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Spectrophotometric Method for the Determination of Ciprofloxacin in Pure and Pharmaceutical Preparations: Development and Validation †

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Abstract: Ciprofloxacin (Cip) is spectrophotometrically identified through the formation of a colored charge-transfer complex that exhibits a maximum absorbance at 440 nm. This complex is generated by the reaction of the drug's secondary amine with sodium nitroprusside (SNP) in an alkaline medium in the presence of hydroxylamine (NH_2OH). Classical univariate analysis is employed to optimize the experimental conditions affecting the formation of the charge-transfer (CT) complex. The method presented herein offers a straightforward and sensitive approach for quantifying ciprofloxacin within a concentration range of 50.0–250.0 µg/mL. The method exhibits a molar absorptivity of 364.4817 L/mol·cm and a coefficient of determination (r^2) of 0.997. Validation of the method is achieved through determination of the regression equation, accuracy, precision, and detection limit. The procedure is successfully applied to the quantification of ciprofloxacin in pharmaceutical formulations and demonstrates satisfactory recovery and precision. Statistical validation corroborates the reliability and repeatability of the obtained results.

Keywords: ciprofloxacin determination; spectrophotometry; charge transfer

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1. Introduction

Ciprofloxacin hydrochloride is a fluoroquinolone antibiotic with a broad spectrum of activity against bacterial infections [1]. It is commonly prescribed for a variety of infectious diseases, including chronic inflammation of the large intestine, also known as Crohn's disease. Chemically, ciprofloxacin is described as 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl) quinolone-3-carboxylic acid, with a molar mass of 331.347 g/mol [2]. The structural formula of ciprofloxacin is provided in Figure 1 [3].

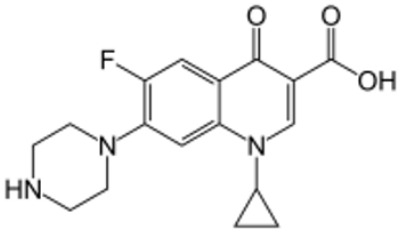

Figure 1. Chemical structural formula of ciprofloxacin.

Several analytical methods have been reported for the quantification of ciprofloxacin, including high-performance liquid chromatography (HPLC) [4,5], spectrofluorometry [6], flow injection analysis [7], voltammetry [8], derivative spectrophotometric methods [9],

titrimetric and spectrophotometric techniques using cerium (IV) sulfate [10], and various other spectrophotometric techniques [11,12].

Some studies have focused on the development of spectrophotometric methods based on charge-transfer complexation techniques [13], indirect spectrophotometric determination using N-Bromosuccinimide and Janus Green B dye [14], and the use of capillary electrophoresis with transient pseudo-isotachophoresis [11,12,15]. The objective of this study is to develop a spectrophotometric analytical method for the quantification of ciprofloxacin hydrochloride in pharmaceutical preparations. The method employs sodium nitroprusside as a reagent in conjunction with hydroxylamine in a basic medium. The absorbance of the resulting dye is measured at 440 nm. This approach aims to establish a colorimetric method for detecting ciprofloxacin in both raw and formulated medications. The method's sensitivity is attributed to the formation of a charge-transfer complex between ciprofloxacin, sodium nitroprusside (SNP), and hydroxylamine (NH_2OH) in an alkaline environment. The stoichiometric ratio of the reactants was determined, optimal reaction conditions were established, and the method was applied for ciprofloxacin quantification in pharmaceutical manufacturing.

2. Materials and Methods

Absorption measurements utilized a pg T80+ UV–visible double-spectrophotometer (UK) with 1.0 cm quartz cells. The device was interfaced with a Windows-compatible computer running UV Win 5 software v5.2.0.1104. Weighing was conducted using a KERN sensitive balance (Germany). Solutions were prepared with a Labtech magnetic stirrer (Korea), and heating processes used a Labtech water bath (Korea). All reagents and solvents were of analytical grade. Reference ciprofloxacin with a purity of 99.99% was provided by the Samara Drug Company in Iraq. Other chemicals included 99%-pure sodium nitroprusside (Merck), hydrochloride of hydroxylamine (Chemapol), and hydrated sodium carbonate (SEELEZE-HANOVER), among others. An 8% (w/v) sodium carbonate hydrate solution was prepared by dissolving approximately 8 g of $Na_2CO_3.H_2O$ in 0.1 L of distilled water. Similarly, 0.4% (w/v) solutions of sodium nitroprusside and hydroxylamine hydrochloride were prepared. Stock solutions (10,000 µg/mL) of glucose, starch, lactose, sucrose, and acacia were also prepared [16]. A standard ciprofloxacin solution (1000 µg/mL) was created by dissolving 0.1 gm of the drug in 100 mL of distilled water. From this stock, dilutions were made to obtain solutions with lower concentrations. A 5.8×10^{-3} M solution of ciprofloxacin and a similar concentration of sodium nitroprusside (SNP) were also prepared. For sample stock solutions, the contents of 10 units (tablets or capsules) of each ciprofloxacin medication were weighed, crushed, and mixed. A weight of powder equivalent to 0.1 gm of ciprofloxacin was dissolved in distilled water, and the volume was adjusted to 100 mL. Insoluble substances were removed by filtration using Whatman No. 41 filter paper, and subsequent solutions were prepared from this stock. Based on univariate optimization results, 1 mL of SNP solution and 1 mL of hydroxylamine solution were added to a series of 10 mL volumetric bottles, each containing 50 to 250 µg of ciprofloxacin. Additionally, 0.8 mL of Na_2CO_3 solution was added to each bottle. The reaction mixture was shaken and left to stand for five minutes at 2 ± 24 °C. The volume was then adjusted with distilled water and mixed well, and the absorbance was measured at 440 nm relative to a blank solution. The method was validated in accordance with the International Conference on Harmonization (ICH) guidelines [17]. Validation parameters included specificity, linearity, recovery values, limits of detection (LODs), and limits of quantification, along with precision and accuracy assessments.

3. Result and Discussion

3.1. Absorption Spectra

The maximum absorption of the yellow color relative to the reagent blank was observed at 440.0 nm for the reaction between ciprofloxacin and sodium nitroprusside in the presence of hydroxylamine under alkaline conditions. This observation is illustrated in

Figure 2. It is noteworthy that at this specific wavelength, neither ciprofloxacin nor sodium nitroprusside exhibited significant absorption.

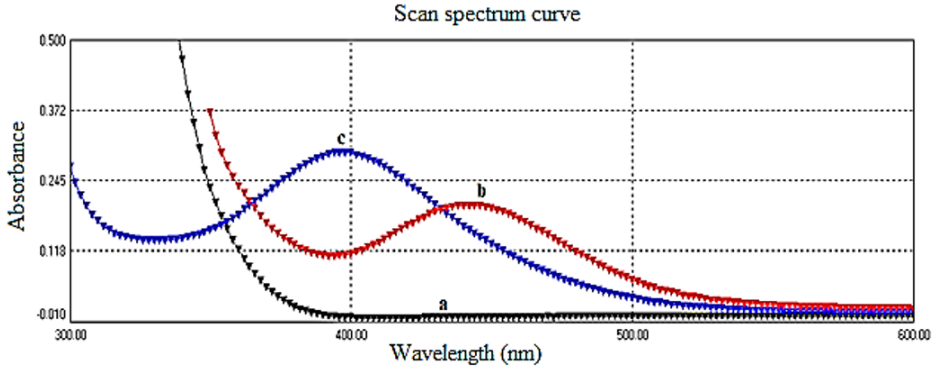

Figure 2. (a) Absorption spectrum of ciprofloxacin vs. D.W., (b) absorption spectrum of charge-transfer complex for ciprofloxacin (200.0 µg/mL) and sodium nitroprusside vs. blank, and (c) absorption spectrum of reagent blank (SNP + NH_2OH + Na_2CO_3) vs. D.W.

3.2. Optimization of Reaction Conditions

Experiments were conducted in 10 mL volumetric flasks, each containing 1 mL of a 200 gm/mL drug compound solution. Factors such as SNP, hydroxylamine hydrochloride, Na_2CO_3, temperature, and reaction time were evaluated for their impact on the formation of the colored complex. The color intensity was found to be maximized with 1 mL of SNP solution (Figure 3) and 1 mL of NH_2OH (Figure 4). A 0.8 mL volume of $Na_2CO_3H_2O$ solution was sufficient for achieving constant maximum absorption (Figure 5). The color intensity reached its peak after five minutes (Table 1), and the reaction was most stable at room temperature (2 ± 24 °C) (Table 2). The compound's absorption remained unchanged for over 5 h.

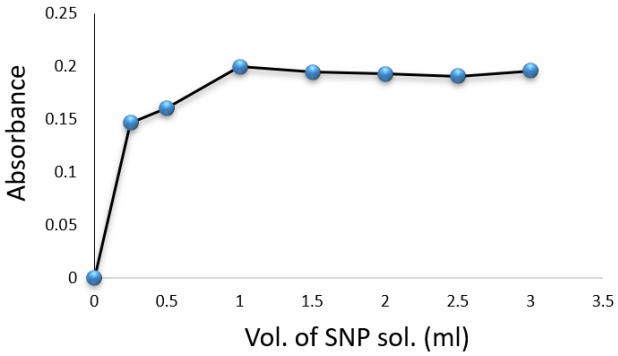

Figure 3. Effect of volume of 0.4% (*w*/*v*) SNP solution on the formation of Cip–SNP complex.

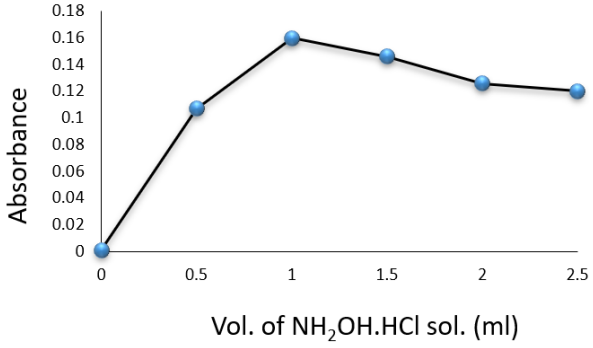

Figure 4. Effect of volume of 0.4% (*w*/*v*) $NH_2OH.HCl$ solution on the formation of Cip–SNP complex.

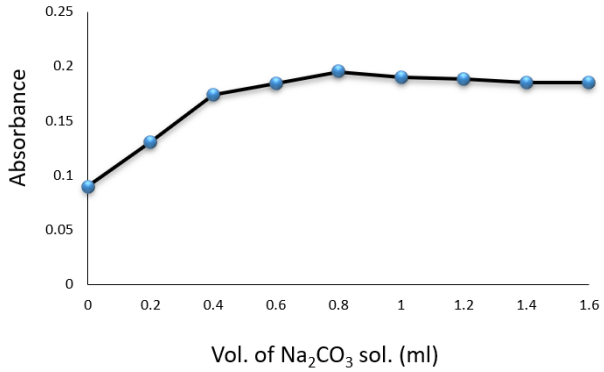

Figure 5. Effect of volume of 8% $Na_2CO_3.H_2O$ on the formation of Cip–SNP complex.

Table 1. Effect of reaction time on the color development for the estimation of 200.0 µg/mL Cip.

Time (min)	Absorbance
3	0.185
5	0.201
6	0.202
7	0.203
8	0.197
10	0.199
15	0.200

Table 2. Effect of temperature on the color development for the determination of 200.0 µg/mL Cip.

Temperature (°C)	Absorbance
15	0.154
20	0.182
25	0.202
30	0.198
35	0.183
40	0.172
45	0.165

3.3. Chemical Reactions of the Method

In this method, ciprofloxacin (Cip) serves as an electron donor to SNP in the presence of alkali hydroxylamine, forming aqua ferricyanide $[Fe(CN)_5H_2O]^{3-}$. The resulting color is attributed to the formation of $[Fe(CN)_5M]^{3-}$, where M is the drug compound. The proposed reaction between ciprofloxacin and SNP is illustrated in Figure 6 [18].

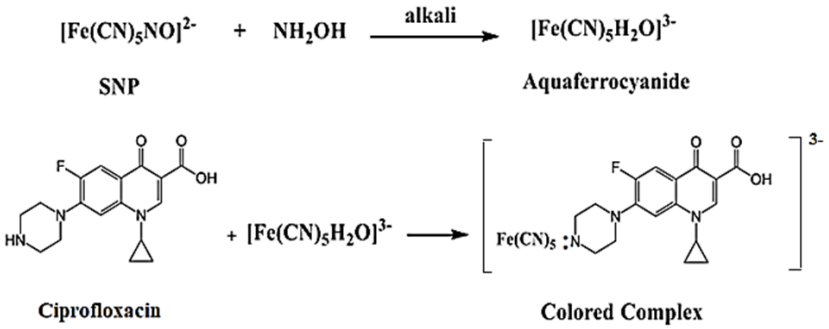

Figure 6. The possible sequence of reaction between ciprofloxacin and SNP.

3.4. Calibration Curves and Analytical Data

Calibration curves were generated by plotting the absorbance values of a series of solutions with varying Cip concentrations against the corresponding reagent blank at 440 nm. These curves, depicted in Figure 7, were linear over the 50.0–250.0 $g \cdot mL^{-1}$ range of Cip concentration, as confirmed by linear regression.

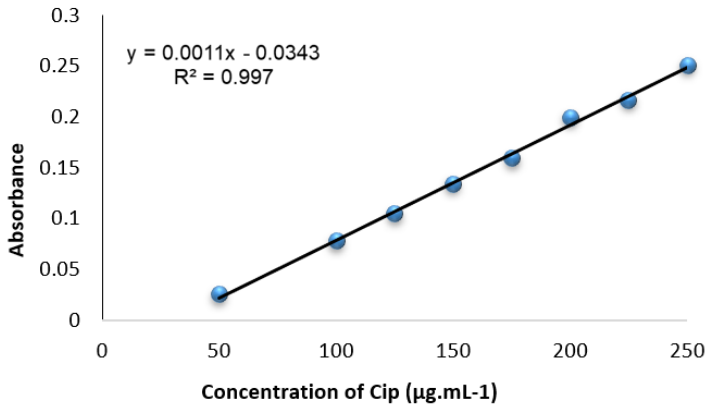

Figure 7. Standardization plot for Cip developed via univariate optimization under ideal circumstances.

Various optical and statistical properties of the Cip–SPN complex are summarized in Table 3 [19]. The high correlation coefficient (r) values of the regression equation confirm the linearity of the curves. The method's sensitivity, as indicated by the molar absorbance, limits of detection, and quantification, as well as Sandel's sensitivity, is deemed acceptable for the determination of the drug compound under study.

Table 3. Optical and regression data of the proposed method for ciprofloxacin.

Parameter	Value
λ max (nm)	440.0
Color	Yellow
Linearity range (µg/mL)	50.0–250.0
Regression equation	$y = 0.0011[\text{Cip } \mu g/mL] - 0.0343$
Calibration sensitivity (µg/mL)	0.0011
Correlation coefficient (r)	0.99849
Correlation of linearity (R^2)	0.997
Molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$)	364.4817
Sandell's sensitivity ($\mu g/cm^2$)	0.90909
Detection limit (µg/mL)	12.57
Quantification limit (µg/mL)	41.909
Mole ratio (R/D)	2:1

3.5. Equivalent of the Reactions

The mole-ratio method was employed to ascertain the nature of the colored complex. Under optimal conditions of hydroxylamine concentration, alkalinity, and temperature, the stoichiometry of the interaction between Cip and SNP was investigated. The equivalence ratio between the reagent and the drug was found to be 2:1, as shown in Figure 7.

3.6. Accuracy and Precision

To assess the method's accuracy, five replicates of the pure drug were analyzed at three different concentration levels. Precision was evaluated by calculating the coefficient of variation (C.V. %) for identical drug sample solutions.

The results, summarized in Table 4, indicate low C.V. % values, ranging from 0.08911% to 0.6823%, and relative error percent values not exceeding 0.1%. These findings attest to the high precision and accuracy of the proposed method.

Table 4. The accuracy and precision of the method.

Conc. of Chloramphenicol (μg/mL)		Recovery (%)	Relative Error (%)	C.V. * (%)
Taken	Found			
100.000	99.899	99.899	0.101	0.6823
150.000	149.965	99.976	0.024	0.2314
200.000	199.891	99.945	0.055	0.08911

* Average of five determinations.

3.7. Interference Study

The method's analytical capability was further evaluated by investigating the influence of common excipients such as acacia, sucrose, glucose, lactose, and starch, which are often found in pharmaceutical formulations. These compounds were tested in the presence of 200 g/mL of Cip. According to the data in Table 5, none of the examined excipients significantly interfered with the detection of Cip.

Table 5. Recovery of ciprofloxacin in the presence of different excipients.

Excipients	Concentration (μg/mL)	Ciprofloxacin Conc. Taken (200.0 μg/mL)	
		Conc. Found * (μg/mL)	Recovery * (%)
Acacia	1000	199.52	99.76
Glucose		197.97	98.985
Lactose		199.71	99.855
Starch		198.98	99.49
Sucrose		200.11	100.055

* Average of three determinations.

3.8. Effect of Mixing Sequence

The optimal sequence for mixing the reagents to achieve maximum consistent absorbance was determined to be (Drug–SNP–NH2OH–Base), as indicated in Table 6. Subsequent experiments adhered to this mixing sequence.

Table 6. Effect of reagent addition sequence on the color development of 200.0 μg/mL Cip.

Order	1st	2nd	3rd	4th	Abs.
Order 1	Cip.	SNP	NH_2OH	Base	0.201
Order 2	SNP	Cip.	NH_2OH	Base	0.200
Order 3	Base	Cip.	SNP	NH_2OH	0.166
Order 4	SNP	NH_2OH	Cip.	Base	0.158
Order 5	SNP	Base	Cip.	NH_2OH	0.163
Order 6	SNP	NH_2OH	Base		

3.9. Application of the Method

The proposed method was applied to an analysis of several pharmaceutical formulations to evaluate its suitability for determining Cip in real-world samples. The aggregated results, presented in Table 7, show that the recovery percentage values range between 97.885% and 99.047%, and the C.V. % values do not exceed 1.833%.

These findings confirm that the developed method is highly accurate and aligns well with the original dosages of the active ingredient in the pharmaceutical preparations.

Table 7. Application of the proposed method under conditions obtained via univariate optimization for ciprofloxacin determination in pharmaceutical samples.

Marketed Formulation	Certified Value ($\mu g \cdot mL^{-1}$)	Found Value ($\mu g \cdot mL^{-1}$)	Recovery %	Relative Error %	C.V. * %
Cipro-Denk 500 mg/tablets. Denk Pharma—Germany.	200.00	195.77	97.885	2.115	1.833
Ciproxin 500 mg/capsule. Karachi Sindh.	200.00	198.094	99.047	0.953	1.366

* Average of five determinations.

4. Conclusions

The present study successfully developed and validated a visible spectrophotometry method for the determination of ciprofloxacin (Cip) in both its pure form and in pharmaceutical formulations. The method exhibited high sensitivity, with a molar absorptivity of 364.4817 L/mol·cm and a correlation coefficient (r^2) of 0.997, making it a robust analytical tool. One of the significant advantages of the proposed method is its simplicity and cost-effectiveness. Unlike other analytical techniques such as high-performance liquid chromatography (HPLC) and spectrofluorometry, the developed method does not require specialized equipment or temperature-controlled environments. This makes it particularly useful for routine analyses in settings with limited resources. Moreover, the method demonstrated high levels of accuracy and precision, as evidenced by the low coefficients of variation and relative error percentages in the validation studies. The technique was further validated by its successful application to various pharmaceutical formulations, showing recovery percentages ranging from 97.885% to 99.047%, thereby confirming its applicability for quality control in pharmaceutical manufacturing. Additionally, the method showed excellent specificity, as common excipients like acacia, glucose, lactose, starch, and sucrose did not interfere with Cip detection. This is particularly important for ensuring the reliability of the method in complex sample matrices. In summary, the proposed visible spectrophotometry method offers a straightforward, sensitive, and accurate approach for the routine determination of Cip. Its simplicity and high level of accuracy make it a viable alternative to more complex and resource-intensive methods, and potential applications may extend to other quinolone antibiotics. Future work could focus on further method optimization and its applicability to a broader range of pharmaceutical compounds.

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