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# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE QUANTITATIVE ESTIMATION OF CEFADROXIL MONOHYDRATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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## ABSTRACT

A simple, precise, specific and accurate RP-HPLC method has been developed for the determination of cefadroxil monohydrate in bulk and pharmaceutical dosage forms. Chromatography was performed on a supelco RP C-18 column (250 mm × 4.6 mm) with 5 µm particle size. The mobile phase consists of two solvents methanol and 0.05M disodium hydrogen orthophosphate buffer (60 : 40 v/v) and with pH 3.0 adjusted with orthophosphoric acid. At a flow rate of 0.75 mL/min. Detection was performed at 264 nm. The retention time of cefadroxil monohydrate was found to be 4.108 min. By adoption of this procedure cefadroxil monohydrate is eluted completely. Linear calibration plots were obtained between 20-100 µg/mL. The method of analysis was used for quantification in pharmaceutical preparations with a coefficient of variation < 2%. Results of analysis were validated statistically and by recovery studies. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

**Key words**: Cefadroxil monohydrate, RP-HPLC, Precision, Accuracy.

## INTRODUCTION

Cefadroxil monohydrate is a first generation cephalosporin antibiotic indicated for the treatment of urinary tract infections, upper respiratory tract infections, and skin and soft tissue infections in patients[1]. Cefadroxil monohydrate is chemically (6R, 7R)-7-[(R)-2-amino-2-(p-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid monohydrate, and its structural formula is shown in Fig. I. The

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molecular formula is $C_{16}H_{17}N_3O_5S.H_2O$ and molecular weight is 381.40 g/mol. It is soluble in methanol. It is official drug in United States Pharmacopoeia[2] 2004 and British Pharmacopoeia[3] 2005. Literature survey reveals that, HPLC[4-7], chemiluminescence[8] determination were found and few spectrophotometric methods for the quantitative estimation of cefadroxil monohydrate in bulk and pharmaceutical formulations[9-13] have been developed. The proposed method describes a sensitive, simple, precise and accurate RP-HPLC method for the estimation of cefadroxil monohydrate in bulk and dosage pharmaceutical formulations form with subsequent validation as per ICH guidelines.

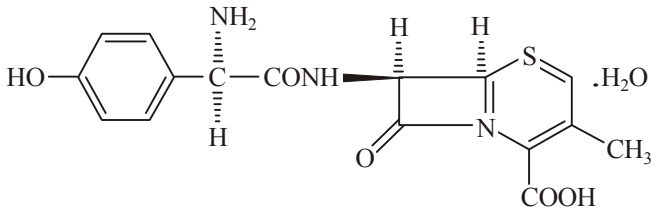


**Fig. 1: Chemical structure of cefadroxil monohydrate**

## EXPERIMENTAL

### Materials and methods

A gradient HPLC (Shimadzu, class VP-Series) equipped with Supelco Reverse Phase C-18 Column (25 cm x 4.6 mm i.d., particle size 5 µm) was used, LC-10 AT VP pump, UV/VIS detector SPD-10A VP with N-2000 CHROMTECK (Shimadzu) software, ortho-phosphoric acid AR grade, double distilled water and methanol HPLC grade. The optimized chromatographic conditions are summarized in Table 1.

**Table 1: Optimized chromatographic conditions for the proposed method**

| Parameters | Optimized condition |
|---|---|
| Linearity range (µg/mL) | 20-100 |
| Detection wavelength (nm) | 264 |
| Temperature | $25^0$C |
| Retention time (t) (min) | 4.108 |
| Run time (min) | 10.0 |
| Limit of detection (ng/mL) | 1.77 |
| Limit of quantification (ng/mL) | 5.376 |

**Preparation of mobile phase**

600 mL of HPLC grade methanol was mixed with 400 mL of 0.05M of disodium hydrogen orthophosphate buffer, which was prepared in double distilled water and its pH was adjusted to 5.5 using ortho-phosphoric acid. Then it was ultrasonicated for 20 minutes and then filtered through 0.4 µm membrane filter paper**.**

**Preparation of standard stock solution of cefadroxil monohydrate**

25 mg of standard cefadroxil monohydrate was weighed accurately; transferred to 25 mL volumetric flask and dissolved in 10 mL of mobile phase and then volume was made up to the mark with mobile phase to get 1000 µg/mL of standard stock solution 'A' of the drug. These stock solutions were filtered through 0.4 µm membrane filter paper.

**Preparation of marketed formulations**

Cefadroxil monohydrate equivalent to 100 mg was weighed; transferred to 100 mL volumetric flask and dissolved in sufficient quantity of mobile phase. The contents were ultrasonicated for 20 minutes and the final volume was made up to the mark with mobile phase to get 1000 µg/mL of standard stock solution 'B' of the drug. Then the above prepared solution was filtered through 0.4 µm membrane filter paper.

**Chromatographic condition**

The mobile phase containing methanol and disodium hydrogen orthophosphate buffer in the ratio of (60 : 40) was selected as the optimum composition of mobile phase, because it was found that this solvent system eluted the drug with good resolution. The flow rate was set to 0.75 mL/min and UV detection was carried out at 264 nm. The mobile phase and samples were degassed by ultrasonication for 20 min and filtered through 0.4 µm membrane filter paper. All determinations were performed at constant column temperature ($25^{o}$C).

**Analysis and preparation of calibration curve for cefadroxil monohydrate**

Appropriate aliquots were pipetted out from the standard stock solution 'A' (1000 µg/mL) in to a series of 10 mL volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 20-100 µg/mL of cefadroxil monohydrate. 20 µL of each solution were injected into the HPLC system and their chromatograms were recorded under the same chromatographic conditions as described above. The cefadroxil monohydrate was eluted at 4.108 min as shown in Fig. 2. The calibration curve was constructed by plotting average peak area versus concentration

and is presented in Fig. 3. The method was extended for determination of cefadroxil monohydrate in pharmaceutical dosage form. The linearity range was found to be 20-100 µg/mL.

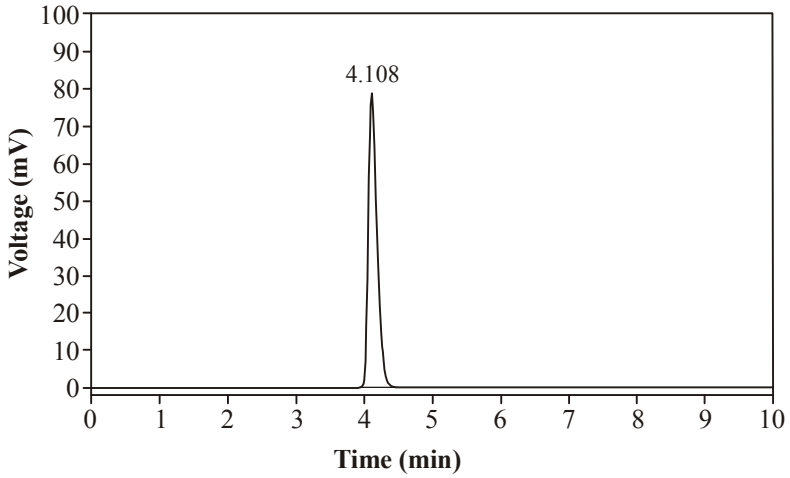


**Fig. 2: Chromatogram of cefadroxil monohydrate by RP-HPLC method**

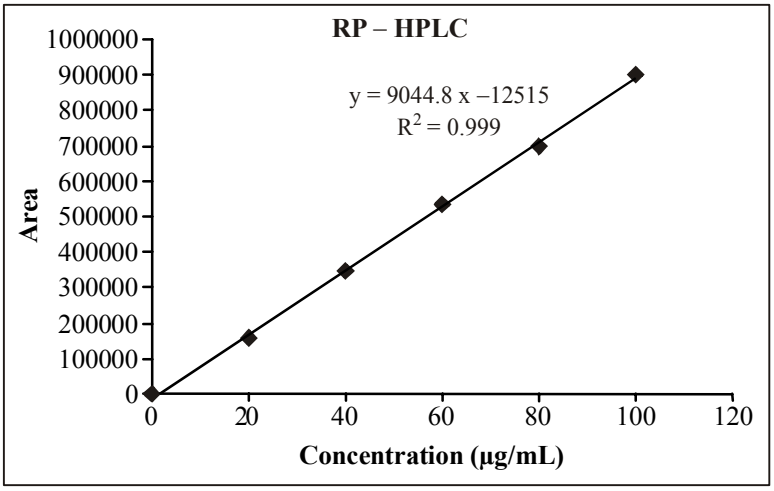


**Fig. 3: Calibration curve of cefadroxil monohydrate at 264 nm by RP−HPLC method**

## Analysis of cefadroxil monohydrate in formulations

From this stock solution 'B', various dilutions of the sample solution were prepared and analyzed. A 20 µL volume of each sample solution was injected in to the sample

injector of HPLC system and their chromatograms were recorded under the same chromatographic conditions as described above. The area of each peak was determined at 264 nm and the amount of drug present in the sample was determined. The proposed methods were validated as per the ICH guidelines.

## Method validation[14-17]

### Accuracy

The procedure for the preparation of solutions for accuracy determination at 80%, 100% and 120% levels were prepared in the same manner as explained earlier. The solutions were filtered through 0.4 µm membrane filter paper and then these were subjected to analysis by RP-HPLC method under the same chromatographic conditions as described earlier. At each level, six determinations were performed. The results obtained were compared with expected results and were statistically validated.

### Precision

Intraday and inter-day precision were carried out for the various concentrations of the sample at different time intervals in the same day and at same time on different days. The concentration of the sample solution was determined as per the procedure given for the tablet formulation by determining peak area at selected analytical wavelength 264 nm. The variation of the results within the same day was analyzed and statistically validated.

### Linearity and range

Appropriate aliquots were pipetted out from the standard stock solution 'A' in to a series of 10 mL volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 20-100 µg/mL of the drug.

The solutions were injected using a 20 µL fixed loop in to the chromatographic system at the flow rate of 0.75 mL/min and the effluents were monitored at 264 nm, chromatograms were recorded.

### Robustness

The evaluation of robustness showed the reliability of analysis with respect to deliberate variations in method parameters. The various concentrations were prepared and injected into sample injector of HPLC six times under different parameters like deliberate variations in flow rate, detection (nm).

## RESULTS AND DISCUSSION

In this method the conditions were optimized to obtain elution of cefadroxil monohydrate. Mobile phase and flow rate selection was based on peak parameters (height, tailing factor, theoretical plates, capacity or asymmetry), run time and resolution. The system with disodium hydrogen orthophosphate buffer : methanol (40 : 60 v/v) with pH 3 with system suitability parameters are shown in Table 2.

**Table 2: System suitability test parameters for the proposed method**

| Parameters | Optimized condition |
|---|---|
| Retention time (t) (min) | 4.108 |
| Theoretical plates (N) | 5255.466 |
| Peak asymmetry | 1.467 |

The run time was set at 10 min and $R_t$ for cefadroxil monohydrate was found 4.108 ± 0.0155 min with standard deviation less than 1% with a good linear relationship ($r^2 = 0.9999$) was observed between the concentration of cefadroxil monohydrate and the respective peak areas in the range 20-100 µg/mL. The regression of cefadroxil monohydrate was found to be Y = 9044.8 x –12515, where 'Y' is the peak area and 'X' is the concentration of cefadroxil monohydrate (Table 3).

**Table 3: Regression analysis of the calibration curve for the proposed method**

| Parameters | Optimized condition |
|---|---|
| Linearity range (µg/mL) | 20-100 |
| Regression equation (Y = mx +c) | |
| Slope (m) | 9044.8 |
| Intercept (c) | 12515 |
| Correlation coefficient ($r^2$) | 0.999 |
| Relative standard deviation (%) | 1.2489 |
| Retention time (min) | 4.108 |

The proposed RP-HPLC method was validated for intra and inter-day precision with % RSD, 0.4588 and 0.5520, respectively. A known amount of the pure drug solution (80, 100 and 120 %) was added to the powder sample of the formulation and subjected for the

recovery studies. High recovery was obtained indicating that the proposed method is highly accurate. Robustness was determined by changing the parameters like flow rate and detection using similar operational and environmental conditions (Table 4).

**Table 4: Summary of validation parameters for the proposed method**

| Parameters | Values |
|---|---|
| Limit of detection (ng/mL) | 1.77 |
| Limit of quantitation (ng/mL) | 5.376 |
| ***Accuracy (% RSD)** | |
| 80% | 0.9021 |
| 100% | 0.4232 |
| 120 % | 0.3460 |
| ***Precision (% RSD)** | |
| Intra day | 0.45888 |
| Inter day | 0.55200 |
| ***Robustness (% RSD)** | |
| Change in flow rate | |
| 0.7 mL/min | 1.400 |
| 0.8 mL/min | 1.408 |
| **Change in detection wavelength** | |
| 262 nm | 1.406 |
| 266 nm | 1.413 |
| *Mean of six determinations, RSD indicates relative standard deviation | |

The proposed method was validated in accordance with ICH parameters and applied for analysis of the same in marketed formulations.

## CONCLUSION

Thus, it can be concluded that the method developed in the present investigation is simple, sensitive, accurate, robust, rapid and precise. Hence, the above said method can be successfully applied for the estimation of cefadroxil monohydrate in pharmaceutical dosage forms.

## ACKNOWLEDGEMENT

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