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# A Validated Reversed Phase HPLC Method Development for the Assay of Ciprofloxacin in Oral Suspension

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**Keywords:**
Ciprofloxacin, RP-HPLC

**ABSTRACT**

A simple Reverse phase liquid chromatographic method has been developed and subsequently validated for the determination of Ciprofloxacin in oral suspension. The separation was carried out using a mobile phase consisting of buffer of pH 2.0 and Acetonitrile in the ratio of 87: 13. The column used was Inertsil ODS-3 4.6×250mm, 5µ. with a flow rate of 1.5 ml / min by detection at 278 nm. The described method was linear over a concentration range of 25-150%. The retention time of Ciprofloxacinwas found to be 9.4min. Results of analysis were validated statistically and by recovery studies. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Ciprofloxacinin its pharmaceutical dosage forms.

## 1. INTRODUCTION

Ciprofloxacin is a broad-spectrum antimicrobial carboxyfluoroquinoline. The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercooling repair, and recombination. [1]

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Ciprofloxacin is a broad-spectrum anti-infective agent of the fluoroquinolone class. Ciprofloxacin has in vitro activity against a wide range of gram-negative and gram-positive microorganisms. The mechanism of action of quinolones, including ciprofloxacin, is different from that of other antimicrobial agents such as beta-lactams, macrolides, tetracyclines, or aminoglycosides; therefore, organisms resistant to these drugs may be susceptible to ciprofloxacin. There is no known cross-resistance between ciprofloxacin and other classes of antimicrobials. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian. The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercooling repair, and recombination.

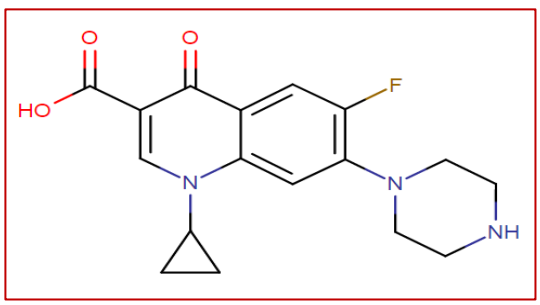


**Figure. 1. Molecular structure of Ciprofloxacin**

## 2. Materials and Methods:

### 2.1 Chemicals and Reagents:

Standard bulk drug sample Ciprofloxacin was provided by Chandra labs, Hyderabad. All the chemicals used were of analytical and HPLC grade procured from Qualigens, India Ltd. The chemicals used for this study were Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (HPLC grade), Ortho phosphoric acid (Analytical grade). Waters HPLC 2695with UV detector was used for the analysis.

### 2.2. Preparation of Mobile Phase:

**Mobile phase-A:** (Buffer) Pipette out 10ml of Methanol in to 1000ml of and mix. Adjust to pH 2.0 with Orthophosphoric acid, then filter through 0.45µ filter paper and sonicate for 2minutes.

**Mobile phase-B:** Acetonitrile

**Preparation of Mobile Phase**:

Mix the mobile phase-A and mobile phase-B in the ratio in the ratio 87:13%v/v.

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**2.3. Preparation of Stock and Standard Solutions:**

**Preparation of Standard solution:**

Weigh and transfer 25mg ciprofloxacin working standard into a 50mL volumetric flask. Add 7ml of acetonitrile andsonicate for 2minutes then add 30ml of pH2.00buffer, sonicate for 10minutes then make up to the mark with diluent. Further pipette out 5ml of the above solution in to 20ml volumetric flask, add 2.8ml of acetonitrile mix well, then add 12ml of pH2.00 buffer, then make up to the mark with diluent.

**Preparation of Test Solution:**

Shake the bottle 10minutes immediately prior to sampling in order to accomplish homogeneity of suspension. Weigh and transfer 5.5g ciprofloxacin suspension into a 500mL volumetric flask. Add 70ml of acetonitrile sonicate for 10 minutes, then add 250ml of pH 2.00 buffer, sonicate for 20minutes then make up to the mark with diluent. Further pipette out 5ml of the above solution in to 20ml volumetric flask, add 2.8ml of acetonitrile mix well, then add 12ml of pH2.00 buffer, then make up to the mark with diluent.

**2.4. Optimized Chromatographic Conditions:**

Column: Inertsil ODS-3 4.6×250mm, 5µ.

Flow rate : 1.5 mL /min.

Wavelength : 278 nm

Column temperature : 40°C

Injection Volume : 10 µL

Run Time : 15 minutes

Retention time: Ciprofloxacin, RT about 9.4min

**3. Method Validation Parameters:**

**Linearity:**

A series of Ciprofloxacin solutions were prepared in the concentration ranging from 25% to 150% of specification level and injected into the HPLC system as per the test method. The square of the correlation coefficient, intercept and residual sum of squares were calculated.

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**Accuracy:**

A series of solutionswereprepared in triplicatetest preparation at the specification limit in the range of about 25%to 150% of test concentration and injected into HPLC system and analyzed as per the test method. Individual % recovery, mean % recovery, %RSD and linearity of the testmethodwere calculated at each level.

**Intermediate Precision:**

To evaluate the intermediate precision for assay method, six samples were prepared and analyzed as per test method by using different column, by different analysts on different days. Intermediate precision was calculated and found to be within the acceptable limits. The overall % RSD of six samples in method precision, intermediate precision (n=6 and n=12) were calculated.

**Filter Validation:**

A study was conducted to evaluate the filter suitability by using two different types of filters namely0. 45 µm PVDF and 0.45µm Nylon filters. Standard solution was prepared in single and test solution was prepared in duplicate as per the test method. Portion of standard and test solutions were filtered through 0.45 µm PVDF, 0.45 µm nylon filter and some portion of standard and sample solutions were centrifuged and analyzed as per test method.

**Robustness:**

**Flow Rate Variation:**

A study was conducted to determine the effect of variation in flow rate. Blank, Standard and sample (at the specification level) were prepared as per the test method and injected into the HPLC system with flow rates of 1.4ml/minute and 1.6ml/minute. The system suitability parameters sample was evaluated and found to be within the specified limits as per test method.

**Column Oven Temperature Variation:**

A study was conducted to determine the effect of variation in Column oven Temperature. Standard and test preparations (at the specification level) were prepared as per the test method and injected into the HPLC system with a column oven temperature of 35°C and 45°C. System suitability parameters and sample were evaluated and found to be within the specified limits as per test method.

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**Effect of Variation In Mobile Phase Composition:**

A study was conducted to determine the effect of variation in mobile phase composition. Two different mobile phases of Buffer and Acetonitrile were prepared in the ratio of 855:145%v/v and 885:115%v/v as per the test method. Standard and test preparations with specification level were prepared as per the test method and injected into the HPLC system.

**Effect of pH Variation in Mobile Phase:**

A study was conducted to determine the effect of variation in pH in the mobile phase. Two mobile phases of pH 2.80 and 3.20 were prepared as per the test method. Blank, Standard and test preparations were prepared as per the test method and injected into the HPLC system with System suitability parameters and sample were evaluated and found to be within the specified limits as per test method.

**The Effect of Wavelength Variation:**

A study was conducted to determine the effect of variation in wavelength. Standard and test preparations (at the specification level) were prepared as per the test method and injected into the HPLC system with wavelength of -Ciprofloxacin280nm and 276nm.System suitability parameters and sample were evaluated and found to be within the specified limits as per test method.

## 4. Results and Discussion:

The solution of Ciprofloxacin was scanned in the range of 200-400nm and 278nm was selected as detection wavelength by RP-HPLC method with an isocratic elution technique. The optimization was done by changing the composition of the mobile phase, ratio and flow rate. Finally the mobile phase with buffer (pH 2): ACN in the ratio 87:13v/v% was optimized for the estimation of Ciprofloxacin and the column used for separation is Inertsil ODS-3 4.6×250mm,5µ. [2]

The chromatographicparameters of system suitability such as %RSD, standard recovery, Tailing factor, Theoretical plates were found to be satisfactory. The values of these parameters are tabulated in Table-1.

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**Table.1. System suitability data for Ciprofloxacin**

| System suitability parameters for Ciprofloxacin | Method Precision | Intermediate precision | Acceptance Criteria |
|---|---|---|---|
| %RSD | 0.3 | 0.3 | Not more than 2.0 |
| Standard recovery (%) | 101.4 | 99.5 | Between 98.0 to 102.0 |
| Tailing factor | 1.1 | 1.1 | Not more than 2.0 |
| Theoretical plates | 9925 | 9271 | Not less than 2000 |

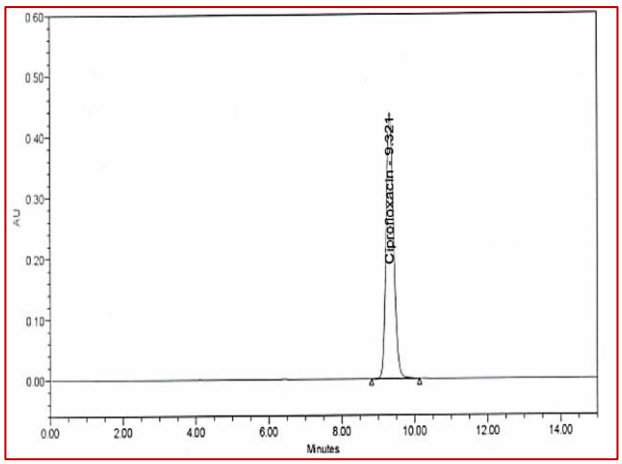


**Figure.2.Typical chromatogram of Standard solution**

The linearity of the developed method was determined by analyzing different concentrations of the standard solution containing a concentration range from 25% to 150%. The response factor of the standard solutions was calculated. The ratio of peak areas of ciprofloxacin was plotted against the concentration to obtain the calibration graph (Fig. 3) and was found to be linear over the concentration range from 25% to 150%. The data were analyzed by linear regression, least-squares method and the corresponding equation are given by Y = BX + c, where 'Y' is the ratio of the peak areas values of Ciprofloxacin, 'b' is the slope, 'c' is the intercept and 'X' is the concentration of the analyte. Linear regression, least squares fit data are given in (Table 2). [3] The percentage purity was found to be 99.3%. The precision of the method was confirmed by the repeatability of formulation for six times. The accuracy of the method was confirmed by recovery studies and the data was given by (Table 3). [4] Similarity factors were calculated for the filtered standards against unfiltered standard (Centrifuged) and found to be within the specified limit. The difference in the % between unfiltered (centrifuged) and filtered samples were calculated and found to be

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meeting the acceptable limit. Both PVDF and Nylon filters were suitable for the intended purpose.

**Table.2.Linearity of detector response for Ciprofloxacin**

| % Linearity level | Concentration (ppm) | Response | Acceptance criteria |
|---|---|---|---|
| 25 | 31.0875 | 1536480 | Square of Correlation co-efficient should not be less than 0.999 |
| 50 | 62.175 | 3107355 | |
| 75 | 93.2625 | 4623963 | |
| 100 | 124.35 | 6039873 | |
| 150 | 186.525 | 9110398 | |
| Square of correlation coefficient : 0.999<br>Slope: 48483.76574<br>Intercept : 60448.78378<br>Residual sum of squares: 45710.56736 | | | |

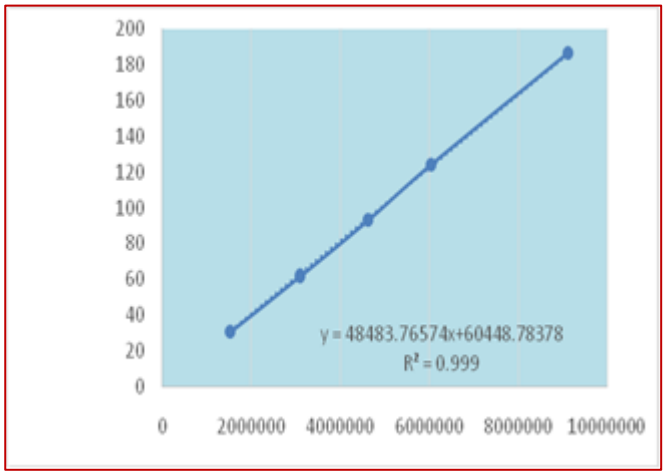


**Figure.3.Linearity of detector response graph for Ciprofloxacin.**

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**Table.3.Accuracy data of Ciprofloxacin**

| S. No. | % spike level | Amount added (%w/w) | Amount recovered (%w/w) | % Recovery | % Mean recovery | % RSD |
|---|---|---|---|---|---|---|
| 1. | 25% | 62.1229 | 62.37308 | 100.4 | 99.8 | 0.6 |
| 2. | | 63.0287 | 62.40228 | 99.0 | | |
| 3. | | 63.0885 | 62.62924 | 99.3 | | |
| 4. | | 63.1979 | 62.72669 | 99.3 | | |
| 5. | | 62.2424 | 62.36469 | 100.2 | | |
| 6. | | 62.3220 | 62.55297 | 100.4 | | |
| 1. | 100% | 250.8408 | 247.83270 | 98.8 | 99.3 | 0.4 |
| 2. | | 248.9894 | 247.87577 | 99.6 | | |
| 3. | | 249.1088 | 247.78317 | 99.5 | | |
| 1. | 150% | 378.5307 | 377.14133 | 99.6 | 100.6 | 0.5 |
| 2. | | 374.4894 | 377.08205 | 100.7 | | |
| 3. | | 374.7781 | 377.28255 | 100.7 | | |
| 4. | | 373.8921 | 377.45520 | 101.0 | | |
| 5. | | 373.2252 | 376.76809 | 101.0 | | |
| 6. | | 374.1609 | 376.10577 | 100.5 | | |

**Specificity:**

Chromatogram of blank and placebo should not show any peak at the retention time of Ciprofloxacin peak and known impurity peaks.

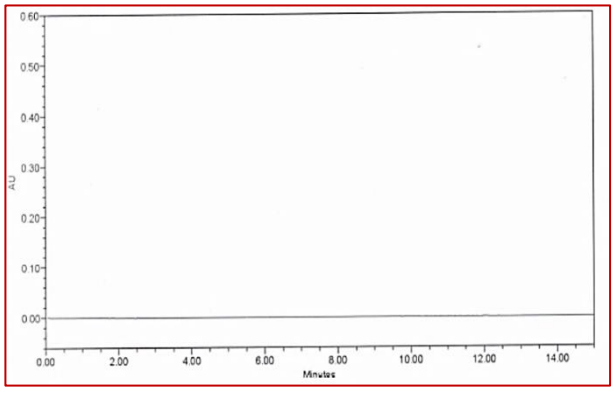


**Figure.4.Typical chromatogram of Blank**

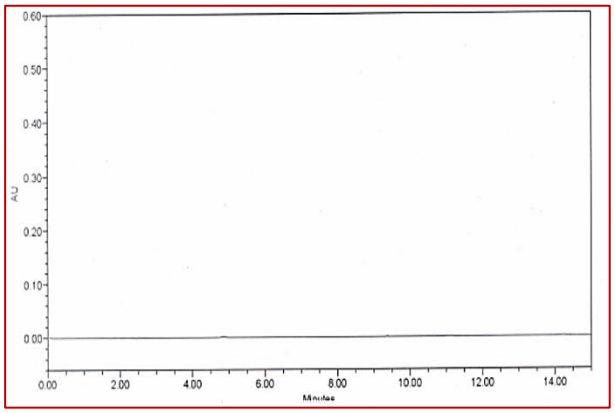


**Figure.5.Typical chromatogram of placebo**

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**Table.4.Method Precision data for Ciprofloxacin**

| Sample No. | Ciprofloxacin content (%) |
|---|---|
| 1 | 100.9 |
| 2 | 100.2 |
| 3 | 100.0 |
| 4 | 99.4 |
| 5 | 101.1 |
| 6 | 100.5 |
| **Mean** | **100.4** |
| **% RSD** | **0.6** |

## 5. Conclusion:

This study showed that the antibiotic drug, Ciprofloxacin can be precisely and accurately determined in pure and pharmaceutical dosages. The proposed method is simple and requires less time for analysis. System performance parameters revealed that the method is ideal for the assay ofCiprofloxacin.

Hence, the developed chromatography method was applied for routine analysis and can be used for the intended purpose.

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