

# Determination of Cefadroxil in Tablet/Capsule formulations by a validated Reverse Phase High Performance Liquid Chromatographic method

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**Abstract:** An innovative, selective and rapid reversed phase High Performance Liquid Chromatographic (RP-HPLC) method for the analysis of cefadroxil in bulk material and oral solid dosage forms has been developed and validated. The chromatographic system consisted of Sil-20A auto sampler, LC-20A pump and SPD-20A UV/visible detector. The separation was achieved by C<sub>18</sub> column at ambient temperature with a mobile phase consisting of methanol: Phosphate buffer (10: 90) at a flow rate of 1.5 ml/min. The method is reproducible, repeatable (%RSD for intra-day and inter-day ranged between 1.75-5.33% and 0.58-2.69%) and linear (R<sup>2</sup>=0.9935). The LOD and LOQ of the method were 0.5 and 1.0 µg/ml, respectively. The present RP-HPLC method was found to be sensitive, accurate, precise, rapid and cost effective that can be efficiently used in QC/QA laboratories for routine analysis of the raw materials as well as oral dosage formulations of cefadroxil.

**Keywords:** Cefadroxil, QC/QA laboratories, RP-HPLC method.

## INTRODUCTION

Cefadroxil is parahydroxy derivative of cephalixin having molecular formula of C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>·H<sub>2</sub>O and a molecular weight of 381.4 with a biological half-life of 1.5 hrs and 85% bioavailability. It has a pronounced oral absorption and used as drug of choice for the treatment of mild to moderate infections of soft tissues like skin, upper respiratory tract, pharyngitis and urinary tract. The daily recommended dose of cefadroxil is 500-1000 mg/day (Shetty, Shulmant *et al.*, 1999). Cefadroxil is a broad spectrum antibiotic that acts against both Gram positive and Gram negative organisms, including *Staphylococcus aureus*, *Proteus spp.*, *Bacillus spp.*, *Escherichia coli* and more potent to *Klebsiella spp.* It is less sensitive to β-lactamase produced by *Staphylococcus aureus* and *Bacillus subtilis* as compared to penicillins (Block and Beale, 2004). Cefadroxil remains as free drug in plasma because its protein binding is less than 20%. It is mainly excreted unchanged in urine. Structure of cefadroxil is given in fig. 1.

Cefadroxil monohydrate is available in different dosage forms including capsules, tablets, and dry powder for oral suspension, produced by both local and multinational companies. In the literature, spectrophotometric analysis has been reported for the quantitative estimation of cefadroxil monohydrate in pharmaceutical preparations including tablets/capsules (El-Gindy, El Walily *et al.*, 2000, Rageh, El-Shaboury *et al.*, 2010, Shukla, Pandey *et al.*, 2008, Shukla, Patel *et al.*, 2008). Many scientists have

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adopted HPLC for the quantitatively estimating cefadroxil in bulk and pharmaceutical preparations (Anjum, Shetty *et al.*, 2012, Devaliya and Jain, 2009, Dhoka and Chopade, 2012, Hsu, Chang *et al.*, 1992, Madhuri, Spandana *et al.*, 2012, Nahata and Jackson, 1990, Parasrampurua and Gupta, 1990, Patil, Patil *et al.*, 2011, Samanidou, Hapeshi *et al.*, 2003, Ting, 1988, Wu, Guo *et al.*, 1999). However, no simple and cost effective high performance liquid chromatographic (HPLC) method has been reported for the determination of cefadroxil in bulk and pharmaceutical preparations. The present study was conducted with the aim to focus on the development and validation of an accurate, rapid as well as cost effective HPLC method for the quantification of cefadroxil in tablets/capsules.

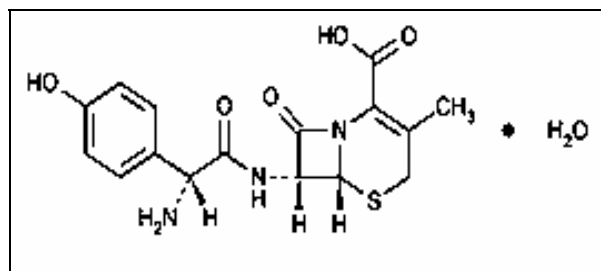


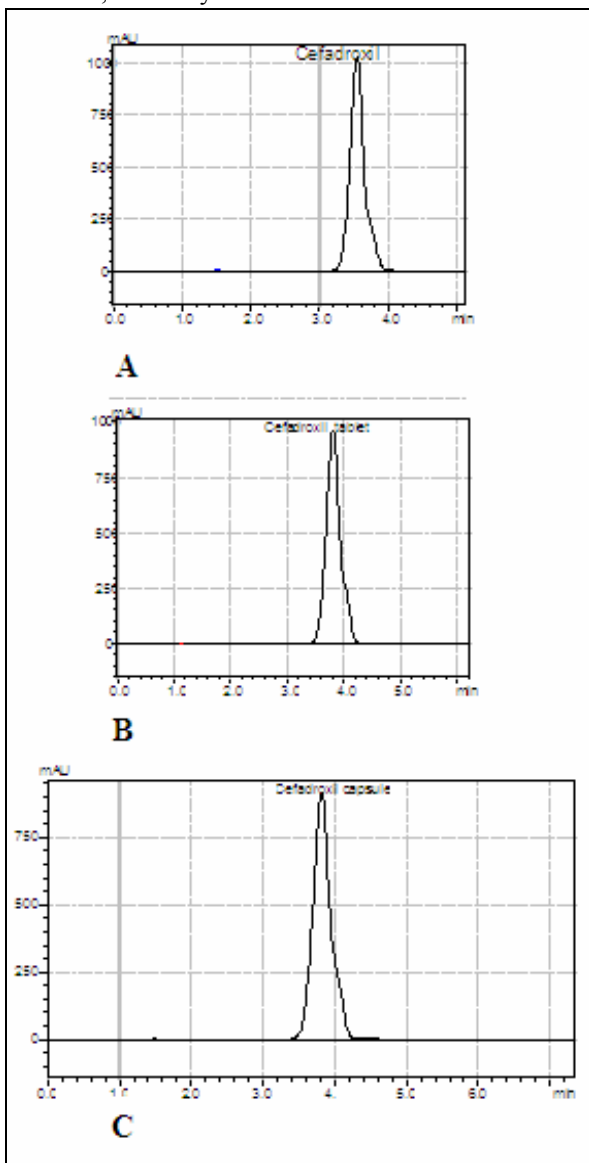
Fig. 1: Structure of Cefadroxil.

## MATERIALS AND METHODS

### Materials/reagents

Reference standard of cefadroxil was manufactured by ACS Dobfar, S.P.A, Italy and provided by BASIL Biopharm (Pvt) Ltd. All chemicals and reagents used

were of analytical grade and purchased from Merck Chemicals, Germany.



**Fig. 2:** Chromatograms of Cefadroxil pure (a), tablet (b) and capsule (c).

The method was an HPLC, with C<sub>18</sub> column and a mobile phase of methanol: phosphate buffer of pH 4.0 at ratio of 10:90, at flow rate of 1.5 ml/min and UV detection at 260 nm.

#### Equipment

Chromatographic studies were carried out by using the HPLC system (Shimadzu Corporation, Japan) equipped with Sil-20A series auto sampler, LC-20 AT pump and SPD-20AUV-VIS detector. Chromatographic system was integrated via Shimadzu model CBM-20A with LC solution software. C<sub>18</sub> column (5 $\mu$ m, 150mm $\times$ 4.5mm) (Water corporation, USA) and auto sampler was used. In addition, electronic balance, micro liter syringe, micropipette and micropore filtration assembly were used.

#### Chromatographic condition

The mobile phase consisted of methanol: phosphate buffer of pH 4.0 at ratio of 10:90 and filtered before use through 0.45 $\mu$ m membrane filter and was pumped at a flow rate of 1.5ml/min. The separation was carried out on a C<sub>18</sub> column at a temperature maintained at 25°C. The sample of 20 $\mu$ l was injected and analyzed under isocratic conditions. Chromatograms were recorded at  $\lambda = 260$  nm using SPD- 10A VP Shimadzu UV-VIS detector.

#### Preparation of cefadroxil reference standard solution

Standard solution of cefadroxil monohydrate was prepared by dissolving 25mg of reference standard of cefadroxil in 25ml of mobile phase. The resulting concentration was 1mg/ml or 1000 $\mu$ g/ml. This solution was further diluted as required for calibration and linearity.

#### Preparation of sample solutions from tablets

A total of 20 tablets of 200mg cefadroxil were carefully weighed, triturated with the help of mortar and pestle to get a fine powder and the amount of powder equivalent to 200mg of cefadroxil was transferred to a 200ml volumetric flask. It was dissolved in the buffer pH 4.0 by shaking for about 15min. The final concentration of sample solution was kept equivalent to 1mg/ml or 1000 $\mu$ g/ml.

#### Preparation of sample solutions from capsules

A total of 20 capsules of 200mg cefadroxil were weighed intact and emptied with the help of soft brush and the shells were weighed individually to calculate average content weight. The amount of capsule content equivalent to 200mg cefadroxil was transferred to a 200ml volumetric flask and the volume was made up with buffer pH 4. Both the sample solutions were filtered through 0.45 $\mu$ m HV Millipore membrane filter and injected to the HPLC system.

#### Validation procedure

Present study was conducted to obtain an innovative, simple, rapid and affordable method for the determination of cefadroxil. The HPLC method development and validation was performed according to the official specifications of Centre of Drug Evaluation and Research (CDER-1994), International Conference on Harmonization and United State Pharmacopeias (ICH, 1997, USP, 2008). The method validation parameters included system suitability, linearity, specificity, accuracy, limit of detection, limit of quantification, precision and ruggedness/robustness.

#### System suitability

The system suitability was judged by injecting six replicates of the drug at a concentration of 60 $\mu$ g/ml and was used to verify that the resolution and reproducibility of the chromatographic system sufficient for the analysis to be done. This was assessed by analyzing tailing factor,

**Table 1:** System suitability of HPLC method for determination of Cefadroxil in tablet/capsules

	Retention time (min)	Peak area (uv)	Peak height	USP tailing factor	Theoretical plates	Resolution
1	3.772	1159516	70844	1.261	1129.96	6.611
2	3.762	1158552	70737	1.31	1121.88	6.655
3	3.904	1168483	70286	1.229	1259.01	7.607
4	3.906	1163255	82175	1.401	1250.19	7.099
5	3.986	1162967	82001	1.416	1165.75	5.694
6	3.745	1148493	80987	1.411	1278.01	5.6
Mean	3.846	1160211	76171.67	1.338	1200.8	6.544
SD	0.099	6720.623	6095.39	0.082	69.66	0.783
% COV	2.57	0.58	8.002	6.16	5.8	11.9

retention time, peak height, peak area, theoretical plates and resolution.

#### **Selectivity**

The selectivity of the chromatographic method was determined as it is the significant basis for analytical procedures. Chromatographic method developed should ensure separation of active pharmaceutical ingredient (cefadroxil) in the presence of excipients used in tablets/capsules. Chromatograms were observed for no peak interference.

#### **Linearity**

A linear relationship must exist over the range of the analytical procedure. The range of an analytical method is the gap between the highest and lowest analytical concentrations of a sample while the method has shown satisfactory accuracy, precision and linearity. To evaluate the linearity, limit of detection and limit of quantification (LOQ) of the method, serial dilutions of reference drug i.e. cefadroxil were prepared from the standard stock solution (1000µg/ml) in the range of 0.5-90µg/ml (n=8) and resolved in a C<sub>18</sub> column with UV detector at 260 nm.

**Table 2:** Linearity of the proposed HPLC method

Concentration (µg/ml)	Peak area (n=3)
90	1536157
60	1162183.66
30	570804.33
15	330753.7
10	182349.67
5	97305.67
1	40039
0.5	18110.67
Pearson correlation (R <sup>2</sup> )	0.9935
Slope	17436.35
Intercept	31239.34

#### **Recovery**

Percentage recovery of active drug in pharmaceutical dosage form was evaluated for further validation by

preparing known amount of drug at three concentration levels (in triplicate) i.e. 70%, 100% and 130% levels of the target cefadroxil amount and injecting to HPLC.

#### **Precision/accuracy**

The precision, accuracy, interday and intraday reproducibility of the method was established. Cefadroxil standard solutions were prepared as 90, 60 and 15µg/ml. These concentrations were examined at three different times of the same day (intraday precision). Interday precision was verified by injecting three concentrations (60, 30 and 10µg/ml) on three consecutive days. % Precision and % accuracy were evaluated using back-calculated concentration.

#### **Robustness/ruggedness**

To assess the ruggedness of the method, the procedure was repeated in the Institute of Pharmaceutical and Environment Research, Dow University of Health Sciences, Karachi. Robustness studies performed on method precision, using a sample concentration (60µg/ml) by creating slight variations in flow rate, change in pH, detection wave length and proportion of methanol.

#### **Determination of cefadroxil in tablets/capsules**

The different formulations of 200mg cefadroxil (tablets/capsules) were assayed using the developed HPLC method. All the results were calculated as percentage of label claim of the manufacturer to detect whether these were in good agreement with the label claims.

## **RESULTS**

The present work was intended to develop simple, quick and precise method for the quantitative analysis of cefadroxil in marketed formulations (tablets/capsules). The developed method was validated keeping in consideration the FDA and ICH guidelines (FDA, 1996, ICH, 1997).

Percent Relative Standard Deviation, %RSD in this method for the retention time, peak area, peak height, tailing factor, theoretical plates and resolution were 2.57,

**Table 3:** Precision and accuracy of proposed method

	Concentration ( $\mu\text{g/ml}$ )	Mean $\pm$ SD	Precision	Accuracy
Precision Interday (n=3)	60	62.69 $\pm$ 1.69	2.69	4.48
	30	30.36 $\pm$ 0.76	2.5	1.2
	10	8.67 $\pm$ 0.05	0.58	13.3
Precision Intraday (n=3)	90	84.98 $\pm$ 1.86	2.18	5.57
	60	62.5 $\pm$ 1.09	1.75	4.16
	15	14.85 $\pm$ 0.79	5.33	0.97
Recovery	Concentration spiked at three level (%)	Mean Recovery %	SD	% COV
	70	106.56	0.49	0.46
	100	95.96	0.24	0.25
	130	103	0.3	0.29

**Table 4:** Method robustness/ruggedness

Changes in chromatographic conditions	Peak area	Peak height	Retention time	Tailing
Flow rate (ml/min)	1.4	4.44	0.24	0.32
	1.6	0.02	0.16	0.09
Proportion of methanol (%)	12	0.11	1.26	0.61
	8	0.01	0.03	0.20
pH of buffer	3.8	0.40	1.18	0.62
	4.2	0.42	0.45	0.19
Detection wavelength (nm)	255	0.03	0.15	0.28
	265	0.01	0.14	0.54

0.58, 8.002, 6.16, 5.8 and 11.9, respectively (table 1). The chromatograms of cefadroxil raw material were compared with that of cefadroxil tablets/capsules to prove no peak interference. Comparison proved the selectivity of the method (fig. 2). Linearity was observed in a range of 0.5 to 90 $\mu\text{g/ml}$  (fig. 3). Linear regression by least square method was performed for determination of coefficient of linearity that was 0.9935 (limit is >0.990). The linear equation was  $y=mx+b$  where m (slope) was 17436 and b (intercept) was 31239. LOD and LOQ of the proposed method were 0.5 and 1.0 $\mu\text{g/ml}$ , respectively (table 2). The accuracy was examined by analyzing drug content from cefadroxil capsules at three levels (i.e. 70%, 100% and 130%) (table 3). The percent Coefficient of Variance (%COV) was less than 0.5 at all three levels. Five brands of cefadroxil 200mg including tablets (n=2) and capsules (n=3) were assayed using the above method. The results revealed that %RSD was as low as 0.08% and as high as 0.39%.

The concentrations of 60, 30 and 10 $\mu\text{g/ml}$  were injected for inter-day and 90, 60 and 15 $\mu\text{g/ml}$  for intraday for repeatability and reproducibility. The percent RSD observed was in a range between 0.58-2.69% and 1.75-5.33% for inter-day and intra-day respectively (table 3). The method confirmed to be reasonably stable and robust as there was no significant changes in the % RSD as illustrated in table 4.

## DISCUSSION

The HPLC methods for the quantitative determination of drugs have become a regular contemplation to assess the

quality of different pharmaceutical products. The HPLC method employed previously for the quantification of cefadroxil were complex, time consuming and expensive. System suitability is the test employed to authenticate the resolution and reproducibility of the chromatographic system, which should be adequate for the analysis to be done. It was demonstrated that the method is appropriate for quantitative analysis of cefadroxil in oral solid dosage forms. The run time was less than 6 min with the retention time of approximately 4 mins (mean  $\pm$ SD=3.846 $\pm$ 0.099) showing that the method is quick enough to suite the requirement of QA/QC laboratory. The  $C_{18}$  column was used in the current method, similar column was utilized in some reports in the literature (Anjum, Shetty *et al.*, 2012, Dhoka and Chopade, 2012, Shukla, Pandey *et al.*, 2008). The method was proved to be linear and accurate (table 2 and 3). Repeatability, reproducibility and intermediate precision are considered to be the major component of precision as per the ICH guidelines (ICH, 1997). Therefore, the precision was determined at three time points on the same day and on three alternative days. Hence, it was concluded that the method is reproducible and repeatable. Robustness of the method was proved by premeditated variation made to the method parameters, such as pH of the buffer solution, flow rate, proportion of methanol and detection wavelength. Therefore, three replicates of prepared samples were injected under small variations of each parameter. Anjum, in 2011 documented a simple, precise and specific validated RP-HPLC method for the quantitative estimation of cefadroxil monohydrate in bulk and pharmaceutical formulations. Supelco  $C_{18}$  column was used, with the similar mobile phase

consisting of methanol and disodium hydrogen orthophosphate buffer in the ratio of 60: 40v/v (Anjum, Shetty *et al.*, 2012). The methanol content used in current study was much less than content of methanol in mobile phase used by Anjum, in 2011, which makes the present method more economical. The proposed method for assay of cefadroxil was, thus, found validated according to the ICH guidelines with respect to selectivity, linearity, accuracy, ruggedness and robustness.

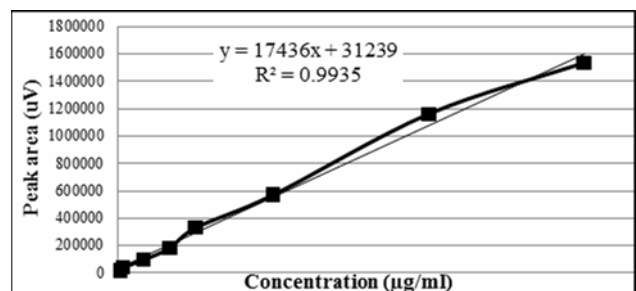


Fig. 3: Calibration curve showing linearity

## CONCLUSION

The proposed RP-HPLC method was sensitive, accurate, precise, simple, rapid and linear with acceptable LOD, LOQ and goodness of fit ( $R^2$ ) for the quantitative analysis of cefadroxil. Hence, the mentioned technique has the prospective application in Quality control laboratories for the routine analysis of cefadroxil in raw materials as well as for the content uniformity of cefadroxil tablets/capsules.

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