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Quantitative Analysis of Ciprofloxacin Sodium Chloride Pharmaceutical Infusions Using Ultraviolet-visible Spectroscopy

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ABSTRACT

This work was undertaken to develop a rapid and low cost ultraviolet-visible spectrophotometric method for determining the amount of ciprofloxacin in ciprofloxacin sodium chloride pharmaceutical infusions. Ultraviolet-visible spectrophotometric analysis was performed at a pre-determined maximum wavelength of 326 nm with water as diluent and 0.018 mg/ml sodium chloride solution as blank. The method was validated for linearity, accuracy, precision and reproducibility. The method was used to estimate the amount of ciprofloxacin in four infusion samples on the Ghanaian market. The regression data for the calibration curve exhibited a good linear relationship (R^2 = 0.999) having a regression equation, y =74.554 x + 0.019 over a concentration range of 0.002 - 0.012 mg/ml. The amount of ciprofloxacin in samples A, B, C and D were 216.8227 mg/100 ml ± 0.0002, 239.4238 mg/100 ml ± 0.0002, 271.1457 mg/100 ml ± 0.0002 and 252.1662 mg/100 ml respectively. These concentrations were greater than that indicated on the packs which were 200 mg/100 ml for all the samples. When the method was tested with a prepared standard of concentration 0.2 mg/ml, the result obtained was 0.2068 mg/ml ± 0.0002 representing an increment of 3.4 % more than expected. Hence, the method could only account for 3.4 % of the increase in the sample concentration. The proposed method gave good validation results and the statistical analysis performed proved that the method is precise, accurate and reproducible and hence can be employed for routine analysis of ciprofloxacin in intravenous infusions.

Keywords: *Absorbance, ciprofloxacin, fluoroquinolone, ultraviolet-visible spectroscopy*

1. INTRODUCTION

The quinolone antimicrobials embrace a group of synthetic substances that possess a basic nucleus of an *N*-1-alkylated-3-carboxypyrid-4-one ring fused to another aromatic ring with a substituent on it. The first group of these quinolones surfaced in the 1960's for treatment against certain gram negative bacteria. They were considered as minor urinary tract disinfectants: an example is nalidixic acid. Further work resulted in the discovery of the fluoroquinolones which had a fluoro group on position six of the basic nucleus and were more active biologically [1]. Fig.1 gives the general structure of fluoroquinoline.

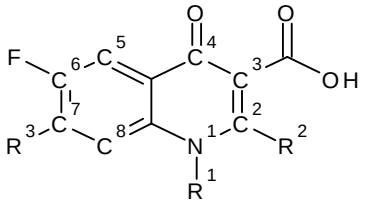

Fig 1: General structure of the fluoroquinolones

Ciprofloxacin chemically is 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid [2]. It is a broad spectrum antibacterial agent which belongs to the fluoroquinolone family. The fluoroquinolones (cinoxacin, ciprofloxacin, nalidixic acid and norfloxacin) act by inhibiting DNA gyrase and thus preventing bacteria DNA replication. These chemotherapeutic agents are used particularly in infections caused by gram-negative and gram-positive bacteria. The gram bacteria are the main ways of grouping bacteria into two large groups according to their ability to retain the crystal violet dye in gram staining protocol or not [3]. Bacteria that retain the dye are stained blue or violet and are called gram positive bacteria; examples are Staphylococcus, Streptococcus, Enterococcus, Bacillus, Clostridium and Listeria [4, 5] . Those that are not stained are gram negative, examples are Neisseria gonorrhoeae, Neisseria meningitides, Moraxella catarrhalis, Hemophilus influenza, Klebsiella pneumonia, Legionella pneumophila, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Enterobacter cloacae, Serratia marcescens, Helicobacter pylori, Salmonella enteritidis, Salmonella typhi, etc. [6].

Ciprofloxacin is active against infections that are caused by most of the above mentioned gram bacteria. The fluoroquinolones are selective against the bacterial enzyme because the bacterial enzyme is structurally different from the mammalian enzyme. [7, 8] Ciprofloxacin is used to treat urinary tract infections, typhoid fever, etc. [9]. Ciprofloxacin has no chiral center in its structure as shown in Fig. 2.

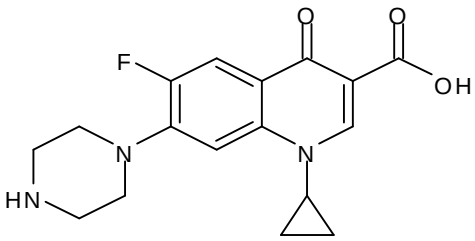

Fig 2: Structure of Ciprofloxacin

Intravenous ciprofloxacin is most frequently associated with side effects such as nausea, diarrhea, central nervous system disturbance, infusion site reactions, hepatic enzyme abnormalities, eosinophilia, headache, restlessness, and rash. The majority of these effects are of mild to moderate severity. An overdose of ciprofloxacin can lead to the following effects: headaches, dizziness, drowsiness, disorientation, slurred speech, tremors, nausea and vomiting, diarrhea, abdominal (stomach) pain, kidney problems, etc; [10]

A variety of analytical methods have been developed for the analysis of drugs of the 4-quinolones [11], some of which are High Performance Liquid Chromatography (HPLC) recommended by the British pharmacopoeia [12], High Performance Thin Layer Chromatography (HPTLC), Spectrofluorimetry and Atomic Absorption Spectrometry (AAS). These methods are expensive and mostly unavailable in many laboratories in developing countries including Ghana.

There is, therefore, the need to develop a method that is less expensive and with equipment that is widely available. Ultraviolet-visible spectroscopy was considered and it is one method that is less expensive, easily available and effective for analyzing drugs belonging to the quinolone group. It involves absorption of radiation in the range of 200 nm to 800 nm within the electromagnetic spectrum [13]. Absorption occurs at a specific part of the molecule - the chromophore and absorption is highest at the maximum wavelength of 326 nm [14]. Absorption that leads to electronic transitions from non-bonding (n) to pi-antibonding (π*) and from pi-bonding (π) to π* are of great diagnostic value in the ultraviolet-visible region. Ultraviolet-visible spectroscopy has been better applied in assaying than identification, especially in research. The amount of metals in certain sample substances can be determined by this method. This principle is also applied in drug metabolites, where samples are taken from various sites of the body and analyzed to determine the amount of metabolites at those sites. In reactions leading to the formation of products that can absorb within the UV-visible region, UV-visible spectroscopy can be used to study their rate of reaction [15].

The objective of this study is to employ UV-visible spectroscopic methods to develop an accurate, precise, reproducible and economic routine method for the estimation of ciprofloxacin intravenous infusions on the Ghanaian market.

2. MATERIALS AND METHODS

2.1 Purity and Identification Test

Crystalline ciprofloxacin was obtained and its purity checked. Thin layer chromatography (TLC) analysis was carried out using silica plates with a mixture of methanol, chloroform, ammonia and hexane in ratios: 35 %: 43 %: 17 %: 5 % v/v respectively as the eluent and developed with iodine vapour. Infra-red (IR) spectrum of the material was superimposable with the spectrum of ciprofloxacin in the Aldrich Chemical Catalogue. The melting point of the crystalline material was also obtained.

2.2 Stock Preparation

A stock solution of concentration 0.05 mg/ml was prepared by first dissolving 0.02 g of crystalline ciprofloxacin hydrochloride with 10 ml of distilled water in a 100 ml beaker. The resulting solution was then transferred quantitatively into a 100 ml volumetric flask, swirled for complete dissolution and topped to the mark with distilled water. A 25 ml aliquot of this solution was taken into another 100 ml volumetric flask, topped to the mark with distilled water, corked and labeled stock A. The procedure was repeated to obtain stock B.

2.3 Preparation of Standards

Standard solutions of concentrations ranging from 0.002 mg/ml to 0.012 mg/ml at intervals of 0.002 mg/ml were prepared. From each of the stock, 2 ml, 4 ml, 6 ml, 8 ml, 10 ml and 12 ml were taken into separate 50 ml volumetric flasks using a 10 ml graduated pipette and topped to the mark with distilled water. These were corked and labeled $\mathbf{A_1 - A_6}$ and $\mathbf{B_1 - B_6}$ to obtain two sets of standards from the respective stock.

2.4 Preparation of the Sample

Four brands of ciprofloxacin sodium chloride pharmaceutical infusions were obtained from various pharmacies in Accra. Sample solutions of concentration 0.004 mg/ml were prepared. A 20 ml aliquot of the drug sample was transferred into a 100 ml volumetric flask and topped to the mark with distilled water. A 1 ml aliquot of the resulting solution was taken into another 100 ml volumetric flask and distilled water added to the mark.

2.5 Blank Preparation

I. Sample blank

Sodium chloride solution with a concentration of 0.018 mg/ml was prepared as sample blank- by dissolving 0.4500 g of sodium chloride with 10 ml of distilled water in a 50 ml beaker. The resulting solution was transferred into a 50 ml volumetric flask and topped to the mark with distilled water. A 10 ml aliquot of the resulting solution was taken into a 50 ml volumetric flask and distilled water added to the mark. Finally, 0.5 ml of this solution was taken into another 50 ml volumetric flask and topped to the mark with distilled water. It was corked and labeled sample blank.

II. Standard blank

Distilled water was used as blank for the standards.

All solutions prepared were used within twelve hours after preparation.

2.6 Measurement of Absorbance

The HACH DR 3800 single beam ultraviolet spectrophotometer was used for the measurement of absorbance. The equipment was warmed for 5 minutes,

after which a wavelength scan was done to obtain the wavelength of maximum absorption. The wavelength of maximum absorption obtained was 326 nm. The spectrophotometer was then set to a single wavelength mode, which is 326 nm. The spectrophotometer was zeroed using distilled water. The cuvette was rinsed and filled with standard A_1 and the absorbance recorded. This was repeated for the remaining standards A_2 - A_6 and B_1 - B_6. The cuvette was rinsed with methanol followed by distilled water in-between measurements. The cuvette was then rinsed and filled with the first sample prepared from the ciprofloxacin sodium chloride pharmaceutical infusion and absorbance taken. The absorbance of all the samples including the sample blank (0.018 mg/ml sodium chloride) was measured.

2.7 Data Analysis

The data obtained for the standards were taken through mathematical calculations to obtain their respective concentrations. The concentrations together with their corresponding absorbance were taken through least square analysis (Skoog *et al*., 2004) to obtain the least square line for the calibration curve given in Fig. 3 and represented by Eq. (1). All associated errors of the samples were computed at a t = 0.05.

$$y = 74.55x + 0.019,\ R^2 = 0.9999; \quad (1)$$

Where, y is absorbance and x is concentration of ciprofloxacin in mg/ml.

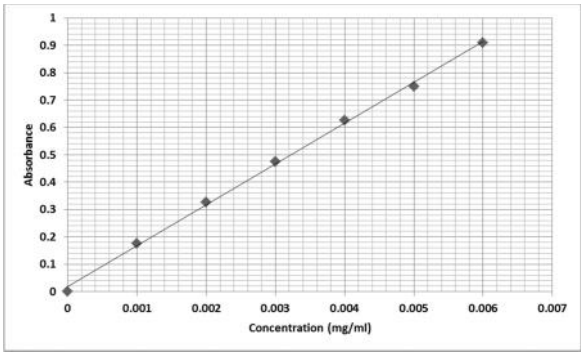

Fig 3: Calibration curve for ciprofloxacin

3. RESULTS

3.1 Purity and Identity Analysis

To use the crystalline ciprofloxacin material for the calibration curve it was necessary to determine its purity and identity. The observed melting point was 254 - 256 ˚C. The thin-layer chromatography (TLC) analysis showed a single spot with an R_f value of 0.48. The IR spectrum of the crystalline material was superimposable with the IR spectrum of the ciprofloxacin in the Aldrich Chemical Catalogue. Table 1 gives prominent peaks in ciprofloxacin, whereas Table 2 and Table 3 give the absorbance of standards prepared from crystalline ciprofloxacin material and ciprofloxacin sodium chloride pharmaceutical infusion samples respectively.

From the absorbance of the samples, their corresponding concentrations were interpolated from the calibration curve and subjected to mathematical and statistical calculations to obtain the concentration of the samples analyzed as well as the associated error. Table 4 below shows the concentrations of the samples with their corresponding expiry date and manufacturer's specified concentration, and experimental concentrations of the samples purchased from the market.

4. DISCUSSION

The crystalline ciprofloxacin hydrochloride obtained showed a single spot on TLC, with an R_f value of 0.48. The melting point was found to be 254 - 256 °C (literature value 255 – 257 °C). The IR spectrum was identical to that of ciprofloxacin in the Aldrich Chemical Catalogue. A calibration curve Eq. (1) (Fig. 1); with a corresponding regression coefficient R^2 = 0.999 was obtained with high linearity which conformed to the Beer-Lamberts' law and this also conformed to the specification of the United States pharmacopoeia. The sodium chloride used in this study had some level of absorption (0.001), which was factored in the calculation and it is necessary to consider such compounds in similar experiments.

Ciprofloxacin sodium chloride pharmaceutical infusions from four different manufacturing companies sold on the Ghanaian market were sampled. The samples were labeled A - D. The concentrations of all the samples analyzed were greater than that specified by the manufacturers. On validating the method with a standard solution of 0.2 mg/ml ciprofloxacin, an increase of 3.4 % was observed whilst the sample concentration gave an average increment of 22.5 %. This implies that the method can only account for 3.4 % of this increment and the remaining attributed to the content of the drug.

5. CONCLUSION

The proposed UV-visible spectrophotometric method is thus, simple, rapid, selective, precise, reproducible and highly sensitive for the analysis of ciprofloxacin. Therefore, it can be used routinely for the determination of ciprofloxacin in ciprofloxacin-based pharmaceutical formulations on the Ghanaian market.

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Table 1: Prominent Peaks in Ciprofloxacin

Functional groups	Peaks (cm^{-1})	Vibrations
Carboxylic acid group	3000 1500–1550 1400	O–H stretch O=C=O asymmetric stretch O=C=O symmetric stretch
Ketone group	1750	C=O stretch
Fluoro-aryl group	1250	F–Ar stretch
Amine group (secondary)	3400	N–H stretch

Table 2: Absorbance Of Standards Prepared From Crystalline Ciprofloxacin Material

Volume (ml)	Absorbance ± 0.001						Average Absorbance
	Standard A			Standard B			
	1	2	3	1	2	3	
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.172	0.182	0.170	0.180	0.183	0.173	0.177 ± 0.006
4	0.322	0.332	0.318	0.332	0.335	0.321	0.327 ± 0.007
6	0.475	0.478	0.472	0.486	0.474	0.47	0.476 ± 0.006
8	0.632	0.628	0.622	0.632	0.621	0.622	0.626 ± 0.005
10	0.749	0.725	0.772	0.742	0.787	0.732	0.75 ± 0.02
12	0.927	0.917	0.913	0.910	0.895	0.911	0.91 ± 0.01

Table 3: Absorbance Of Ciprofloxacin Sodium Chloride Pharmaceutical Infusion Samples

	Absorbance ± 0.001			**Average absorbance**	**Standard deviation**
Sample A	0.343	0.343	0.344	0.3433	0.0006
Sample B	0.375	0.378	0.378	0.377	0.002
Sample C	0.424	0.425	0.424	0.4243	0.0006
Sample D	0.395	0.398	0.396	0.396	0.002
Blank	0.001	0.001	0.001	0.001	0.000
Corrected Absorbance					
Sample A				0.3423 ± 0.0006	
Sample B				0.376 ± 0.002	
Sample C				0.4233 ± 0.0006	
Sample D				0.395 ± 0.002	

Table 4: Manufacturer's Specified Concentration And Experimental Concentration Data

Sample	**Manufacturing date**	**Expiry date**	**Manufacturer's Concentration (mg/100 ml)**	**Experimental Concentration (mg/100 ml)**
A	May 2010	June 2013	200	216.8227
B	April 2011	May 2014	200	239.4238
C	March 2009	April 2012	200	271.1457
D	Sept. 2011	Oct. 2014	200	252.1662