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Noval estimation of cefadroxil in tablet dosage forms by RP-HPLC

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ABSTRACT

A simple specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Cefadroxil in tablet dosage forms. A Hypersil ODS column (4.6 x 250mm, 5µm) in isocratic mode with mobile phase phosphate buffer pH 5.0 and acetonitrile ratio (96:4) was used. The flow rate was 1.0 ml/min. The detection was carried out at 254 nm and retention time of Cefadroxil was found to be 8.1 min. The method was validated for specificity, linearity, accuracy, precision, and limit of quantification, limit of detection & robustness. The limit of detection and limit of quantification for estimation of Cefadroxil were found to be 0.4 and 1.3 mg, respectively. The recoveries of Cefadroxil in tablet dosage were found to be in the range of 99.03-101.8%. Proposed method was successfully applied for the quantitative determination of Cefadroxil in tablet dosage forms.

Key words: Cefadroxil, β lactum antibiotics, RP-HPLC, validation.

INTRODUCTION

Cephalosporin is β-lactum antibiotics with the same fundamental structural requirements as penicillin[1]. They are used for the treatment of infections caused by Gram-positive and Gram-negative bacteria. They act by inhibiting the synthesis of essential components of bacterial cell wall. They are among the safest and the most effective broad-spectrum ant bactericidal antibiotics. Only cephalosporin, C is found naturally, the remaining semi-synthetic cephalosporin's are derived from 7-amino-cephalosporanic acid, a product obtained from cephalosporin C hydrolysis. Their composition is accomplished by β-lactum ring fusion with a dihydrothiazine ring differing in the nature of the substituent attached at the 3- and/or 7-positions of the cephem ring. The substitution at the 3-position[2] affects the pharmacokinetic properties, whereas the substitution at the 7-position affects the antibacterial spectrum of the cephalosporin[2]. The drug is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia(USP) the chemical name of Cefadroxil is 7 (R) – 2 amino – 2 (4 hydroxyphenylmethyl)acet-amido]-3–methyl, 3-cephem, 4-carboxylic acid monohydrate. Molecular Formula : $C_{16}H_{17}N_3O_5$ S H_2O Molecular Weight : 381.40

Various methods and reviews have been published covering the analysis of cephalosporins in biological matrices and pharmaceuticals. which include the use of LC[3-12] with UV Detection, LC with electrospray ionization mass spectrometry, and also determination, LC with Microbiology Assay, Determition of five orally active in human in serum, RP-LC analysis of cephalosporins, LC with fluorimetric detection, LC with derivative spectrophotometric in urine, determination by differential pulse voltammetry, rapid and sensitive high–performance liquid chromatographic

determination of cephalosporin antibiotics in pharmaceuticals and body fluids. Among the published methods of determination of cephalosporins, thin layer chromatography, gas chromatography, high performance liquid chromatography (HPLC), and microbiological assays are available. HPLC methods have been described for the determination of cephalosporin's in biological fluids using different stationary phases, mobile phases with different buffer systems, mostly phosphates or ion pairing agents, with detection mode, e.g., UV and electrochemical and sample preparation procedures. One of the review describes general HPLC conditions for simultaneous separation of more than two cephalosporin's.[13] Simultaneous measurement of several cephalosporin's by RP-HPLC, are also reported.
Present study involves development of RP-HPLC method using simple mobile phase contaning Acetonitrile and buffer for quantitative estimation of cefadroxil in tablet dosage forms which is sensitive and require shorter analysis time, The developed method was validated as per ICH guidlines.

MATERIAL AND METHODS

Cefadroxil (99.9% Purity) were recived from Lupin Pharmaceutical Ltd, Mandideep. All reagent used were of analytical grade, Acetonitrile (ACN) and water used was of HPLC grade . The Flow rate 1.0 ml per minute and detection was carried out at 254 were used in the full study. The HPLC was performed on a isocratic HPLC system (Shimadzu Class 10 A series) equipped with LC-10AS pump, a multiwavelenth analysis detector (SPD 10-A) and hypersil ODS C-18 column (250mm X 4.6mm ID, 5 micron). The HPLC system was equipped with software class CR-10 version (Shimadzu).

In addition a pH meter (Thermo Orion) and degasser (X15522050 Millipore), were used in the full study. The chromatographic condition adopted for drug analysis are outlined in table no 1.

Preparation of Phosphate buffer[14] pH 5.0

13.6 monobasic potassium phosphates in water to make 2000 ml solution adjust with 10 N potassium hydroxide to pH 5.0 buffers and mixed.

Mobile phase

A suitable mixture of pH 5.0 buffer and ACN (96:4) then filtered through 0.45 μ m filter paper under vacuum filtration and degassed.

Preparation of standard solution

50 mg of Cefadroxil working standard was accurately weighed and transferred in to 50 ml volumetric flask then 25 mL of phosphate buffer was added to it and sonicated for 15 min. and volume was made up to 50 mL with phosphate buffer. Solution was filtered using 0.45 µm HVLP filter. 5 ml of this solution was diluted to 50 ml with mobile phase and mixed so that concentration obtained was 100 µg/ml.

Preparation of test solution

20 tablets were weighed and crushed and further powdered drug equivalent to about 50 mg Cefadroxil was weighed and transferred to 50 ml volumetric flask. Test solution with concentration of 100 µg/mL was prepared similarity as with standard solution

Method validation of Cefadroxil

The analytical method was validated as per the recommendation of ICH for the parameters like specificity, range, accuracy, precision, and limit of quantitation, limit of detection, robustness and system suitability. Commonly used excipient such as starch, micro crystalline cellulose, lactose, magnesium stereate was also used to demonstrate the specificity.

Specificity study

[15]Commonly used excipient such as present in selected tablet formulation were spiked in to pre weighed quantity of the drug. The chromatogram was taken by appropriate dilution and quantities of drug were determined. The HPLC chromatogram of the Cefadroxil drug 100 µg/ml was compared with that of blank solution.

The linearity of the method was determined at the six concentration level ranging from 25-125 µg/mL. The standard deviation of y intercepts of regression line was determined and kept in following equation for the determination of detection limit and quantitation limit. Detection limit

is 3.3 σ/s and quantitation limit is 10 σ/s, where σ is the standard deviation of reading of lowest concentration range and s is the slope of the calibration curve.

The accuracy of the method[16] was determined by calculating recovery of Cefadroxil by method of standard addition. Working standard of the drug were prepared triplicate at three concentration level 50 µg/mL, 100 µg/mL and 150 µg/mL & analyzed.

Precision of analytical method was assessed in terms of repeatability and intermediate precision repeatability was checked by analysing six independent sample of Cefadroxil at 100% con level and calculating their relative standard deviation (%RSD)

The intermediate precision was determined by analyzing the standard solution of Cefadroxil at five concentration level. three times within same day (intraday variation) and Three different days within same day (inter-day variation) were observed.

Robustness of the method was studied by introducing small deliberate change in the flow rate composition of mobile phase, temperature, and pH the change flow rate was done by 1 ± 0.1% and percentage of acetonitrile in mobile phase 96 ± 2% and temperature was altered 30 ± 2% and pH was changed in phosphate buffer to 5 ± 2%.

RESULT AND DISCUSSION

Variation of analytical method assures the confidence of generated data for its purported results against method instrument analyst or any other variation. An analytical method is said to be specific as it can detect the analyte in the presence of expected impurities or additives in test sample. The representative chromatogram for blank as well as drug analysis showed that the blank solution (placebo) did not interfere with the drug peak. Retention time for Cefadroxil was found to be 8.1 min. the result are shown in figure-1

The linearity of analytical method signifies direct proportionality between quantifiable response and the concentration of analysts with a given range (bounded by upper and lower limits.

The linearity plot shown in [Figure 2] Calibration curve for Cefadroxil was obtained by the plotting the peak area ratio verses the concentration of Cefadroxil over the range of 25 to 125 µ/ml. Slope and intercept value for calibration was y=5.2797x+5.1941 and it was found to be linear over entire calibration range study with r^2 value of 0.99 the data of regression analysis of the calibration curve shown in the table-3. The detection limit was

Table 1: HPLC Parameter for Cefadroxil

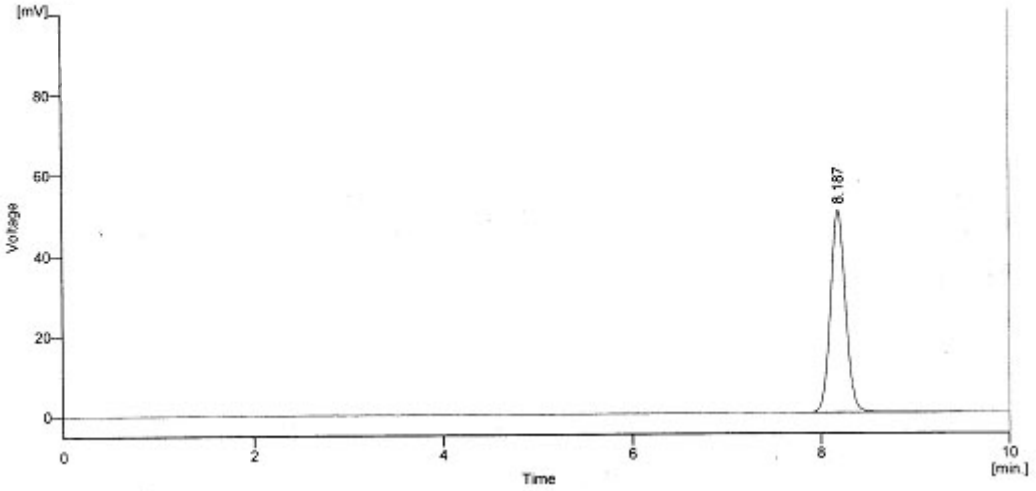
Parameters / conditions	Description / values
Column name	Hypersil ODS 5 microns (4.6 × 250mm, 5µm)
Flow rate	1.0 ml per minute
Injection volume	20 µL
Detector	254 nm
Temperature	30 °C
Mobile phase	Phosphate Buffer {pH 5.0} : Acetonitrile(96:4)

Table 2 : Analysis of RP- HPLC cefadroxil dosage form

Tablet (brand)	Label (Claim)	Assay (%)
1	500mg	100.6
2	500mg	99.3

Table 3: Linearity and range

sample	Range (µg/ml)	Goodness-of fit (r2)	Slope	Intercept
Cefadroxil	25-125	0.99	5.2797	5.1941



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	8.189	558.487	50.154	100.0	100.0
	Total	558.487	50.154	100.0	100.0

Fig. 1: RP HPLC chromatogram of Cefadroxil

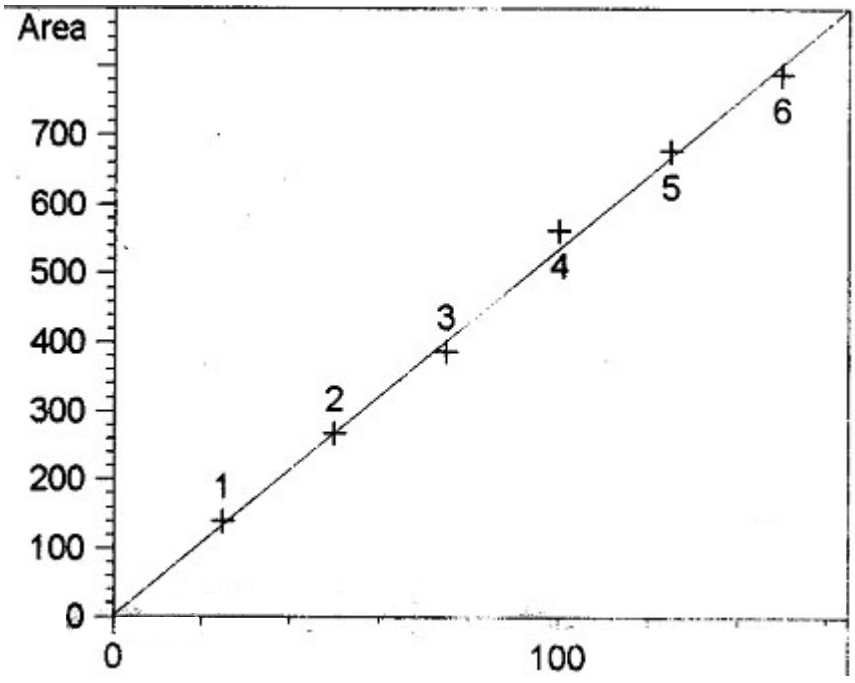


Fig. 2: Calibration curve of cefadroxil

found 0.4 mg and limit of quantification was found 1.3 mg.

The accuracy of analytical method the exactness in analytical response of the analyte to its true value. the result of accuracy Studies are shown in table-4 and it is evident method is accurate with in desired range.

The precision of analytical method certified the exactness of analytical response when tested for multiple sampling of homogenous sample. Repeatability refers to the use of the analytical procedure with in laboratory over a short period of time using same analyst with same equipment and expressed as % RSD. The % RSD values for six independent injection of Cefadroxil at 100% test concentration was 1.4%. The result of Precision Studies are shown in table 4

20 tablet were weighed and crushed and powdered drug equivalent about 50 mg Cefadroxil was weighed and transferred to 50 mL volumetric flask then 25 mL phosphate buffer was added to it and sonicated for 15 min and made up the volume with phosphate buffer. The 5 ml of this solution was diluted to 50 mL with mobile phase and mixed so that concentration was 100 µg/mL.

Table 4: Summary of validation parameters

Pricision	Result	Acceptance NMT 2%
Intraday(N=5)	0.8	RSD
Inter day(N=5)	0.7	
Repeatability(n=6)	1.4	
Accuracy(50%-150%)µg/ml	99.3-101.2	98-102%
Detection limit	0.4	NMT 2% RSD
Quantitation limit	1.3	NMT 2% RSD
System Suitability		NMT 2% RSD
Retention time	8.1	
Theoretical plate	1200	
Tailing factors	1.14	
RSD%	1.3	
Specificity	Specific	
Robustness	Robust	

Accurate with in desired range

The result (Table 1) describes, new RP-HPLC method using simple mobile phase for Cefadroxil in tablet formulation. The result of Analysis of different Brand tablet Studies are shown in table 2. The method was validated and found simple sensitive accurate and precise, percentage of recovery shows that the method is free from interference of the excipient used in the formulation. Therefore the new method can be used for routine analysis and estimation of Cefadroxil in tablet formulation

CONCLUSION

The aim of this study was to develop a simple fast and sensitive method for the determination of Cefadroxil antibiotic agents in Tablet form. The method is validated for various parameters as per ICH guidelines specificity, linearity, precision, accuracy, robustness, system suitability the result obtained are within the acceptance criteria.

Since the result are within acceptance criteria for all validation parameters. Therefore the method considered as validated and suitable for intended used also the method is specific for Cefadroxil 500 mg tablet.

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