Development of a simple UV spectrophotometric method for analyzing Ciprofloxacin HCl

A dissertation submitted to the Department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy.

Submitted by

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Declaration by the Candidate

I, **Romen Royhan**, hereby declare that the dissertation entitled ***"Development of a simple UV spectrophotometric method for analyzing Ciprofloxacin HCl"*** submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by me during the period 2017 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of **Dr. Tasnuva Haque**, Assistant Professor, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

This is to certify that the thesis entitled ***"Development of a simple UV spectrophotometric method for analyzing Ciprofloxacin HCl"*** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Romen Royhan, ID: 2014-1-70-056, during the period 2017 of his research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Dedication

This research paper is dedicated to my beloved Parents and my family members

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List of Abbreviations

Serial number	Abbreviations	Elaboration
1	Conc.	Concentration
2	Abs.	Absorbance
3	Morn.	Morning
4	Eve.	Evening
5	Obt.	Obtain
6	Bet.	Between

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 Harmonisation (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 0.1 µg/mL and 2 µg/mL, respectively. The developed method was applied successfully for the analysis of ciprofloxacin HCl with good accuracy and precision.

Chapter One
Introduction

1. 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 (Olaniyi et al., 2001).

Drug products that are chemically and biopharmaceutically 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 inequivalences have been reported from the use of some generic brands of drug products such as tolbutamide (Adegbolagun et al., 2002).

There are several brands of Ciprofloxacin hydrochloride tablets available within the drug delivery system globally as well as in Bangladesh.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 Bangladesh.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 (Adegbolagun et al., 2007).

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 (Nijhu et al., 2011). 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.

1.1 UV spectroscopy

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Absorption of the ultra-violet radiations results in the excitation of the electrons from the ground state to higher energy state. The energy of the ultra-violet radiation that are absorbed is equal to the energy difference between the ground state and higher energy states. Generally, the most favoured transition is from the highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO). For most of the molecules, the lowest energy occupied molecular orbitals are s orbital, which correspond to sigma bonds. The p

orbitals are at somewhat higher energy levels, the orbitals (nonbonding orbitals) with unshared paired of electrons lie at higher energy levels. The unoccupied or antibonding orbitals (π^* and σ^*) are the highest energy occupied orbitals. In all the compounds (other than alkanes), the electrons undergo various transitions. Some of the important transitions with increasing energies are: nonbonding to π^*, nonbonding to σ^*, π to π^*, σ to π^* and σ to σ^* (indiastudychannel.com, 2017).

1.2 Principle of UV spectroscopy

UV spectroscopy obeys the Beer-Lambert law, which states that: when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.
The expression of Beer-Lambert law is-

$$\mathbf{A = \log (I_0/I) = Ecl}$$

Where, A = absorbance
I_0 = intensity of light incident upon sample cell
I = intensity of light leaving sample cell
C = molar concentration of solute
L = length of sample cell (cm.)
E = molar absorptivity.

From the Beer-Lambert law it is clear that greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption. This is the basic principle of UV spectroscopy (indiastudychannel.com, 2017).

1.3 Instrumentation of UV spectroscopy

Instrumentation and working of the UV spectrometers can be studied simultaneously. Most of the modern UV spectrometers consist of the following parts-

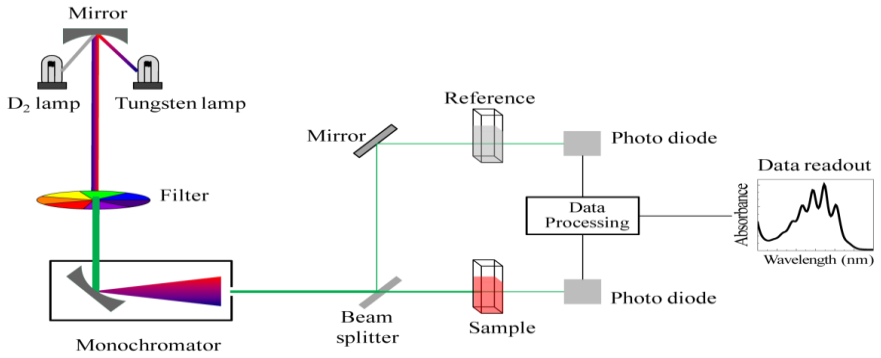

Figure 1.1: Instrumentation of UV Spectroscopy (Chem.libretexts.org, 2017).

Light Source: Tungsten filament lamps and Hydrogen-Deuterium lamps are most widely used and suitable light source as they cover the whole UV region. Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen-Deuterium lamps falls below 375 nm.

Monochromator: Monochromators generally composed of prisms and slits. The most of the spectrophotometers are double beam spectrophotometers. The radiation emitted from the primary source is dispersed with the help of rotating prisms. The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wavelength to pass through the slits for recording purpose. The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

Sample and reference cells: One of the two divided beams is passed through the sample solution and second beam is passé through the reference solution. Both sample and reference solution are contained in the cells. These cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UV region.

Detector: Generally two photocells serve the purpose of detector in UV spectroscopy. One of the photocell receives the beam from sample cell and second detector receives the beam from the reference. The intensity of the radiation from the reference cell is stronger than the beam of sample cell. This results in the generation of pulsating or alternating currents in the photocells.

Amplifier: The alternating current generated in the photocells is transferred to the amplifier. The amplifier is coupled to a small servometer. Generally current generated in the photocells is of very low intensity, the main purpose of amplifier is to amplify the signals many times so we can get clear and recordable signals.

Recording device: Most of the time amplifier is coupled to a pen recorder which is connected to the computer. Computer stores all the data generated and produces the spectrum of the desired compound (indiastudychannel.com, 2017).

1.4 Concept of Chromophore and Auxochrome in the UV spectroscopy

1.4.1 Chromophore: Chromophore is defined as any isolated covalently bonded group that shows a characteristic absorption in the ultraviolet or visible region (200-800 nm). Chromophores can be divided into two groups- a) Chromophores which contain p electrons and which undergo π to π^* transitions. Ethylenes and acetylenes are the example of such chromophores. b) Chromophores which contain both p and nonbonding electrons. They undergo two types of transitions; π to π^* and nonbonding to π^*. Carbonyl, nitriles, azo compounds, nitro compounds etc. are the example of such chromophores.

1.4.2 Auxochromes: An auxochrome can be defined as any group which does not itself act as a chromophore but whose presence brings about a shift of the absorption band towards the longer wavelength of the spectrum. –OH,-OR,-NH_2,-NHR, -SH etc. are the examples of auxochromic groups (indiastudychannel.com, 2017).

1.5 Absorption and intensity shifts in the UV spectroscopy

There are four types of shifts observed in the UV spectroscopy-

a) **Bathochromic effect:** This type of shift is also known as red shift. Bathochromic shift is an effect by virtue of which the absorption maximum is shifted towards the longer wavelength due to the presence of an auxochrome or change in solvents. The nonbonding to π^* transition of carbonyl compounds observes bathochromic or red shift.

b) **Hypsochromic shift:** This effect is also known as blue shift. Hypsochromic shift is an effect by virtue of which absorption maximum is shifted towards the shorter wavelength. Generally it is caused due to the removal of conjugation or by changing the polarity of the solvents.

c) **Hyperchromic effect:** Hyperchromic shift is an effect by virtue of which absorption maximum increases. The introduction of an auxochrome in the compound generally results in the hyperchromic effect.

d) **Hypochromic effect:** Hyperchromic effect is defined as the effect by virtue of intensity of absorption maximum decreases. Hyperchromic effect occurs due to the distortion of the geometry of the molecule with an introduction of new group (indiastudychannel.com, 2017).

1.6 Applications of UV spectroscopy

Detection of functional groups: UV spectroscopy is used to detect the presence or absence of chromophore in the compound. This is technique is not useful for the detection of chromophore in complex compounds. The absence of a band at a particular band can be seen as an evidence for the absence of a particular group. If the spectrum of a compound comes out to be transparent above 200 nm than it confirms the absence of – a) Conjugation b) A carbonyl group c) Benzene or aromatic compound d) Bromo or iodo atoms.

Detection of extent of conjugation: The extent of conjugation in the polyenes can be detected with the help of UV spectroscopy. With the increase in double bonds the absorption shifts towards the longer wavelength. If the double bond is increased by 8 in the polyenes then that polyene appears visible to the human eye as the absorption comes in the visible region.

Identification of an unknown compound: An unknown compound can be identified with the help of UV spectroscopy. The spectrum of unknown compound is compared with the spectrum of a reference compound and if both the spectrums coincide then it confirms the identification of the unknown substance.

Determination of configurations of geometrical isomers: It is observed that cis-alkenes absorb at different wavelength than the trans-alkenes. The two isomers can be distinguished with each other when one of the isomers has non-coplanar structure due to steric hindrances. The cis-isomer suffers distortion and absorbs at lower wavelength as compared to trans-isomer.

Determination of the purity of a substance: Purity of a substance can also be determined with the help of UV spectroscopy. The absorption of the sample solution is compared with the absorption of the reference solution. The intensity of the absorption can be used for the relative calculation of the purity of the sample substance (indiastudychannel.com, 2017).

1.7 Method development parameter

Lamda max (λ_{max}) : Lambda max refers to the wavelength in the absorption spectrum where the absorbance is maximum. Generally molecules absorb in a wavelength range cantered around the lambda max. It acts as a single quantitative parameter to compare the absorption range of different molecules. It is like finger print as it differs with the different compound. The maximum wavelength (λ_{max}) is selected by scanning the compound between 200-400 nm regions (quora.com, 2017).

1.8 Ciprofloxacin

Ciprofloxacin is a second generation fluoroquinolone antibiotic that is widely used in the therapy of mild-to-moderate urinary and respiratory tract infections caused by susceptible organisms. Ciprofloxacin has been linked to rare but convincing instances of liver injury that can be severe and even fatal (HJ, 1999).

1.8.1 Background

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 Neisseria 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 (Slama TG, 1990). 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 (Wolfson JS, et al., 1991).

1.8.2 Pharmacodynamics

Ciprofloxacin is a broad-spectrum anti-infective agent of the fluoroquinolone class. Ciprofloxacin has in vitro activity against a wide range of gram-negative and gram-positive microorganisms. The mechanism of action of quinolones, including Ciprofloxacin, is different from that of other antimicrobial agents such as beta-lactams, macrolides, tetracyclines, or aminoglycosides; therefore, organisms resistant to these drugs may be susceptible to Ciprofloxacin. There is no known cross-resistance between Ciprofloxacin and other classes of antimicrobials. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian (Drusano, G.L, et al., 1986).

Ciprofloxacin is a synthetic broad spectrum fluoroquinolone antibiotic. Ciprofloxacin binds to and inhibits bacterial DNA gyrase, an enzyme essential for DNA replication. This agent is more active against Gram-negative bacteria than Gram-positive bacteria (Ciprofloxacin properties, 2017).

1.8.3 Physicochemical parameter

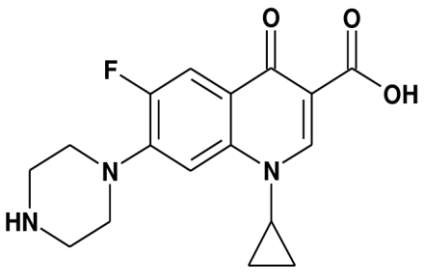

Figure 1.2: Ciprofloxacin

(Diab N, et al., 2014)

IUPAC Name:

1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid.

Molecular Formula: $C_{17}H_{18}FN_3O_3$

Molecular Weight: 331.34

Physical state: Solid

Color: Faint to light yellow crystalline powder (Medical Economics Co, p. 852; 2001).

Melting Point: 255-257 °C (Merck and Co., Inc., 2006).

Water Solubility: In water, 30,000 mg/L at 20° C

Soluble in dilute (0.1N) hydrochloric acid ; practically insoluble in ethanol (Nowara A. et al., 1997).

Stability:

Ciprofloxacin hydrochloride tablets should be stored in tight containers at a temperature <30°C.The drug should be protected from intense UV light. Ciprofloxacin microcapsules for oral suspension and the diluent provided by the manufacturer should be stored at <25°C and protected from freezing (McEvoy, G.K, 2001).

Following mixture with the dilutent, ciprofloxacin oral suspension should be stored at <30°C and protected from freezing, and is stable for 14 days when stored at room temperature or in a refrigerator (American Society of Health-System Pharmacists, 2001).

1.8.4 Mechanism of action

Ciprofloxacin like other fluoroquinolones inhibits the enzyme bacterial DNA gyrase that produces cuts in the double-stranded DNA, leading to negative supercoiling and then re-ligation of the cut ends. This helps in averting positive supercoiling which may occur in excess. The DNA gyrase consists of two A and two B subunits: The A subunit brings about cutting of DNA, the B subunit causes negative supercoiling and then the A subunit causes resealing. Ciprofloxacin binds to A subunit with great affinity and restricts the nicking and resealing action. In gram-positive bacteria the major target of action is an analogous enzyme topoisomerase IV which nicks and separates daughter DNA strands once the DNA replication is complete. Ciprofloxacin is more potent against gram positive bacteria due to higher affinity for topoisomerase IV. The damaged DNA leads to formation of exonucleases resulting in digestion of the DNA and this possibly contributes to the bactericidal action of Ciprofloxacin. The mammalian cells possess an enzyme topoisomerase II instead of DNA gyrase or topoisomerase IV that has very low affinity for Ciprofloxacin - thus the low toxicity to host cells.

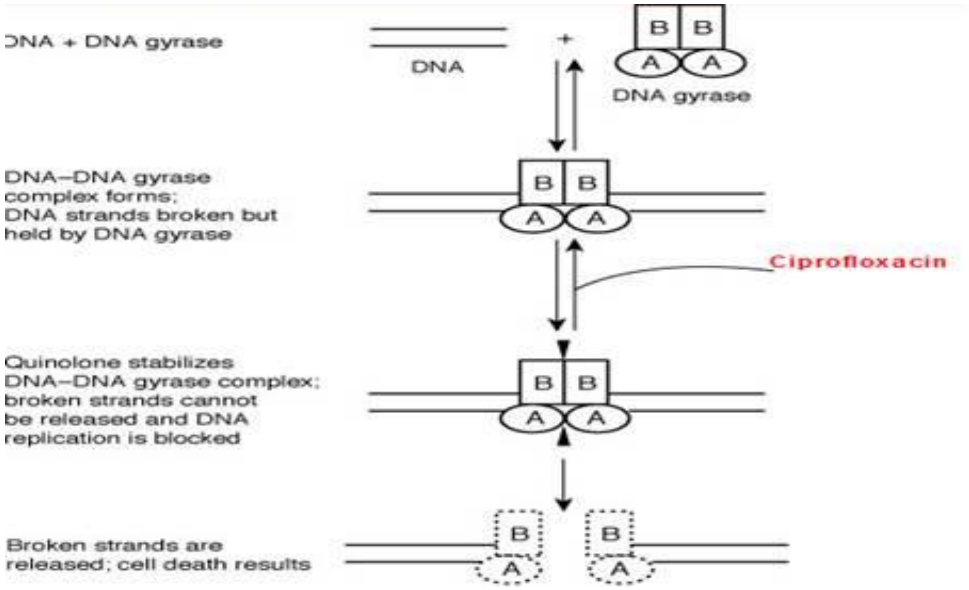

Figure 1.3 Mechanism of action of Ciprofloxacin

(Antibiotic Drugs, 2016)

1.8.5 Microbiology

Ciprofloxacin is active against-

Gram-positive bacteria

- Staphylococcus aureus
- Enterococcus faecalis
- Staphylococcus epidermidis
- Saprophyticus
- Pneumococci
- Streptococcus pyogenes

Gram-negative bacteria

- Proteus (vulgaris and mirabilis)
- Campylobacter
- Citrobacter freundii
- Providencia (stuartii and rettgeri)
- E. coli, Klebsiella pneumoniae
- Haemophilus influenzae and parainfluenzae
- P. aeruginosa
- Salmonella typhi
- Neisseria gonorrhoeae
- Serratia marcescens
- Shigella species

- Moraxella catarrhalis
- Morganella morganii

The prominent microorganism which are resistant are: Bacteroides fragilis, Clostridia, anaerobic cocci.

The noteworthy microbiological features of Ciprofloxacin are:

- Ciprofloxacin has highly potent action with swift bactericidal action.
- Plasmid resistant mutants are not easily selected.
- Anaerobes and streptococci present in the intestine which are useful are not affected.
- It is effective against the bacteria which are not sensitive to the aminoglycosides and beta lactam group of antibiotics.
- The bactericidal action is very less at decreased pH (Anti Biotic Drugs, 2016).

1.8.6 Pharmacokinetics

After oral intake, the fluroquinolones have good oral absorption. Food delays absorption. It is extensive tissue distribution. The plasma t1/2 is about 3 to 5 hours. Plasma protein binding is 20-35%. It is excreted primarily in urine, both by glomerular filtration and tubular secretion. Urinary and biliary concentrations are10-50 folds higher than plasma. Plasma concentrations of orally given dose match with those of an IV given dose (Antibiotic Drugs, 2016).

1.8.7 Drug Interactions

- Plasma concentration of theophylline, caffeine and warfarin are increased by Ciprofloxacin due to inhibition of metabolism: toxicity of these drugs can occur.
- NSAIDs may augment the CNS toxicity of Ciprofloxacin; seizures are reported.
- Antacids, sucralfate and iron salts given concomitantly decrease absorption of fluroquinolones.
- Concomitant administration of omeprazole reduces plasma concentration of Ciprofloxacin.
- Both reduced and enhanced serum levels of phenytoin may be seen in persons taking simultaneous Ciprofloxacin.
- Concurrent prescription of Ciprofloxacin with glyburide has, rarely can lead to severe hypoglycaemia.
- The excretion of Ciprofloxacin through the kidney is affected negatively by probenecid leading to enhanced serum levels of Ciprofloxacin.
- The oral absorption of Ciprofloxacin is enhanced by metoclopramide.
- Ciprofloxacin increases the serum levels of ropinirole, lidocaine, sildenafil and clozapine.
- Excretion of methotrexate by the kidney is inhibited by Ciprofloxacin.

Precaution should be taken when using Ciprofloxacin concomitantly with class IA or III antiarrhythmics as Ciprofloxacin may have an additive effect on the QT interval (Antibiotic Drugs, 2016).

1.8.8 Special consideration

Renal Impairment

Alteration of the dosage regimen is necessary for patients with impairment of renal function.

Elderly population

Ciprofloxacin can lead to enhanced risk of developing severe tendon disorders including tendon rupture which is enhanced in persons taking corticosteroids.

Children

Ciprofloxacin, like other quinolones, causes arthropathy and histological changes in large joints which bear the weight of juvenile animals resulting in lameness.

Pregnancy

It is a Category C drug. There is not much data available about the effect of Ciprofloxacin in pregnancy.

Lactation

Ciprofloxacin is secreted during lactation (Antibiotic Drugs, 2016).

1.8.9 Contraindications

Ciprofloxacin is contraindicated in persons with a history of hypersensitivity to Ciprofloxacin or any member of the quinolone class of antimicrobial agents.

Concomitant administration with tizanidine is contraindicated (Antibiotic Drugs, 2016).

1.8.10 Adverse effects

The commonly observed adverse effects are:

- GIT: Vomiting, altered taste , decreased appetite
- CNS: Giddiness, headache, anxious behaviour, restlessness, decreased sleep, impairment of concentration, tremor. Seizures are rare; occur only at high doses or when predisposing factors are present.

Other rare reactions are:

- Hypersensitive: skin rashes, increased itching ,photosensitivity
- Tendonitis and tendon rupture
- Vasculitis, joint pain , muscle pain
- Allergic pneumonitis;
- Acute kidney insufficiency or failure;
- Hepatitis; jaundice; acute necrosis of liver or failure;
- Anemia, including haemolytic and aplastic; decreased platelet count, thrombotic thrombocytopenic purpura; decreased WBC count, agranulocytosis; pancytopenia (Antibiotic Drugs, 2016).

1.9 Literature Review

In Bangladesh, there are different Ciprofloxacin hydrochloride drugs available and they are marketed as different brands. From available brands one brand that is "Ciprocin®" was chosen for analyzing. This is why a "literature review" was performed to evaluate the previous UV method development related works that were done on the Ciprofloxacin hydrochloride. It was observed that the studies done on the Ciprofloxacin hydrochloride were not similar to this research project. But those studies helped to find the information's that help in the research work and also helped to compare this research work with other research projects. Jeast of some studies are given below:

In 2014, Safila Naveed et al., conducted a research on "Simple UV-Spectrophotometric Assay of Ciprofloxacin". Their objective was to develop a rapid, simple, accurate, and economical least time consuming spectrophotometric method for assaying Ciprofloxacin and then compare it with the assay of different brands available in Pakistan. The assay was determined by measuring the absorbance of stock solution against the solvent blank and comparing with the absorbance of available brands of Ciprofloxacin at the wavelength of 278 nm by spectrophotometer (Naveed et al., 2014).

For developing a simple, fast and reliable spectrophotometric method in 2015, Rekha k. et al., conducted a research on ''Estimation of Ciprofloxacin Hydrochloride in Bulk and Formulation by Derivative UV-Spectrophotometric Methods''. The quantitative determination of the drug was carried out using the zero, first, and second order method values measured at 264, 273 and 273 nm respectively. . Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Ciprofloxacin Hydrochloride using 2-10 µg/ml (r^2=0.9991, r^2=0.9993, r^2=0.9955) for zero, first and second order spectrophotometric method. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations (Rekha k. et al., 2015).

In 2012, Rakesh s.k et al., experimented a research on "A Simple UV Spectrophotometric Method Development and Validation for Estimation of

Ciprofloxacin Hydrochloride in Bulk and Tablet Dosage Form". In their experiment they the wave length they used was 274 nm and the range was 2-10 ppm. The proposed method has been applied successfully for the analysis of Ciprofloxacin hydrochloride either in bulk or pharmaceutical tablet dosage form with good accuracy and precision (Rakesh S.K et al., 2012)

Krishna J.R et al., in 2014 conducted an experiment on "Development and Validation of UV Spectrophotometric method for the Simultaneous estimation of Ciprofloxacin Hydrochloride and Ornidazole in Combined Pharmaceutical Dosage Form". The aim of their study was to develop a simple, precise, accurate and economical UV Spectrophotometric method for the simultaneous estimation of Ciprofloxacin and ornidazole in combined pharmaceutical dosage form using simultaneous equation method. In their study the wave length they selected was 270 nm and 319 nm for Ciprofloxacin and ornidazole respectively .The concentration range was 2-12 µg/ml for Ciprofloxacin and 6-16 µg/ml for Ornidazole.The correlation coefficient of Ciprofloxacin HCl and Ornidazole was found to be 0.999 and 0.997 respectively. The method was statistically validated as per the ICH guidelines (Krishna J.R et al., 2014).

For developing a simple, precise and accurate difference spectroscopic method a research had been conducted on ''Difference Spectroscopic Method for the Estimation of Ciprofloxacin Hydrochloride in Bulk and in its Formulation'' by Rekha k. et al., in 2015. According to their study Ciprofloxacin hydrochloride exhibited maximum absorbance at about 272nm and 278nm in acidic and basic solution respectively. Beer's law was obeyed in the concentration range of 2-10 µg/ml in both the cases. The regression of coefficient was found to be r^2=0.9982. The LOD and LOQ value were found to be 0.5140 ppm and 0.5577 ppm, respectively. As per ICH guidelines the result of the analysis were validated statistically and were found to be satisfactory (Rekha k. et al., 2015).

Chapter Two
Materials and Method

2.1 Materials

2.1.1 Sample Collection

For the purpose of analysing Ciprofloxacin HCl, reference standard was collected from the Eskayef Bangladesh 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 Banasree, Dhaka, Bangladesh (Table 2.1).

Table 2.1: Materials used for the experiment

Sample name	Source(Supplier Name)
Ciprofloxacin HCl (Raw)	Eskayef Bangladesh Limited
Ciprocin® 250mg	Square Pharmaceutical Limited
Ciprocin® 500mg	Square Pharmaceutical Limited

2.1.2 Sample

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

Table 2.2: Solvents used for developing UV method for Ciprofloxacin HCl

Reagents Name	Source (Supplier Name)
Methanol (HPLC grade)	Active Fine Chemicals
Distilled Water/De-ionized Water	Total Water Purification

2.1.3 Equipments& Instruments

The equipments and instruments used for the study were included in Table 2.3.

Table 2.3: Lists of equipments used for the experiment

Equipment	Source (supplier name)	Origin
UV Spectrophotometer	Shimadzu UV1800	Japan
Electronic Balance	Shimadzu ATX 224	Japan
Sonicator	HWASHIN Power sonic 520	Korea

2.2 Methods

In the following diagram the complete UV method development and validation process has been mentioned

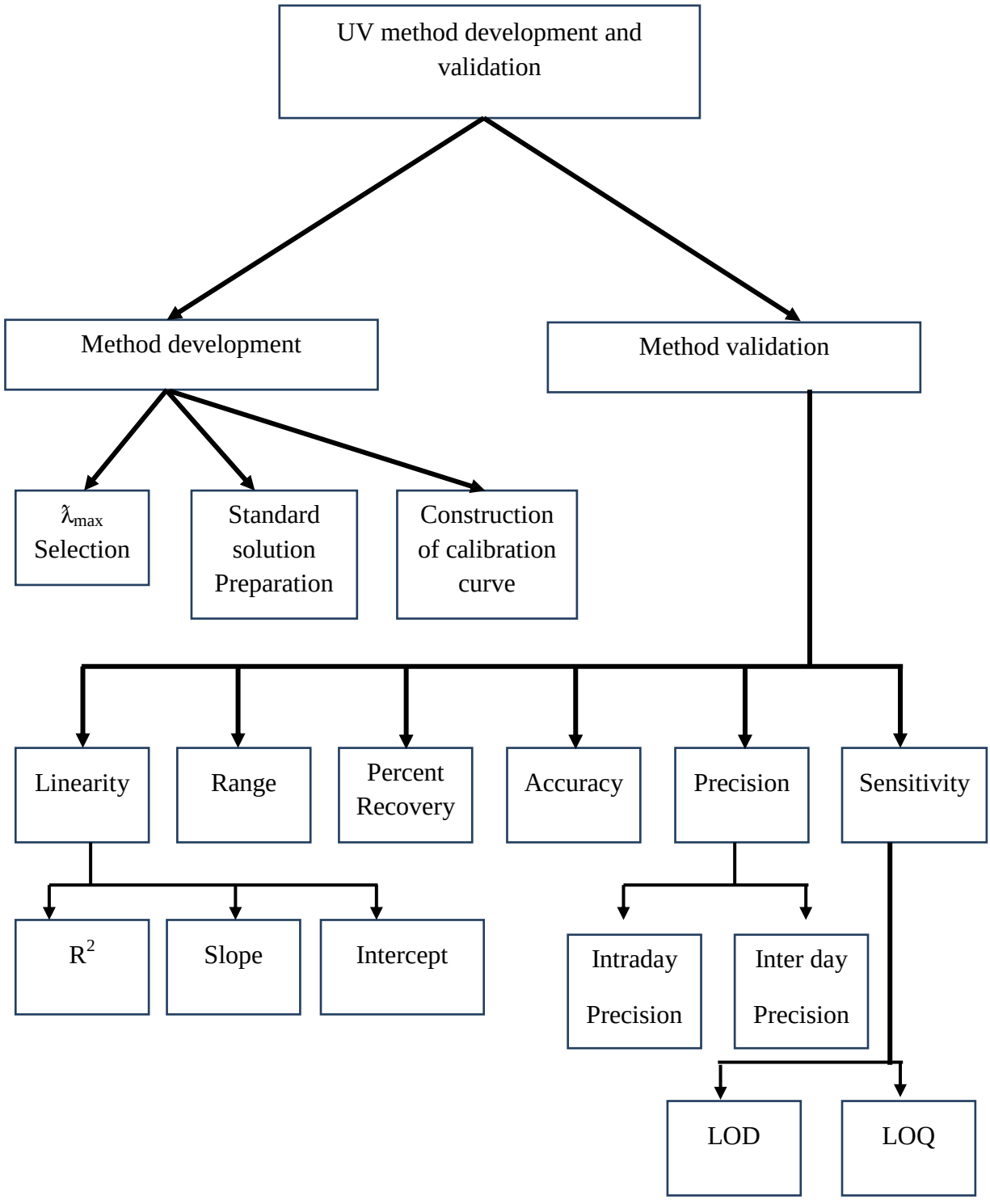

Figure 2.1: Schematic diagram of UV method development and validation methods

2.2.1 Wavelength Selection

Ciprofloxacin 100 µg/ml stock solution was accurately prepared by dissolving 5 mg of Ciprofloxacin HCl 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 (λ_{max}) was observed at 272 nm with the absorbance value of 0.658.Therefore, 272 nm was selected as the λ_{max} for Ciprofloxacin HCl .However, in different papers the λ_{max} of Ciprofloxacin HCl were found to be slightly different (Nijhu et al., 2011).

2.2.2 Preparation of standard solution of Ciprofloxacin in water

Ciprofloxacin HCl is freely soluble in water (Solubility is 10-30 mg/ml) at acidic pH (<5.0) (Varanda,F. et al., 2006). 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 µ/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 HCl

2.2.4.1 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" (USP 34, 2011).

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.

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 a

suitable level of precision, accuracy, and linearity using the procedure as written. The range is normally expressed in the same units as test results (e.g., percent, parts per million) obtained by the analytical procedure" (USP 34, 2011).
The concentration range between which Ciprofloxacin HCl obeyed Beer-Lambert's law was taken as the range.

2.2.4.3 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 (USP 34, 2011).

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. The accuracy was calculated using equation 1.

$$\text{Accuracy (\%)} = \frac{Observed\ conc. - Theoritical\ conc.}{Theoritical\ conc.} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq(1) (USP 34, 2011).}$$

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 calculated using equation 2.

$$\text{\% Recovery} = \frac{\text{Observed concentration} \times 100}{\text{Original concentration}} \ldots\ldots\ldots\ldots\ldots\ldots \text{Eq (2) (Iqbal, 2017).}$$

2.2.4.5 Precision

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical procedure is usually expressed as 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 this

context, 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 (USP 34, 2011).

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" (USP 34, 2011).
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.

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" (USP 34, 2011).
In order to find out LOQ, concentrations of Ciprofloxacin HCl distilled water from 0.1 to 2 µg/mL was prepared and absorbance values were recorded. The concentration which showed the minimum absorbance value with acceptable recovery and preceision values was selected as the LOQ of this UV method.

2.2.4.7 Assay of Ciprofloxacin HCl

Three tablets of two strengths (250 and 500 mg) of Ciprofloxacin 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. The accuracy value was again calculated using equation 1.

2.2.5 Statistical analysis

All the data were analysed using MS Excel 2007.Most of the cases the results were represented as mean±standard deviation (SD). In some cases relative standard deviation (RSD) was calculated using equation 3.

Relative standard deviation (RSD %) = $\frac{\text{SD}}{\text{Mean}} \times 100$ ... ... Eq(3) (Pearson, 1896).

Chapter Three
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 µ/ml concentration). The absorbance values of these solutions were noted at 272 nm wavelength (λ_{max} for Ciprofloxacin HCl). Data was collected and plotted (Table 3.1) 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 3.1: Absorbance data of standard solutions of Ciprofloxacin at 272 nm

Conc. (µg/mL)	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.

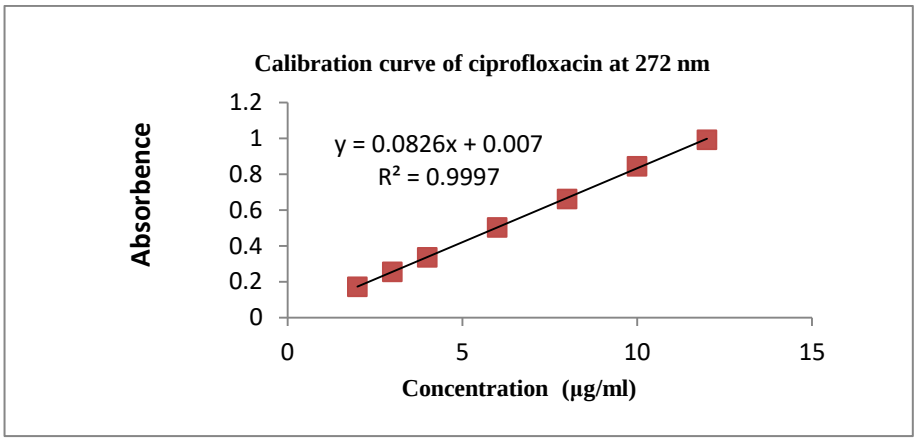

Figure 3.1: Calibration curve of Ciprofloxacin in distilled water at 272 nm

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 3.2: 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	0.999888	0.998574	0.999932	0.999465	0.0008	0.08
Slope (m)	0.083855	0.080843	0.08298	0.082559	0.0015	1.88
Intercept	0.000647	0.012724	0.007703	0.007024	0.0061	

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 3.3.The table s hows 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. 3.3: Accuracy data of Ciprofloxacin HCl

Absorbance	Dilution factor	Observed conc. (µg/mL)	Original conc. (µg/mL)	% Recovery	Mean	SD
0.898	10	107.87	100.00	107.87	103.36	7.93
0.935	10	112.35	100.00	112.35		
0.885	10	106.30	100.00	106.30		
0.778	10	93.34	100.00	93.34		
0.137	1	1.57	1.62	96.93		

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 label claim (standard addition method) was added to pre-analyzed sample of tablet. The acceptable value of recovery is 80-110%. From the recovery data table 3.4 we can see that the mean recovery was 100.69%. Therefore, the value was in recommended range.

Table 3.4: 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 HCl 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 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 3.5 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 3.5.

Table 3.5: 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. conc. in the eve.)	Mean conc. (bet. morn. & eve.)	SD of conc. (bet. morn. & eve.)	RSD	Avg. RSD
	0.188	0.19	2.21	2.40						
	0.186	0.187	2.18	2.37						
2	0.19	0.191	2.23	2.41	2.21	2.39	2.30	0.13	5.75	
	0.51	0.51	6.13	6.30						
	0.51	0.514	6.13	6.35						3.13
6	0.509	0.51	6.12	6.30	6.13	6.32	6.23	0.14	2.17	
	1.025	1.031	12.41	12.66						
	1.016	1.023	12.30	12.56						
12	1.01	1.019	12.23	12.51	12.32	12.58	12.45	0.18	1.48	

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 3.6. 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 3.6.

Table 3.6: Inter day (1 day) precision data of Ciprofloxacin HCl

Conc. (µg/mL)	Abs. (Day 0)	Abs. (Day 2)	Conc. (Day 0)	Conc. (Day 2)	Avg. conc. in Day 0	Avg. conc. in Day 2	Mean conc. (bet. Day 0 & Day 2)	SD of conc. (bet. Day 0 & Day 2)	RSD	Avg.RSD
	0.248	0.247	2.94	3.10						
	0.313	0.317	3.73	3.95						
2	0.38	0.385	4.55	4.78	3.74	3.94	3.84	0.14	3.74	
	0.566	0.574	6.82	7.09						
	0.538	0.553	6.48	6.83						2.75
6	0.518	0.533	6.23	6.59	6.51	6.83	6.67	0.23	3.45	
	0.912	0.912	11.04	11.21						
	0.927	0.927	11.22	11.39						
12	0.934	0.934	11.30	11.48	11.19	11.36	11.27	0.12	1.07	

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 and12 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. The RSD value was given in the table 3.6.

Table 3.7: Inter day (4 days) precision data of Ciprofloxacin HCl

Conc.(µg/mL)	Abs. (Day 0)	Abs. (Day 2)	Conc. (Day 0)	Conc. (Day 2)	Avg. conc. in Day 0	Avg. conc. in Day 2	Mean conc. (bet. Day 0 & Day 2)	SD of conc. (bet. Day 0 & Day 2)	RSD	Avg. RSD
	0.248	0.249	2.94	3.12						
	0.313	0.382	3.73	4.74						
2	0.38	0.321	4.55	4.00	3.74	3.96	3.85	0.15	3.96	
	0.566	0.536	6.82	6.62						
	0.538	0.581	6.48	7.17						4.73
6	0.518	0.551	6.23	6.80	6.51	6.87	6.69	0.25	3.78	
	0.912	0.841	11.04	10.34						
	0.927	0.829	11.22	10.20						
12	0.934	0.821	11.30	10.10	11.19	10.21	10.70	0.69	6.45	

3.2.6 Sensitivity of the method

The LOD of Ciprofloxacin was found to be 0.1 µg/ml and LOQ was found to be 2 µg/. The results are given in Table 3.8.

Table 3.8: LOD and LOQ data of Ciprofloxacin HCl

Conc. (µg/mL)	Abs.	Conc.(µg/mL)	%Recovery	Intraday precision	Inter day precision (1 day)	Inter day precision (4 day)
0.25	0.018	0.13	53.66			
0.1	0.007	0	0			
0.5	0.029	0.27	53.66			
1	0.058	0.62	62.20			
2	0.167	1.95	97.56	5.75	3.75	3.96

LOD = 0.1 µg/mL; LOQ = 2 µg/mL

3.2.7 Assay testing of 250 and 500 mg of Ciprofloxacin HCl tablet

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

Table 3.9: %Recovery of 250 mg of Ciprofloxacin HCl tablet

Dilution factor	Absorbance	Concentration (µg/mL)	Known concentration (µg/mL)	% Recovery
50	0.467	278.45	319.69	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 3.10: %Recovery of 500 mg of of Ciprofloxacin HCl tablet

Dilution factor	Absorbance	Concentration (µg/mL)	Known concentration (µg/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.

3.2.8 Summary of the method validation parameters

The total method validation parameter data is summarized in the following table 3.11.

Table 3.11: Summary of UV method validation parameter of Ciprofloxacin HCl

Parameter	Unit		
	µg/mL	**mean±SD**	**RSD (%)**
Range	2 to 12		
Linearity (n=3)			
R^2		0.9995±0.0008	0.08
Slope		0.083±0.0015	1.88
Intercept		0.007±0.0061	
Recovery (n=7)		100.68±0.73	0.73
Accuracy (n=5)		103.36±7.93	
Precision (n=9)			
Intraday			3.13
Interday (1 days variation)			2.75
Interday (4 days variation)			4.73
Sensitivity			
LOD(µg/mL)	0.1		
LOQ (µg/mL)	2		
Assay			
250 mg Tablet (n=1)		87.1	
500 mg Tablet (n=1)		88.55	

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.

3.2.10 Conclusion

The statistical analyses showed that the data from the proposed methods are in good agreement for the estimation of Ciprofloxacin hydrochloride in bulk drug. 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.

Chapter Four
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