blood as an active cefditoren. Cefditoren Pivoxil is used in the treatment of mild to moderate pharyngitis, tonsillitis, uncomplicated skin, skin structure infections, and acute exacerbations of chronic bronchitis [1]. Molecular formula and the molecular weight of Cefditoren Pivoxil are $C_{25}H_{28}N_6O_7S_3$ and 620.73 respectively.

Literature review reveals that very few analytical methods were evoked for the determination of Cefditoren Pivoxil by RP-HPLC method for the determination of Cephalosporins (Cefditoren Pivoxil and Cefdinir) in pharmaceutical dosage forms[2], UV-Visible Spectrophotometric determination of Cefditoren Pivoxil in pharmaceutical formulations[3], Water vapour adsorption properties of amorphous cefditoren Pivoxil were evaluated by adsorption isotherms and microcalorimetry[4], determination of Cefditoren Pivoxil in bulk by RP-HPLC in presence of its degradation products[5]. The present study was aimed to develop a simple and reliable RP-HPLC for the determination of Cefditoren Pivoxil in their tablet dosage forms.

2. MATERIAL AND METHODS

2.1. Chemicals and Reagents

Cefditoren Pivoxil (99.999% purity) was obtained as a gift sample from Dr. Miltons Laboratories, Pondicherry. Methanol, acetonitrile were of HPLC grade obtained from Merck Research Laboratory, Mumbai, India. Sodium dihydrogen ortho phosphate and ortho phosphoric acid was also obtained from Merck Research Laboratory, Mumbai, India. Water (HPLC Grade) obtained from Himedia Pvt. Ltd, India.

2.2. Instrumentation

The instrument used was Shimadzu UFLC with the SPD-M20A High Performance Liquid Chromatography with PDA detector which provides advanced level of sensitivity and stability was used.

2.3. Chromatographic Conditions

Isocratic elution of mobile phase consisting of phosphate buffer (pH-3), acetonitrile and methanol in the ratio 50:25:25 v/v with the flow rate of 1.0 ml/min was performed on C_{18} Nucleosil, 5µm; 150x4.6mm i.d, ODS analytical column. The run time was set at 10 minutes. The column was equilibrated for 30 to 40 minutes with mobile phase before injecting the analyte. 20 µl of the analyte was injected and the detection was carried out at 230 nm at 25±1°C.

2.4. Chromatographic Method

2.4.1. Preparation of Mobile Phase

For the preparation of phosphate buffer (pH 3.0), 3.12 gm of sodium dihydrogen orthophosphate was dissolved in 1000 ml of water and the pH was adjusted to 3.0 with orthophosphoric acid. To this buffer, acetonitrile and methanol were added and mixed to get the ratio of 50:25:25 v/v respectively and were used as the mobile phase. The mobile phase were filtered through 0.45 µ membrane filter and degassed by ultrasonication before use.

2.4.2. Preparation of Standard Stock Solutions

100 mg of Cefditoren Pivoxil working standard was transferred into a 100 ml volumetric flask and dissolved in a mixture of 25 ml of methanol and 25 ml of acetonitrile and the volume was made up to 100 ml with buffer solution. 10 ml of this stock solution was transferred into a 50 ml volumetric flask

and the final volume was made up to 50 ml with buffer solution.

2.4.3. Preparation of Test solutions

Twenty tablets were weighed and powdered. A quantity of the powdered tablets equivalent to 100 mg was calculated, weighed and transferred into a 100 ml volumetric flask. A mixture of 25 ml methanol and 25 ml acetonitrile was added to dissolve the drug and the volume was made up to 100 ml with buffer solution and filtered. 10 ml of the above filterate solution was transferred into a 50 ml volumetric flask and the final volume was made up to 50 ml with buffer solution.

2.4.4. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample [6]. Here the linearity range of Cefditoren Pivoxil was found to be 40 to 360 µg/ml.

2.5. Statistical analysis

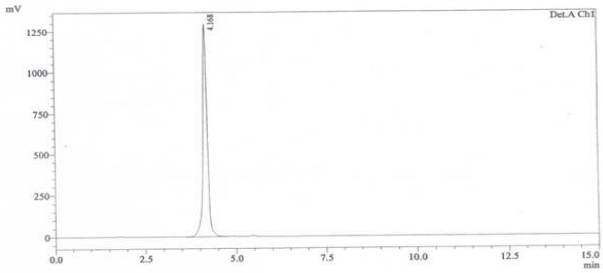
Statistical analysis and significance was carried out using correlation coefficient, standard deviation and percentage relative standard deviation (% RSD) with the help of Microsoft excel, 2007.

3. RESULTS AND DISCUSSION

3.1. Method Development

The above RP-HPLC procedure was developed for the determination of concentration of Cefditoren Pivoxil in tablet dosage form. The chromatographic conditions were optimized by changing the mobile phase composition and pH and buffers. Different ratios of solvent were used to get optimized mobile phase. Finally a mixture of buffer (pH-3), acetonitrile and methanol in the ratio of 50:25:25 v/v was optimized. A typical chromatogram was obtained by using the above mentioned mobile phase which was illustrated in Fig.1 and the retention time was found to be 4.16 minutes.

Fig.1: Chromatogram of Cefditoren Pivoxil



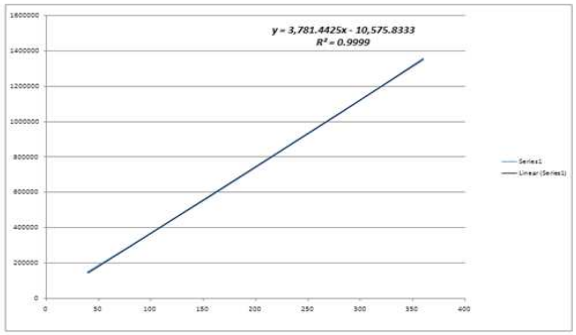
3.2. Method Validation

After the development of RP-HPLC method, it has been validated in terms of parameters like specificity, precision, accuracy, linearity, range, ruggedness, robustness and stability. For all the parameters, percentage relative standard deviation values were calculated. The proposed RP-HPLC method was validated as per ICH guidelines [7].

3.2.1. Linearity and Range

The linearity was evaluated by analysing different concentrations of the standard solutions of Cefditoren Pivoxil. The Beer's Lambert's concentration was found to be 40 to 360 µg/ml. Calibration curve was drawn by plotting average peak area against concentration and regression equation was calculated. The graph was given in Fig. 2.

Fig. 2: Calibration curve of Cefditoren Pivoxil



From the graph, it was noted that an excellent correlation exists between peak area and concentration of drug. Slope and the r^2 value of Cefditoren Pivoxil were found to be 3781.44 and 0.9999 respectively. It was observed that the regression value obtained was found to be within the limit.

3.2.2. Precision

Precision is the degree of agreement among individual test results when the procedure is applied repeatedly with multiple samplings of a homogenous sample[6]. Precision of Cefditoren Pivoxil was evaluated and the percentage relative standard deviation (% RSD) was found to be less than 1% which proves that the method was precise. Results were given in Table 1.

Table 1: Percentage relative standard deviation of Cefditoren Pivoxil

Sl. No.	Individual area	% Assay Value
1	1294019	99.89
2	1292443	99.14
3	1348169	98.74
4	1332216	98.94
5	1331173	98.79
6	1348169	99.04
Average assay value		99.09
Average % RSD		0.39

3.2.3. Accuracy

Accuracy was established using nine determinations over three concentration levels which cover the specified range in triplicates. Results were given in Table 2.

3.2.4. Specificity

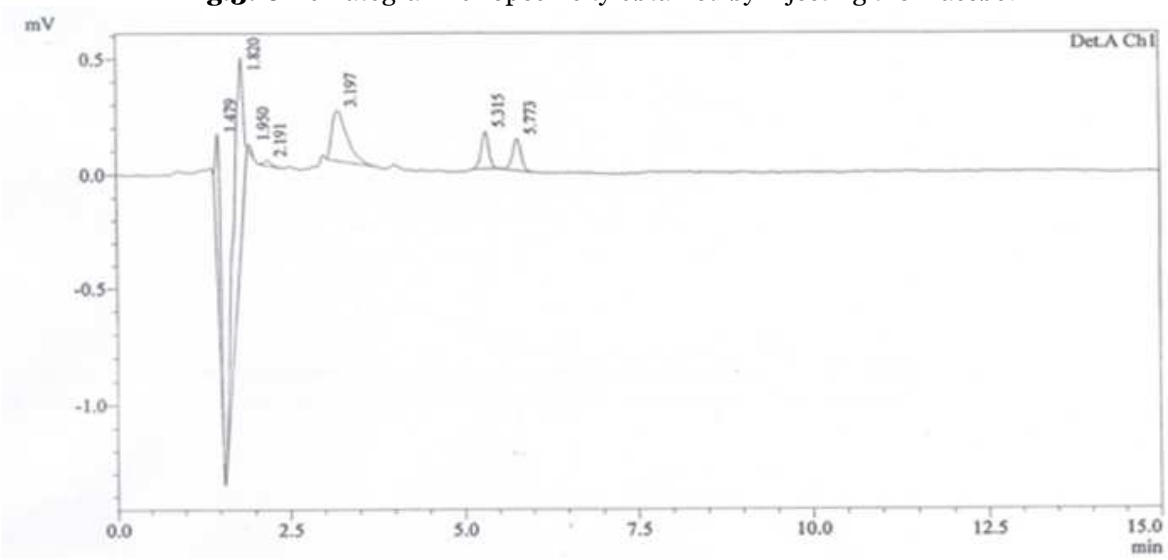
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc[7]. The retention times of various excipients added to the tablet formulation of Cefditoren Pivoxil does not interfere with the retention time of the active ingredient. Specificity of the Cefditoren Pivoxil was shown in fig 3.

Table 2: Accuracy of Cefditoren Pivoxil

Sl. No.	Conc. (µg/ml)	Individual area	% Assay Value
1	50	424446	98.86
		354990	99.42
		375641	99.28
2	200	1348169	98.89
		1329133	99.74
		1311879	99.68
3	350	2289349	98.16
		2300854	99.17
		2281956	99.96
Average assay value			99.21

An acceptance criterion of Assay was 98 to 102 %. Hence the assay value of the present study was fulfilling the acceptance criteria.

Fig.3: Chromatogram for Specificity obtained by injecting the Placebo:



Peak#	Name	Ret. Time	Area	Area %	Tailing Factor	Theoretical Plate#
1	RT1.479	1.479	3323	18.083	1.217	2229.901
2	RT1.820	1.820	9246	50.320	0.677	606.591
3	RT1.950	1.950	94	0.512	1.237	6428.540
4	RT2.191	2.191	151	0.822	1.297	2234.232
5	RT3.197	3.197	3222	17.535	2.031	1194.833
6	RT5.315	5.315	1190	6.474	0.963	10689.451
7	RT5.773	5.773	1149	6.253	0.977	9819.263
Total			18375	100.000		

Retention time of the drug was 4.16 minutes.

3.2.5. Ruggedness

Ruggedness is defined as the reproducibility of results when the method is performed under actual condition. This includes different analysts, laboratory, columns and instruments, sources of reagents, chemicals, solvents and so on[6]. Ruggedness of Cefditoren Pivoxil was performed using different system and different columns. The results were given in Table 3 and 4.

Table 3: Ruggedness values when performed on different column:

Si No.	Individual areas on different column	% Assay Value
1	1032958	98.87
2	1025052	99.00
3	1031395	98.36
4	1028899	99.18
5	1019821	98.64
Average assay value		98.81
Relative Standard Deviation		0.29
Acceptance Criteria:		RSD NMT 2%

Table 4: Ruggedness values when performed on different system:

Si No.	Individual areas on different system	% Assay Value
1	1065779	98.04
2	1064133	98.12
3	1063168	98.46
4	1059428	97.74
5	1053953	98.04
Average assay value		98.08
Relative Standard Deviation		0.23
Acceptance Criteria		RSD NMT 2%

Hence, when ruggedness was performed on different system and different column, the relative standard deviation was found to be within the limits that is not more than 2%.

3.2.6. Robustness

Robustness is the measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. This includes parameters such as pH, temperature, flow rate, mobile phase ratio, etc[6]. Robustness of Cefditoren Pivoxil was determined by changing the flow rate and the value was compared with the actual method parameters. It was observed that there were no marked changes in the chromatograms, which was demonstrated that the developed RP-HPLC method was rugged and robust. Further the method has good system suitability and precision under given set of conditions and also it was noted that robustness of Cefditoren Pivoxil were within the acceptance criteria of % RSD (Not more than 2%). The results were given in Table 5 and 6.

Table 5: Robustness values when the flow rate is varied by - 0.2 ml.

Si No.	Individual area at flow rate 0.8mL/min	% Assay Value
1	1127600	98.05
2	1122159	97.96
3	1116410	97.55
4	1112308	98.18
5	1108570	99.06
Average assay value		98.16
Relative Standard Deviation		0.5
Acceptance Criteria:		RSD NMT 2%

Table 6: Robustness values when the flow rate is varied by + 0.2 ml.

Si No.	Individual area at flow rate 1.2mL/min	% Assay Value
1	1163576	98.96
2	1169986	98.91
3	1169912	98.10
4	1167263	98.81
5	1151032	96.83
Average assay value		98.32
Relative Standard Deviation		0.82
Acceptance Criteria:		RSD NMT 2%

Hence, when the flow rate was varied by +0.2 ml and -0.2 ml, the relative standard deviation was found to be within the limits, that is not more than 2%.

3.2.7. System suitability

System suitability is the test to ensure that the method can generate results of acceptable accuracy and precision. System performance parameters of the developed RP-HPLC method were determined by analyzing standard working solutions. System suitability can be measured by determining the chromatographic parameters such as plate number (N), tailing factor, κ and or α, resolution (Rs) and relative standard deviation of peak height or peak area for repetitive injections[6]. System suitability of Cefditoren Pivoxil is given in Table 7.

Table 7: System suitability of Cefditoren Pivoxil

Sl. No	Parameters	Obtained values	Acceptance Criteria
1	Tailing factor	1.275	NMT 1.5
2	% RSD of peak area	0.68%	NMT 2 %

4. CONCLUSION

A convenient and rapid RP-HPLC method has been developed for the estimation of Cefditoren Pivoxil in tablet dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day % RSD coupled with excellent accuracy values. Hence we conclude that the proposed chromatographic method is simple, rapid, accurate and precise for the estimation of Cefditoren Pivoxil in tablet pharmaceutical dosage forms and can be used for routine quality control of this drug in formulations.

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