American Journal of Pharmacy and Pharmacology
2015; 2(1): 1-8
Published online January 30, 2015 (http://www.aascit.org/journal/ajpp)
ISSN: 2375-3900

American Association for Science and Technology

Keywords
Spectrophotometr,
Ciprofloxaciline,
Method Validation,
Quantification

Received: January 13, 2015
Revised: January 26, 2015
Accepted: January 27, 2015

UV-Visible Spectrophotometric Method Development and Quantification of Ciprofloxaciline in Tablets Dosage Form

Tadesse Haile Fereja*, Muluneh Fromsa Seifu, Tizita Yirga Mola

Department of Pharmacy, College of Medicine and Health Sciences, Ambo University, Oromia region, Ethiopia

Email address

tdss_haile@yahoo.com (T. H. Fereja)

Citation

Tadesse Haile Fereja, Muluneh Fromsa, Tizita Yirga Mola. UV-Visible Spectrophotometric Method Development and Quantification of Ciprofloxaciline in Tablets Dosage Form. *American Journal of Pharmacy and Pharmacology*. Vol. 2, No. 1, 2015, pp. 1-8.

Abstract

Introduction: Spectrophotometeric analysis continues to be one of the most widely used analytical techniques available. Ciprofloxacin, 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1, 4-dihydroquinoline-3-carboxylic acid, is a synthetic broad antibacterial compound belonging to the group of fluoroquinolones. Analytical method development and validation involve a series of activities that are ongoing during the life cycle of a drug product and drug substance. *Objectives:* The study mainly aims to develop and validate spectrophotometeric assay method for Ciprofloxacin tablet. *Instruments chemicals and reagents:* Precision balance (Mettler Toledo, AL204-IC, and Switzerland), UV-Vis spectrophotometer (Shimadzu Corporation, UV-2401PC, China), sonicator, mortar and pestle, different glass wares were used throughout the experimental work. Ciprofloxacin 500 mg film coated tablets, Pharmaceutical grade (reference standards) Ciprofloxacin HCl chemical and Distilled water was used as solvent. *Methods:* The maximum wavelength was taken at which the RS solution showed maximum absorption/peak. The method was validated before according to ICH/USP guidelines for method validation parameters. Quantification was done at selected wavelength using Beer's Law. *Results and Discussion:* The maximum wavelength (λ_{max}) of the drug was obtained at 275 nm. The amount of Ciprofloxacin at the selected maximum wave length was found to be 97.93% of the labeled claim. The percent interference for the placebo was found to be 1.59%. The result reveals that there is a strong linear relationship between the concentration of the test sample and the absorbance values over the concentration range 3 to 7 µg/ml of Ciprofloxacin. With a regression equation Y = 0.105X -0.0035 and; r^2 = 0.9994. The percent mean recovery obtained was between 98 - 102%.

1. Introduction

Spectrophotometeric analysis continues to be one of the most widely used analytical techniques available. The greatest use of UV-Vis absorption spectroscopy lies in its application to quantitative measurements. The reasons for this stem from the ease with which most spectrophotometeric measurements can be made, their sensitivity and precision, and the relatively low cost of instrument purchase and operation (Frank, 1997).

A variety of techniques have been developed for different types of samples to be analyzed. Direct spectrophotometric determinations are made when the analyte molecule

contains a chromophore, thus allowing the direct measurement of its absorbance. Indirect determinations are used commonly when the analyte molecule does not contain a suitable chromophore. In these instances the analyte is made to quantitatively react with a molecule containing a chromophore and correlating the diminution of absorbance with the concentration of the analyte or by reacting with a reagent, which produces a chromophoric group (Frank, 1997).

Ciprofloxacin, 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1, 4-dihydroquinoline-3-carboxylic acid, is a synthetic broad antibacterial compound belonging to the group of fluoroquinolones. Figure1.1shows its structural formula (BP, 2009 and Kassab *et al*., 2005).

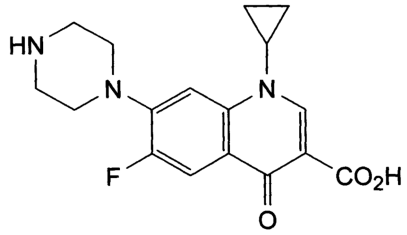


Figure 1.1. *Structural formula of Ciprofloxacin*

It contains chromophore in its structure and hence direct measurement of its UV-Vis absorbance can be made without any modification. Literature revealed that HPLC methods have been reported for the quantification of Ciprofloxacin in pharmaceutical preparations (Kassab *et al*., 2005), but no analytical method using UV spectrophotometer for its quantification is reported yet.

1.1. Analytical Method Development and Validation

Analytical method development and validation involve a series of activities that are ongoing during the life cycle of a drug product and drug substance (Chung *et al*., 2004). Typical method development and establishment for an analytical method include determination of (1) selectivity, (2) accuracy, precision, (3) calibration curve, and (4) stability of analyte in spiked samples (FDA, 2001)

Validation of an analytical procedure is performed in order to demonstrate that the procedure is suitable for its intended use. It means the result(s) generated by a developed analytical procedure are reliable and accurate (BP, 2009). The most important consideration for strategies of method validation is to design experimental work so that the appropriate validation parameters are studied simultaneously, thereby minimizing the number of experiments that need to be done (Chung *et al*., 2004). Accordingly, the following performance characteristics are recommended for assay method validation: specificity, linearity, precision (repeatability, intermediate precision, and reproducibility), accuracy, range and robustness (ICH, 2005). Limit of detection (LOD) and limit of quantification (LOQ) may also be done for quantitative tests like testing for impurities (WHO, 2006).

1.1.1. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present (BP, 2009). Specificity may often be expressed as the degree of bias of test results obtained by analysis of samples containing added impurities, degradation products, related chemical compounds, or placebo ingredients when compared to test results without added substances (BP, 2009).

Specificity is usually demonstrated by measuring the response of the sample and any expected or known species (for example excipients, impurities or degradation products). It would normally be expected that no response would be obtained that interferes with the measurement of the analyte(s) (BP, 2009).

To evaluate specificity in drug product method validation, it is necessary to demonstrate that the results are not affected by placebo constituents, or degradants in the drug product. A proper placebo should consist of everything in the formulation, except the active ingredient, all the excipients and coating materials. For a UV–Vis analysis, the absorption of the placebo solution should not be significant. The interference generally should not exceed 2% in terms of absorbance (Swartz, 2009 and Chung *et al*., 2004).

1.1.2. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. Linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte (BP, 2009)

Linearity is usually demonstrated by the analysis of various concentrations of the analyte(s) across the intended range, and represented graphically. As recommended by the international conference on harmonization (ICH), the usual range for the assay of a drug substance or a drug product should be $\pm$ 20% of the target or nominal concentration. Under normal circumstances, linearity is achieved when the coefficient of determination (r^2) is $\geq$ 0.997. A minimum of five concentrations is recommended (BP, 2009 and ICH, 2005).

1.1.3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample under prescribed conditions. Precision is usually expressed as the relative standard deviation (co-efficient of variation) (BP, 2009 and USP, 2008). Precision should be considered at different levels as follows:

(i) Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time.

Repeatability is also termed intra-assay precision. Repeatability is usually demonstrated by repeated measurements of a single sample (e.g. use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment). Repeatability is determined by replicate measurements of standard and/or sample solutions. A minimum of three determinations at each of three concentrations across the intended range, or a minimum of six determinations at the test concentration is recommended (BP, 2009, Swartz, 2009 and ICH, 2005).

(ii) Intermediate Precision

Intermediate precision expresses within-laboratory variations: different days, different analysts or equipment, etc. Intermediate precision is usually demonstrated by repeated measurements of the sample used in the repeatability experiment within the same laboratory. Usually the repeatability experiment is repeated on the same sample by a different analyst, on a different day, using different equipment if possible (BP, 2009).

(iii) Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology). Reproducibility is usually demonstrated by means of an inter-laboratory trial. It is usually expressed as the concentration of analyte (e.g., percentage, parts per billion) in the sample (BP, 2009)

1.1.4. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Accuracy is usually demonstrated by adding known amounts of analyte(s) to the sample matrix and determining the measured result using the analytical procedure. The recovery of measured against actual amounts is then calculated. Usually a minimum of three determinations at each of three concentrations across the intended range is recommended. The measured recovery is typically 98% to 102% of the amount added (BP, 2009 and ICH, 2005).

1.1.5. Range

The range of an analytical method is the interval between the upper and lower concentration (amounts) of analyte (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. For assays the range is usually not less than 80 to 120% of the test concentration. For determination of content uniformity the range is usually not less than 70 to 130% of the test concentration. For dissolution testing the range is usually +/- 20% over the expected concentrations. Range is usually demonstrated by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range (BP, 2009).

1.1.6. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage (BP, 2009)

The evaluation of robustness should be considered during development of the analytical procedure. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure (Chung *et al*., 2004).

Robustness is usually demonstrated by making small deliberate changes to one of the operating parameters of the method, analyzing samples and comparing the results to those obtained using the prescribed method (BP, 2009).

1.2. Reported Analytical Methods for Quantitative Determination of Ciprofloxaciline in Pharmaceutical Dosage Form

Nájla Mohamad et.al proposed HPLC methods for quantitative determination of ciprofloxacin and norfloxacin in pharmaceutical preparations by high performance liquid chromatography. The method uses water: acetonitrile: triethylamine (80:20:0.3 v/v/v) as a mobile phase where the organic solvelts are not environmentally friendship. Pandey, et al. also proposes quantitative analysis of Ciprofloxacin using FTIR Spectroscopy method. A Validated Method for the Quantitation of Ciprofloxacin Hydrochloride Using Diffuse Reflectance Infrared Fourier Transform Spectroscopy method by Bhoomendra et al., Spectrophotometric and titrimetric Determination of Ciprofloxacin Based on Reaction with Cerium (IV) Sulphate by Kanakapura Basavaiah et al., has been proposed.

1.3. Official Method of Ciprofloxacin Tablet

There are two common ways of analyzing assay of drug products, spectrophotometeric (UV) determinations and high performance liquid chromatography (HPLC). Spectrophotometeric determinations are usually faster, simpler, and require less solvent than HPLC (Swartz, 2009). HPLC is the official method for many of pharmacopoeial drug products. However, it is not simple, and is time consuming and expensive since it uses mostly organic solvents which are rarely available and costly. Among those many products Ciprofloxacin tablet is the one whose pharmacopoeial assay method is HPLC (BP, 2009 and USP, 2008). The mobile phase is the mixture of phosphoric acid and acetonitrile composition which are expensive, environmental polutant and not easily available. Hence, it was deemed necessary to provide a simple, quick, inexpensive and readily applicable method for assaying of Ciprofloxacin tablet, which is one of the essential drugs being produced in Ethiopia.

2. Objectives

2.1. General Objective

- The study mainly aims to develop and validate spectrophotometeric assay method for Ciprofloxacin tablet.

2.2. Specific Objectives

- ✓ To identify maximum wave length and quantify Ciprofloxacin in 500 mg Ciprofloxacin tablet.
- ✓ To studies the effect of excipients on assay determination of Ciprofloxacin tablet,
- ✓ To evaluate the linearity, accuracy, precision of the method developed, and to determine the range of the Ciprofloxacin concentration in which the method is reliable

3. Experimental

3.1. Instruments

Precision balance (Mettler Toledo, AL204-IC, and Switzerland), UV-Vis spectrophotometer (Shimadzu Corporation, UV-2401PC, China), sonicator, mortar and pestle, different glass wares were used throughout the experimental work.

3.2. Materials

3.2.1. Test Samples

Ciprofloxacin 500 mg film coated tablets (Table 3.2.1.) were obtained from RX Africa (Ethiopia), Ethio-American pharmaceuticals, Bishoftu, Ethiopia.

Table 1. *Description of sample investigated*

Product name	Batch number	Mfg date	Exp date	Mode of packaging
Ciprofloxacin 500 mg film coated tablet	CP3012	08/2012	08/2015	10 tabs/blister 10 blisters/box

3.2.2. Chemicals and Reagents

Pharmaceutical grade (reference standards) Ciprofloxacin HCl chemical were obtained from RX Africa (Ethiopia), Ethio-American pharmaceuticals, Bishoftu, Ethiopia and Distilled water was used as solvent.

3.3. Methods

3.3.1. Maximum Wavelength Selection

Ciprofloxacin HCl reference standard equivalent to 0.1 g Ciprofloxacin was weighed using precision balance (Mettler Toledo, AL204-IC, and Switzerland) and dissolved in 100 ml of water and this solution was reserved as stock solution. 10 ml of the resulting solution was diluted to 100 ml with water. Then 5 ml of the final solution was diluted to 100 ml with the same solvent (5 µg/ml). Scanning was carried out over the wave length range of 200 – 600 nm using UV-Vis spectrophotometer (Shimadzu Corporation, UV-2401PC, China). The maximum wavelength was taken at which the RS solution showed maximum absorption/peak.

3.3.2. Quantification of Ciprofloxacin in Ciprofloxacin Tablet (500 mg)

Ten Ciprofloxacin tablets were taken and finely powdered after each was weighed separately. Then, a weight of the finely powdered tablets equivalent to 0.1g Ciprofloxacin was taken and dissolved in 100 ml of water by shaking for 5 min. The solution was filtered using Whatman filter paper no. 42, and 10 ml of the filtrate was diluted to 100 ml with water. Then 5 ml of the final solution was diluted to 100 ml with the same solvent (5 µg/ml). Finally, the absorbance of the sample solution and the controlled reference standard (CRS) solution of the same concentration was measured concurrently at the selected maximum wave length. The amount (in percent) of Ciprofloxacin in the Ciprofloxacin tablet was calculated using the following formula:

$$A_s/A_{rs} \times 100,$$

Where A_s and A_{rs} are the absorbances of sample solution and standard solution, respectively.

3.3.3. Specificity

A placebo, consisting of all the excipients and coating material in the same amounts as in the formulation except the active ingredient, was prepared. Then a solution was prepared from the placebo by the same procedure as that under 3.3.2. Finally, the absorbance of the placebo solution and the CRS solution (5µg/ml) was measured concurrently at the selected maximum wave length. The percent placebo interference was calculated using the following formula:

$$A_b/A_{rs} \times 100,$$

Where A_b and A_{rs} are the absorbances of placebo solution and standard solution, respectively. It should not exceed 2%.

3.3.4. Linearity

A stock solution of 100µg/ml was prepared by dissolving a weight of Ciprofloxacin Hydrochloride RS equivalent to 0.1 g of Ciprofloxacin in 100 ml of water and then diluting 10 ml of the resulting solution to 100 ml with water. Five concentration levels were prepared by diluting 3, 4, 5, 6, and 7 ml of the stock solution to 100 ml with water to get 3, 4, 5, 6, and 7µg/ml, respectively. The respective absorbance readings of the five concentration levels were taken at the selected maximum wavelength. Then, the concentration versus the absorbance was plotted to obtain the Beer-Lambert calibration curve.

3.3.5. Repeatability

5 µg/ml, a concentration mid point to the linearity range of

a sample solution, was prepared six times over a short interval of time within one day by one analyst from finely powdered tablet, by the same procedure as that under section 3.3.2. And the absorbance of the solution was taken at each time. Finally, six assay results (n = 6), indicating the amount of Ciprofloxacin in Ciprofloxacin tablet, were obtained and the standard deviation (SD) was calculated using Microsoft Excel 2003 and the corresponding relative standard deviation (RSD) was calculated by the following formula:

$$RSD = SD/X \times 100,$$

Where X is the mean of the six assay results. It should not exceed 2 %.

3.3.6. Intermediate Precision

The same procedure was followed as that under 3.3.2 except that three assay results were obtained on one day by one analyst. And the other three assay results were obtained on the second day (after 24 hrs of the fist test) by another analyst from newly prepared5 µg/ml sample solution. Finally, the standard deviation (SD) of the six assay results (n = 6) was calculated using Microsoft Excel 2003 and the corresponding relative standard deviation (RSD) was calculated by the following formula:

$$RSD = SD/X \times 100,$$

Where X is the mean of the six assay results. It should not exceed 2 %.

3.3.7. Accuracy

A sample was prepared by blending the active pharmaceutical ingredient (Ciprofloxacin HCl) and the excipients and the coating material in the same amount and the same type as present in the formulation (Ciprofloxacin 500 mg film coated tablet). A stock solution of 100µg/ml was prepared by dissolving the sample equivalent to 0.1 g of Ciprofloxacin in 100 ml of water and then diluting 10 ml of filtered solution to 100 ml with water. Three concentration levels ranging from below (3 µg/ml) to above (7 µg/ml) and 5 µg/ml as target concentration were prepared by diluting 3, 7, and 5 ml of the stock solution to 100 ml with water, respectively. The respective absorbance readings of the three concentration levels and that of the corresponding concentration levels of CRS were taken at the selected maximum wavelength. It was repeated three times to get three replicates for each concentration by proceeding as described above by preparing stock solution from the sample. Finally, nine test results were obtained and the percent recovery was determined. It should be between 98 to 102%.

3.3.8. Robustness

(i) Solution Stability

The same procedure was followed to prepare the solutions as described under 3.2.2. The standard and the sample solutions stability was evaluated initially and after 24 hrs and 48 hrs preserved under ambient temperature and light condition by determining the concentration against fresh standard solution. The test result should be between 98.0 and 102.0% of the initial value.

(ii) Wavelength Change

The effect of a small change in wavelength (λ_{max} ±1nm) on the test result was studied. The assay results obtained at λ_{max} - 1nm and at λ_{max} + 1nm were compared with that obtained at λ_{max}.

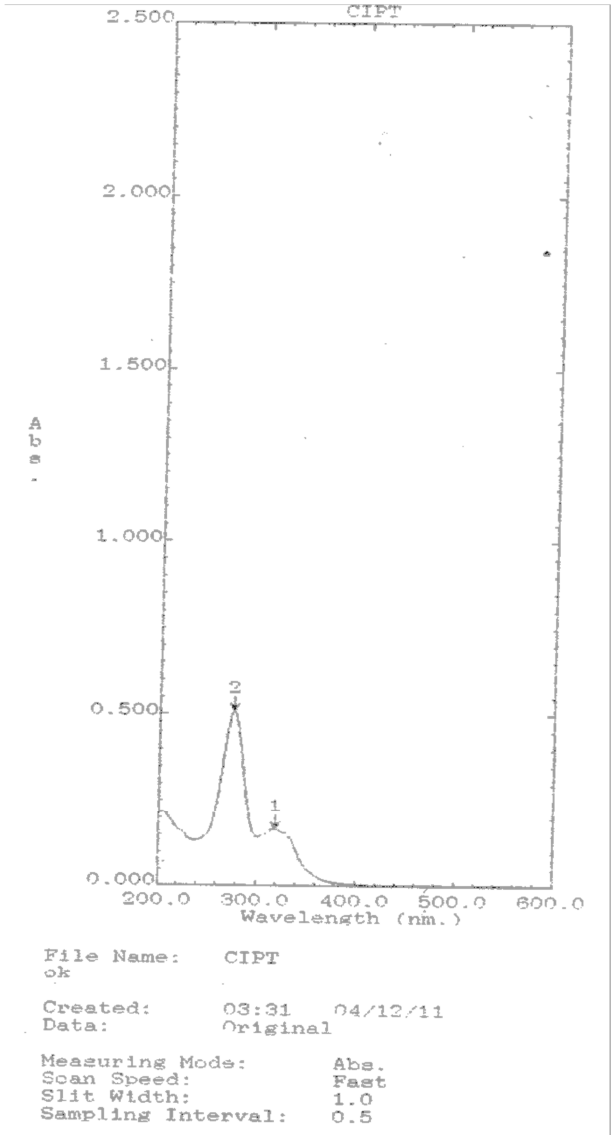
3.3.9. Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification were determined from the calibration curve data obtained by (Instant +) version 3.33 programs using the following formulae:

$$LOD = 3.3\ \sigma/S$$

$$LOQ = 10\ \sigma/S$$

Where, σ is the standard deviation of the response and S is the slope of the calibration curve.



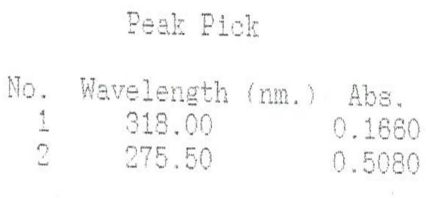


Figure 4.1. *Absorbance spectrum of Ciprofloxacin scanning from 200.00 to 600.00nm*

4. Results and Discussion

4.1. Maximum Wavelength Identification

Distilled water was selected as a solvent as it is easily available and cheaper than other solvents as well as Ciprofloxacin HCl is soluble in it. As shown in Figure 4.1, the maximum wavelength (λ_{max}) was obtained at 275 nm since Ciprofloxacin Hydrochloride CRS solution showed maximum absorption peak at 275 nm upon scanning over the wave length range of 200 – 600 nm using distilled water as a blank.

4.2. Assay of Ciprofloxacin

Ciprofloxacin 500 mg film coated tablet was then analyzed for the quantification of Ciprofloxacin at the selected maximum wave length and found to be 97.93% of the labeled claim. Ciprofloxacin tablet should contain not less than 90% and not more than 110% of the labeled claim of Ciprofloxacin (USP, 2008). The result shows that the assay value lies within the limit specified in the United States pharmacopoeia (USP). Thus, Ciprofloxacin tablet sample met the requirement for assay as specified in the pharmacopoeia.

4.3. Validation of the Proposed Methods

The proposed method was validated for specificity, linearity, accuracy, repeatability, intermediate precision and robustness in accordance with ICH guidelines.

4.3.1. Specificity

The absorbance values of the placebo solution and the CRS solution (5 µg/ml) at 275 nm and the percent placebo interference are depicted below (Table 2).

Table 2. *Absorbance values placebo and test solution*

Test solution	Absorbance
Placebo	0.009
CRS	0.567
% Interference	1.59
Limit in %	Not more than 2

As can be seen from Table 2, the interference of the excipients is found to be 1.59% which is less than 2%, i.e. absorption of the placebo solution is not significant which in turn indicates the insignificance of excipients interference and hence the specificity of the proposed method.

4.3.2. Linearity

The five different concentrations of Ciprofloxacin CRS and the corresponding absorbance readings are indicated in Table 3.

Table 3. *Linearity data indicating concentrations (n=5) and the corresponding absorbance values*

Concentration (µg/ml)	0	3	4	5	6	7
Absorbance (au)	0	0.309	0.407	0.523	0.636	0.728

The absorbance was plotted versus the concentration (µg/ml) to obtain the Beer-Lambert calibration curve (Figure 4.2). The equation for the calibration curve was Y = 0.105X - 0.0035, where Y is the absorbance and X is the concentration in µg/ml; $r^2 = 0.9994$. The value of correlation coefficient (r) was 0.9997. The result reveals that there is a strong linear relationship between the concentration of the test sample and the absorbance values over the concentration range 3 to 7 µg/ml Ciprofloxacin.

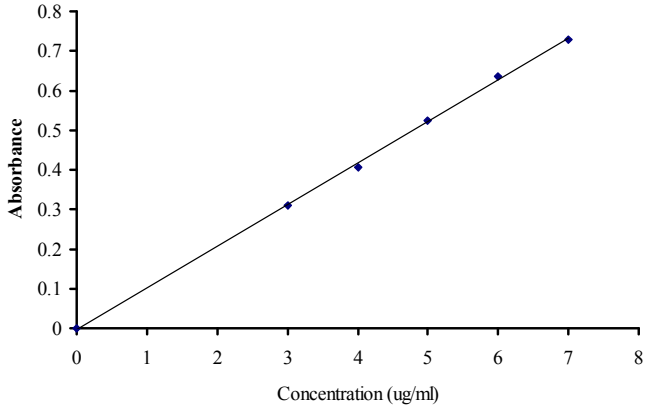


Figure 4.2. *Beer-Lambert calibration curve for Ciprofloxacin Hydrochloride CRS in water and maximum wave length of 275 nm over the range of 3 to 7 µg/ml Ciprofloxacin*

4.3.3. Accuracy

The percent mean recovery obtained from three different concentration levels, 60, 100, and 140% of the test concentration, three replicates each, was 101.57% (Table 4). It should be between 98 - 102% (ICH, 2005). Therefore, the proposed method is accurate as the percent mean recovery lies within the recommended limit.

Table 4. *Assay results (n=9) and % Mean recovery*

%Nominal concentration*	Replicate	Assay (%)
	1	101.67
60	2	101.47
	3	101.50
	1	101.72
100	2	101.89
	3	101.37
	1	101.96
140	2	101.71
	3	100.84
%Mean recovery	101.57	

* = target concentration at which any sample is to be analyzed

4.3.4. Repeatability

The repeatability data indicating assay results (n = 6) of Ciprofloxacin tablet, determined at 100% test concentration, and the percent relative standard deviation (RSD) are shown below in Table 5.

Table 5. *Repeatability at 100% test concentration*

Replicate	Assay (%)
1	101.65
2	101.28
3	100.37
4	102.14
5	101.96
6	102.15
% RSD	0.67

The relative standard deviation value (%RSD) was less than 2.0%, the maximum limit recommended by ICH guidelines, indicating good repeatability of the test results obtained by the proposed method. In other wards, there is no significant variation among the test results generated by the method under development within 12 hrs by one analyst.

4.3.5. Intermediate Precision

The intermediate precision data indicating assay results (n = 6) of Ciprofloxacin tablet, determined at 100% test concentration (5 µg/ml), and the percent relative standard deviation (RSD) are shown below in Table 6.

Table 6. *Intermediate precision (by two analysts on two different days) at 100% test concentration*

Replicate	Assay (%)	
	Analyst 1 (on day 1)	Analyst 2 (on day 2)
1	99.65	97.69
2	98.94	98.99
3	98.8	100.34
%RSD	0.90	

The relative standard deviation value (%RSD) was 0.90, which is less than 2.0% (the maximum limit recommended by ICH guidelines). This means that the variation between the results obtained by two different analysts on two different days is insignificant and therefore the changes in analyst and day (24 hrs) did not affect the consistency of assay results obtained, indicating good precision of the proposed method.

4.3.6. Range

The proposed UV-Vis spectrophotometeric assay method for Ciprofloxacin tablet is accurate, precise and linear within the concentration range of 3 µg/ml (60%) to7 µg/ml (140%) of Ciprofloxacin. Therefore the range of the method is between 60% and 140% of the target concentration (5 µg/ml).

4.3.7. Robustness

(i) Solution Stability

Table 7a shows sample and standard solutions stability performed on first, second and third day after preparation.

Table 7a. *Stability of sample and standard solutions*

Day	% Initial sample	% Initial Standard
1	100.00	100.00
2	96.84	98.62
3	91.70	95.81

As can be seen from table 7a, the amount of Ciprofloxacin declines as the solutions stay for long time for both the sample and the standard. For example, 96.84 and 91.70% of the initial amount remains, after reserving the sample solution for 24 hrs and 48 hrs, respectively. The standard solution also declines to 95.81% of the initial amount after 48 hrs. This suggests that the solutions are not stable after 24 hrs and hence any analysis carried out to determine assay of Ciprofloxacin using this proposed method should be done within 12 hrs.

(ii) Wavelength Change

Table 7 b shows the assay results obtained at $\lambda_{max} \pm 1$ nm to investigate the effect of small change in wave length.

Table 7 b. *Assay results obtained at λ_{max} - 1 nm, λ_{max}, and λ_{max} + 1 nm*

Wave length (nm)	274	275	276
Assay (%)	100.12	99.31	99.87

The assay results obtained at $\lambda_{max} - 1$ nm (274 nm) and at $\lambda_{max} + 1$ nm (276 nm) are almost similar to that obtained at λ_{max} (275 nm), indicating the consistency of test results in spite of small change in wave length.

4.3.8. Limit of Detection and Limit of Quantification

Table 8 shows the calibration curve data used for the determination of LOD and LOQ.

Table 8. *Data obtained from calibration curve*

Parameter	Value
Slope (S) of the calibration curve	79.81
Standard deviation (σ) of the response	0.0029

LOD=3.3 σ/S=3.3X0.0029/79.81=0.00012
LOQ=10 σ/S=10X0.0029/79.81=0.00036

5. Conclusion

In this study, a simple UV-Vis spectrophotometeric assay method for Ciprofloxacin film coated tablet was developed and validated. Distilled water, which is easily available and cheaper than other solvents, was selected as a solvent since Ciprofloxacin HCl is soluble in it. The maximum wavelength (λ_{max}) for Ciprofloxacin HCl was found to be 275 nm. The method was then validated for specificity, linearity, accuracy, repeatability, intermediate precision and robustness in accordance with ICH guidelines.

The interference of the excipients in the formulation of Ciprofloxacin tablet investigated was not significant thereby showing the specificity of the method. The method also indicated good linearity relationship between the concentrations of the test sample and the absorbance values.

It is accurate as the percent mean recovery obtained (101.57%) lies within the recommended limit (98 - 102%). The RSD of assay results (n = 6) of Ciprofloxacin tablet, determined at 100% test concentration was less than 2.0%. In addition, the changes in analyst and day (24 hrs) did not affect the consistency of assay results as the RSD of assay results (n=6) was less than 2.0%. These assured that the proposed method is precise. However, the investigated sample solution was found to be instable after 24 hrs. Unlike solution stability, a small change in wave length ($\lambda_{max} \pm 1$ nm) did not affect the robustness of the method. In general, the proposed method is simple, precise, accurate and specific over the concentration range 3 µg/ml (60%) to 7 µg/ml (140%) of Ciprofloxacin where the target concentration is 5 µg/ml. It is also inexpensive since only water is used as solvent.

Recommendations

- The developed method in this study can be verified by using different Ciprofloxacin tablets manufactured by other pharmaceutical companies.
- The reproducibility (inter-laboratories precision) of the developed method can be done between different laboratories.
- The specificity of the method can be evaluated using Ciprofloxacin related substances and degradable products.
- The linearity, accuracy and precision of the method can be further investigated beyond 60% to 140% of the target concentration studied under this research.

References

[1] BP (British Pharmacopoeia). (2009) *The Pharmaceutical Press*, Her Majesty's Stationery Office, London, Vol. I and II.

[2] Chung Ch, Ch. Herman, L. Lee Y,C and Zhang, X. (2004) *Analytical method validation and instrument performance verification,* John Wiley & Sons, Inc., Hoboken, Publication, New Jersey.

[3] FDA. (2001) *Guidance for Industry: Bioanalytical Method Validation*, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER).

[4] Frank A, S. (1997). *Hand book of instrumental techniques for analytical chemistry,* Prentice Hall, Inc. A Simon & Schuster Company, New Jersey.

[5] International Conference on Harmonization (ICH). (2005) *Validation of Analytical Procedures*, Text and Methodology, Q2 (R1).

[6] Kassab N M, Singh A K, Kedor-Hackmam E R M, and Santoro M I R M. (2005) *Quantitative determination of ciprofloxacin and norfloxacin in pharmaceutical preparations by high performance liquid chromatography*. Brazilian Journal of Pharmaceutical Sciences 41:4.

[7] Swartz M. (2009) *Developing and Validating Dissolution Procedures for Improved Product Quality*, synomics pharm, www.synomicspharma.com

[8] USP (The United States Pharmacopoeia). (2008) USP31/NF26, Rockville, Vol.1, MD, USA.

[9] World Health Organization (WHO). (2006) *Supplementary guidelines on good manufacturing practices: validation;* WHO Technical Report Series, No. 937, 2006.

[10] Nájla M. K., Anil K. S., Erika R. M. K-H. and Maria I. R. M. S.. Brazilian Journal of Pharmaceutical Sciences vol. 41, n. 4, out./dez., 2005.

[11] Pandey S., Pandey P., Tiwari G., Tiwari R. and Rai A. K.. FTIR Spectroscopy: A Tool for Quantitative Analysis of Ciprofloxacin In Pharmaceutical Formulations