Review Article

# Development and validation of simple UV spectrophotometric method for analyzing ciprofloxacin hydrochloride

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**Abstract**

A simple, precise, accurate and rapid UV Spectroscopic method has been developed for the assay of Ciprofloxacin hydrochloride (HCL) in its pharmaceutical tablet dosage form. The UV Spectroscopic method was validated according to the International Conference on Harmonization (ICH) guideline, where range, linearity, accuracy, recovery, precision and sensitivity of the method were examined. Ciprofloxacin HCL showed maximum wavelength of absorbance at 272 nm in water and obeyed linearity or Beer-Lambert's law within the concentration range of 2-12 µg/mL. The regression of coefficient ($R^2$) was found to be greater than 0.99. The limit of detection (LOD)and limit of quantitation (LOQ) values were found to be 0.1µg/mL and 21µg/mL, respectively. The developed method was applied successfully for the analysis of ciprofloxacin HCl with good accuracy and precision.

**Keywords:** International Conference on Harmonization, Ciprofloxacin, Validation, LOD, LOQ

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## Introduction

The introduction of generic drug product from multiple sources into the health care delivery system of many developing countries was aimed at improving the overall healthcare delivery systems in such countries. The need to select one product from among several generic drug products of the same active ingredients during the course of therapy is a cause of concern to a healthcare practitioner. The first stage in ascertaining the therapeutic equivalence of any drug product involves ascertaining the chemical and biopharmaceutical equivalency of such drug products [1]

Drug products that are chemically and bio-pharmaceutically equivalent must be identical in strength, quality, purity as well as content uniformity, disintegration and dissolution rates. Variable clinical response to the same dosage form of a drug product supplied by different manufacturers has been reported (Remington's Pharmaceutical Sciences, 1990). Therapeutic in-equivalences have been reported from the use of some generic brands of drug products such as tolbutamide [2].

There are several brands of Ciprofloxacin hydrochloride tablets available within the drug delivery system globally as well as in India. The increasing level of use of Ciprofloxacin hydrochloride tablets as a result of its versatility in the management of various cases of microbiological infections necessitated the need to evaluate the quality of the Ciprofloxacin hydrochloride tablets available in India. Ascertaining the quality of drug products involves the use of various procedures which includes both biopharmaceutical and chemical assay techniques. Various methods have been reported for the chemical assay of Ciprofloxacin tablets [3].

In our study we used UV spectroscopy for assaying Ciprofloxacin HCl. This study is useful because this drug is commonly administered for various kinds of infections. The UV spectrophotometric analysis is often preferred in quality control testing and ordinary laboratories due to its broader availability, suitability, cost effectiveness and ease of use [4]. The aim of the present investigation is to develop a simple, sensitive and reproducible UV Spectrophotometric method for analysis of Ciprofloxacin HCl in a tablet dosage form and hence an economical method was developed and validated according to the ICH guidelines. Ciprofloxacin (sip" roe flox' a sin) is an oral fluoroquinolone that is used to treat mild to-moderate urinary and respiratory tract infections. Ciprofloxacin is also used for infectious diarrhea, typhoid fever, uncomplicated gonorrhea, treatment of Ncisscria meningitides nasal carriage and prophylaxis against anthrax (RH, 2013). Like other fluoroquinolones, Ciprofloxacin is active against a wide range of aerobic gram-positive and gram-negative organisms. The fluoroquinolones are believed to act by inhibition of type II DNA toposiomerases (gyrases) that are required for synthesis of bacterial mRNAs (transcription) and DNA replication. Ciprofloxacin was approved for use in the United States in 1990 and. currently, approximately 20 million prescriptions are filled yearly [5]. Ciprofloxacin is available in multiple oral formulations, Intravenous formulation. Common side effects include gastrointestinal upset, headaches, skin rash and allergic reactions. Less common, but more severe side effects include prolongation of the QT interval, seizures, hallucinations, tendon rupture, angioedema, Stevens Johnson syndrome and photosensitivity [6].

## Materials and Method

### 2.1 Materials

#### 2.1.1 Sample Collection

For the purpose of analyzing Ciprofloxacin HCL, reference standard was collected from the Eskaycf India Limited and tablets of Ciprofloxacin HCL of two different strengths (i.e.,250 mg ciprocin® and 500 mg ciprocin® tablet of Square Pharmaceutical

Limited) from a reputed pharmaceutical company were purchased from retail pharmacy situated in, India (Table 1).

**Table 1: Materials used for the experiment**

| Sample name | Source(Supplier Name) |
|---|---|
| Ciprofloxacin | Eskayef India Limited |
| Ciprocin® 250mg | Square Pharmaceutical |
| Ciprocin® 500mg | Square Pharmaceutical |

**2.1.2 Sample**

For conducting the UV method development study, the following solvents were used (Table 2).

**Table 2: Solvents used for developing UV method for Ciprofloxacin HCL**

| Reagents Name | Source (Supplier |
|---|---|
| Methanol (Laboratory | Active Fine |
| Distilled Water/De-ionized water | Active Fine Chemicals |

**2.1.3 Equipment's& Instruments**

The equipment's and instruments used for the study were included in Table 3.

**Table 3: Lists of equipment's used for the experiment**

| Equ | Source (supplier | Origin |
|---|---|---|
| UV | Shimadzu UV1800 | Japan |
| Electronic Balance | Shimadzu ATX 224 | Japan |
| Sonicator | HWASHIN Power sonic 520 | Korea |

**2.2 Methods**

**2.11 Wavelength Selection**

Ciprofloxacin 100 µg/ml stock solution was accurately prepared by dissolving 5 mg of Ciprofloxacin reference standard in 50 ml distilled water. A solution of 100 µg/ml concentration was prepared by diluting the stock solution and scanned in the UV regions (200-400 nm). The maximum wavelength of absorbance ($_{max}$) was observed at 272 nm with the absorbance value of 0.658.Therefore, 272 nm was selected as the $_{Max}$ for Ciprofloxacin HCL .However, in different papers the $_{max}$ of Ciprofloxacin HCL were found to be slightly different .[4]

**2.2.2 Preparation of standard solution of Ciprofloxacin in water:** Ciprofloxacin is freely soluble in water (Solubility is 10-30 mg/ml) at acidic pH (<5.0)[7]. Therefore, standard solution of Ciprofloxacin was prepared in water. A stock solution of 100 µg/mL was prepared by accurately weighing 5 mg of Ciprofloxacin powder in to a 50 mL volumetric flask. The drug was then dissolved in distilled water. Required amount of water was added to make it volume. The stock solution was diluted further with distilled water to prepare concentrations of 2, 3, 4,6,8,10,12 µg/ml [no. of replicates (n) were 3 for all cases].

**2.2.3 Construction of calibration curve**

Using UV Spectrophotometer, the absorbance values of these diluted solutions were noted at 272nm wavelength. All the samples were prepared and measured in three replicates. Data then plotted using MS Excel 2007 software. A calibration graph was prepared by plotting the concentration versus the average absorbance values.

**2.2.4 Method validation of Ciprofloxacin Hydrochloride**

**Linearity**

"The linearity of an analytical procedure is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Thus, in this section, "linearity" refers to the linearity of the relationship of concentration and assay measurement. In some cases, to attain linearity, the concentration and/or the measurement may be transformed". The linearity was evaluated by analyzing three different calibration curves of Ciprofloxacin HC1. The regression coefficient and slope were specifically evaluated by using the RSD value.

**2.2.4.2 Range**

"The range of an analytical procedure is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated to be determined with asuitable level of precision, accuracy, and linearity using the procedure as written. Therange is normally expressed in the same units as test results (e.g., percent. parts per million) obtained by the analytical procedure" [8].

The concentration range between which Ciprofloxacin HCI obeyed Beer-Lambert's law was taken as the range.

**2.2.43 Accuracy testing**

Accuracy testing was performed according to USP. Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between the mean and the accepted true value, together with confidence intervals.

For accuracy testing, 10mg of Ciprofloxacin HCL standard was weighed in an electronic balance and then transferred it to a 100 ml volumetric flask and it was then dissolved properly with distilled water. The solution finally was made up to the mark with distilled water. 10 ml of solution was then transferred to another 100ml volumetric flask to dilute the solution 10 times with distilled water. Four replicates were prepared by the same way and the absorbance values were taken at 272 nm.

**2.2.4.4 Recovery Testing**

While making the calibration curve, three replicates of each concentration was prepared and the average absorbance values were calculated. Calibration curve was prepared by constructing the concentration vs. average absorbance values. Again from the average absorbance values, the observed concentration was calculated using the equation of calibration curve. The percentage of recovery was then calculated.

**2.2.4.5 Precision**

The precision of an analytical procedure is the degree of agreement between individual test results when the procedure is applied repeatedly to multiple samplings or a homogeneous sample. The precision of an analytical procedure is usually expressedas the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or of repeatability of the analytical procedure under normal operating conditions. In thiscontext, reproducibility refers to the use of the analytical procedure in different laboratories, as in a collaborative study. Intermediate precision (also known as ruggedness) expresses within-laboratory variation, as on different days, or with different analysts or equipment within the same laboratory. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with same equipment. Three different concentrations within the range highest, middle and the lowest were prepared three times. The solutions were analyzed by its absorbance values keeping them at room temperature two times in a day (intraday precision) and at 1 and 4 days intervals (inter day precision). The values
were compared with the initial absorbance values in terms of RSD .[8]

**2.2.4.6 Sensitivity**
**Limit of detection (LOD)**
The detection limit is a characteristic of limit tests. It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Thus, limit tests merely substantiate that the amount of analyte is above or below a certain level. The detection limit is usually expressed as the concentration of analyte (e.g., percentage, parts per billion) in the sample". In order to find out LOD, concentrations of Ciprofloxacin HCL in distilled water from 0.1 to 2 µg/mL was prepared and absorbance values were recorded. The concentration which showed the minimum absorbance value was selected as the LOD of this UV method.

**2.2.4.7 Assay of Ciprofloxacin HCL**
Three tablets of two strengths (250 and 500 mg) of Ciprolioxacin HCL marketed brand product were purchased and average weights were noted. The three tablets were grinded using mortar and pestle and a definite amount of the tablet powder was transferred in a 100 mL, volumetric flask. Distilled water and methanol at 90:10 ratio was added to dissolve the powder mix. The mixture was then sonicated for 15 mins and then made upto volume. The solution was finally filtered using whatman filter paper. The clear solution was again diluted using the same water methanol (90:10) mixture and absorbance values were recorded.

**2.2.5 Statistical analysis**
All the data were analyzed using MS Excel 2007.Most of the cases the results were represented as mean±standard deviation (SD).

**Limit of Quantitation (LOQ)**
The quantitation limit is a characteristic of quantitative assays for low levels of compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals. It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The quantitation limit is expressed as the concentration of analyte (e.g., percentage parts per billion) in the sample". In order to find out LOQ, concentrations of Ciprofloxacin HCl distilled water from 0.1to 2 µg/mL was prepared and absorbance values were recorded. The concentration which showed the minimum absorbance value with acceptable recovery and precision values was selected as the LOQ of this UV method.

## RESULTS AND DISCUSSION

**3.1 Construction of calibration curve**
To construct the calibration curve seven standard solutions were prepared (2, 3, 4. 6, 8. 10, 12 µg/ml concentration). The absorbance values of these solutions were noted at 272nm wavelength ($_{max}$ for Ciprofloxacin HCL). Data was collected and plotted (Table 4) in a chart to obtain the standard curve (Fig no.3.2). From this curve a regression coefficient value ($R^2$) of 0.9997 was found.

**Table 4:Absorbance data of standard solutions or Ciprofloxacin at 272 nm.**

| Conc. | Standard 1 | Standard 2 | Standard 3 | Average |
|---|---|---|---|---|
| 2 | 0.171 | 0.167 | 0.178 | 0.172 |
| 3 | 0.255 | 0.253 | 0.258 | 0.255 |
| 4 | 0.336 | 0.334 | 0.339 | 0.336 |
| 6 | 0.501 | 0.508 | 0.5 | 0.503 |
| 8 | 0.663 | 0.658 | 0.664 | 0.662 |
| 10 | 0.839 | 0.849 | 0.844 | 0.844 |
| 12 | 1.013 | 0.958 | 1.005 | 0.992 |

By plotting the absorbance against the concentration of Ciprofloxacin a straight line was found. The equation obtained from the calibration curve was Y=0.0826x + 0.007.

**3.2 Method validation parameter**
**3.2.1 Linearity**
The linearity was evaluated by analyzing three different calibration curves of Ciprofloxacin HCL. The regression coefficient and slope were specifically evaluated by using the RSD value.

**Table 5: $R^2$, slope and intercept values of Ciprofloxacin HCL in three standard solutions**

| Conc. (µg/ml) | Standard 1 | Standard 2 | Standard 3 | Average | SD | RSD |
|---|---|---|---|---|---|---|
| $R^2$ | 0.99988 | 0.99857 | 0.99993 | 0.99946 | 0.0008 | 0.08 |
| | 8 | 4 | 2 | 5 | | |
| Slope | 0.08385 | 0.08084 | 0.08298 | 0.08255 | 0.0015 | 1.88 |
| (m) | 5 | 3 | | 9 | | |
| Intercept | 0.00064 | 0.01272 | 0.00770 | 0.00702 | 0.0061 | 0.07 |
| | 7 | 4 | 3 | 4 | | |

**3.2.2 Range**

The method obeyed the Beer-Lambert's law from the concentration range of 2-12 µg/mL.

**3.2.3 Accuracy testing result**

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. Accuracy testing data is shown in Table 6.The table shown that 103.36±7.93% Ciprofloxacin was obtained from the assay testing. 100±5% is an acceptable range. Therefore, the value was slightly higher than the recommended range.

**Table no. 6: Accuracy data of Ciprofloxacin HC1**

| Absorbance | Dilution factor | Observed conc. | Original conc. | % Recovery | Mean | SD |
|---|---|---|---|---|---|---|
| 0.938 | 10 | 107.87 | 100.00 | 107.87 | 103.36 | 7.93 |
| 0.935 | 10 | 112.35 | 100.00 | 112.35 | 102.33 | 6.29 |
| 0.885 | 10 | 106.30 | 100.00 | 106.30 | 103.22 | 5.39 |
| 0.778 | 10 | 93.34 | 100.00 | 93.34 | 101.33 | 8.32 |
| 0.137 | 10 | 98.57 | 100.00 | 96.93 | 100.45 | 6.38 |

**3.2.4 Recovery testing:** The accuracy of the method was determined by recovery experiments. A known amount of standard Ciprofloxacin hydrochloride corresponding to 2, 3, 4, 6 and 8, 10, 12% of the labelclaim (standard addition method) was added to pre-analyzed sample of tablet. The acceptable value of recovery is 80-110%. From the recovery data table 6 we can see that the mean recovery was 100.69%. Therefore, the value was in recommended range.

**Table 6: Recovery data of Ciprofloxacin HCl**

| Conc. (µg/mL) | Standard 1 | Standard 2 | Standard 3 | Average | Observed conc. (µg/mL) | % Recovery |
|---|---|---|---|---|---|---|
| 2 | 0.171 | 0.167 | 0.178 | 0.172 | 2.01 | 100.61 |
| 3 | 0.255 | 0.253 | 0.258 | 0.255 | 3.03 | 100.95 |
| 4 | 0.336 | 0.334 | 0.339 | 0.336 | 4.02 | 100.41 |
| 6 | 0.501 | 0.508 | 0.5 | 0.503 | 6.05 | 100.81 |
| 8 | 0.663 | 0.658 | 0.664 | 0.662 | 7.98 | 99.80 |
| 10 | 0.839 | 0.849 | 0.844 | 0.844 | 10.21 | 102.07 |
| 12 | 1.013 | 0.958 | 1.005 | 0.992 | 12.01 | 100.10 |
| Mean Recovery% | | | | | | 100.69 |
| SD Recovery | | | | | | 0.24 |
| RSD recovery | | | | | | 0.24 |

**3.2.5 Intra and Inter day precision**:
In this step **of** method validation we examined the differences in UV absorbance of Ciprofloxacin standard solutions at different time in a particular day and at different days. To do so 3 sets of 2, 6 and 12 µg/mL solutions were prepared from 100 µg/mL stock solution and the solutions were analyzed in the morning and afternoon and then after 2 days and after 4 days respectively.

**Intraday precision result:**

The concentrations of the drug were measured three times on the same day at intervals of five hours. From the table 7 we can see the average concentration of the drug taken in the morning and the evening was almost same. The average conc. in the morning for 2, 6, and 12 was 2.21, 6.13 and 12.32 respectively and the average conc. in the evening for 2,6,12 was 2.39, 6.32, and 12.58 respectively. A negligible variation was observed. The acceptable RSD value is 5. We got the RSD value 3.13 which is in recommended range. The RSD value was given in the table 7.

**Table 7: Intraday precision data of Ciprofloxacin HCL**

| Conc. (µg/mL) | Abs. (obtd. In The Morn.) | Abs. (obtd. in the eve.) | Conc. (obtd. in the morn.) | Conc. (obtd. in the eve.) | Avg. conc. in The morn.) | Avg. cone. in the eve.) | Mean conc. (bet. mom. | SD of cone. (bet. morn. eve.) | RSD | Avg. RSD |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0.188 | 0.19 | 2.21 | 2.40 | 2.21 | 2.39 | 2.30 | 0.13 | 5.75 | 3.13 |
| | 0.186 | 0.187 | 2.18 | 2.37 | | | | | | |
| | 0.19 | 0.191 | 2.23 | 2.41 | | | | | | |
| 6 | 0.51 | 0.51 | 6.13 | 6.30 | 6.13 | 6.32 | 6.23 | 0.14 | 2.1 | 5.28 |
| | 0.51 | 0.514 | 6.13 | 6.35 | | | | | | |
| | 0.509 | 0.51 | 6.12 | 6.30 | | | | | | |
| 12 | 1.025 | 1.031 | 12.41 | 12.66 | 12.32 | 12.58 | 12.45 | 0.18 | 1.4 | 10.1 |
| | 1.016 | 1.023 | 12.30 | 12.56 | | | | | | |
| | 1.01 | 1.019 | 12.23 | 12.51 | | | | | | |

**Inter day precision result: One day interval:**
The concentrations of the drug was measured on three different days for inter day study. The standard deviation and Relative Standard Deviation were calculated (RSD). Precision data of one day interval is shown in the table 8. The average concentration even after one day interval remained almost same. The average concentration in day zero for 2, 6 and12 was 3.74, 6.51 and 11.19 respectively and the average concentration after one day for 2, 6 and12 was 3.94, 6.83 and 11.36 respectively. The acceptable RSD value is 5. We got the RSD value 2.75 which is in recommended range. The RSD value was given in the table 8.

**Table 8: Inter day (1 day) precision data of Ciprofloxacin HCl**

| Con.(µg/mL) | Abs. (Day 0) | Abs. (Day 2) | Conc. (Day 0) | Conc. (Day 2) | Avg. conc.In Day 0 | Avg.cone. In Day2 | Mean conc. (bet. Day 0 & Day 2) | SD of conc. (bet. Day 0 & Day 2) | RSD | Avg.RSD |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0,248 | 0.247 | 2.94 | 3.10 | 3.74 | 3.94 | 3.84 | 0.14 | 3.74 | 2.75 |
| | 0.313 | 0.317 | 3.73 | 3.95 | | | | | | |
| | 0.38 | 0.385 | 4.55 | 4.78 | | | | | | |
| 6 | 0.566 | 0.574 | 6.82 | 7.09 | 6.51 | 6.83 | 6.67 | 0.23 | 3.45 | |
| | 0.538 | 0.553 | 6.48 | 6.83 | | | | | | |
| | 0.518 | 0.533 | 6.23 | 6.59 | | | | | | |
| 12 | 0.912 | 0.912 | 11.04 | 11.21 | 11.19 | 11.36 | 11.27 | 0.12 | 1.07 | |
| | 0.927 | 0.927 | 11.22 | 11.39 | | | | | | |
| | 0.934 | 0.934 | 11.30 | 11.48 | | | | | | |

**Four days interval:** The concentrations of the drug was measured on three different days for inter day study. The standard deviation and Relative Standard Deviation were calculated (RSD). The average concentration of drug after four days interval remained almost same except the concentration 12. The concentration of 12 after four days was notably changed compared to concentration 2 and 6. The average concentration in day zero for 2,6 andl 2 was 3.74, 6.51 and 11.19 respectively and the average concentration after four days for 2,6 and12 was 3.96, 6.87 and 10.21 respectively. It is also noticed that the concentration of 2 and 6 increased slightly whereas the concentration of 12 decreased slightly. The acceptable RSD value is 5. We got the RSD value 4.73 which is in recommended range.

**3.2.6 Sensitivity of the method:** The LOD of Ciprofloxacin was found to be 0.1 µg/m1 and LOQ was found to be 2 µg/m1.

**3.2.7 Assay testing of 250 and 500 mg of Ciprofloxacin HCl tablet:** To perform the assay testing of Ciprofloxacin HC1 tablet of a renowned pharmaceuticalcompany of India was used as the known standard. Each of this ciprocin®tablet contains 250 and 500 mg of Ciprofloxacin HC1. 3 tablets from each strength were weighed in an electronic balance and the average weight was calculated.

**Table 9: %Recovery of 500 mg of of Ciprofloxacin HCL tablet**

| Dilution factor | Absorb-ance | Concentration (ug/ml) | Known concentration (ug/mL) | % Recovery |
|---|---|---|---|---|
| 50 | 0.467 | 279.06 | 319.13 | 87.10 |

The recommended value of % recovery is 80-110%. From the table we can see the the obtained % recovery of 250 mg of Ciprofloxacin HCL is 87.10 %.Which is within the recommended range.

**Table 10: %Recovery of 500 mg of of Ciprofloxacin HCL tablet**

| Dilution factor | Absorb-ance | Concentration (ug/ml) | Known concentration (ug/mL) | % Recovery |
|---|---|---|---|---|
| 50 | 0.468 | 279.06 | 315.13 | 88.55 |

The recommended value of % recovery is 80-110%. From the table we can see the the obtained % recovery of 500 mg of Ciprofloxacin HCL is 88.55 %.Which is within the recommended range.

**3.2.9 Discussion:** Estimation of Ciprofloxacin Hydrochloride was found to be simple, accurate and reproducible. It follows Beer-Lambert's law in the concentration range of 2-12 µg/ml. The optical characteristics such as percent relative standard deviation and percent range of error were found to be within the limit and satisfactory. All of the analytical validation parameter for the proposed method was determined according to USP guidelines. All validation parameters results were getting within the range of USP standards. The recovery studies showed that the result were within the limit indicating no interference. The proposed method is simple, sensitive, accurate and precise and can be successfully employed for the routine analysis of the Ciprofloxacin hydrochloride in bulk drug and solid dosage forms.

**3.2.10 Conclusion:** The statistical analyses showed that the data from the proposed method are in good agreement for the estimation of Ciprofloxacin hydrochloride in bulk drugand solid dosage forms. The method is economical, rapid and do not require any sophisticated instruments contrast to chromatographic method. Thus it can be effectively applied for the routine analysis of ciprofloxacin hydrochloride in bulk drug and solid dosage forms.

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