1. Introduction

1.1. Drugs:

A **drug** is any substance other than food, that when inhaled, injected, smoked, consumed, absorbed via a patch on the skin or dissolved under the tongue causes a physiological change in the body.[1][2]

In pharmacology, a pharmaceutical drug, also called a medication or medicine, is a chemical substance used to treat, cure, prevent, or diagnose a disease or to promote well-being. Traditionally drugs were obtained through extraction from medicinal plants, but more recently also by organic synthesis. Pharmaceutical drugs may be used for a limited duration, or on a regular basis for chronic disorders.[3][4]

Pharmaceutical drugs are often classified into drug classes—groups of related drugs that have similar chemical structures, the same mechanism of action (binding to the same biological target), a related mode of action, and that are used to treat the same disease.[5][6]

The Anatomical Therapeutic Chemical Classification System (ATC), the most widely used drug classification system, assigns drugs a unique ATC code, which is an alphanumeric code that assigns it to specific drug classes within the ATC system. Another major classification system is the Bio pharmaceutics Classification System. This classifies drugs according to their solubility and permeability or absorption properties.[7]

Psychoactive drugs are chemical substances that affect the function of the central nervous system.

Altering perception, mood or consciousness. They include alcohol, a depressant, and the stimulants nicotine and caffeine. These three are the most widely consumed psychoactive drugs worldwide and are also considered as recreational drugs since they are used for pleasure rather than medicinal purposes. Other recreational drugs include hallucinogens, opiates and amphetamines and some of these are also used in spiritual or religious settings. Some drugs can cause addiction and all drugs can have side effects. Excessive use of stimulants can promote stimulant psychosis. Many recreational drugs are illicit and international treaties such as the Drug sexist for the purpose of their prohibition. (8)(9)(10)(11)(12)

Pharmaceutical or a drug is classified on the basis of their origin.

1. Drug from natural origin: Herbal or plant or mineral origin, some drug substances are of marine origin.
2. Drug from chemical as well as natural origin: Derived from partial herbal and partial chemical synthesis Chemical, example steroidal drugs
3. Drug derived from chemical synthesis.
4. Drug derived from animal origin: For example, hormones, and enzymes.
5. Drug derived from microbial origin: Antibiotics

6. Drug derived by biotechnology genetic-engineering, hybridoma technique for example
7. Drug derived from radioactive substances.

One of the key classifications is between traditional small molecule drugs, usually derived from chemical synthesis, and biologic medical products, which include recombinant proteins, vaccines, blood products used therapeutically (such as IVIG), gene therapy, and cell therapy (for instance, stem cell therapies).

Pharmaceutical or drug or medicines are classified in various other groups besides their origin on the basis of pharmacological properties like modeof action and their pharmacological action or activity, such as by chemical properties, mode or route of administration, biological system affected, or therapeutic effects. An elaborate and widely used classification system is the Anatomical Therapeutic Chemical Classification System (ATC system). The World Health Organization keeps a list of essential medicines [13] .

A sampling of classes of medicine includes:

1. Antipyretics: reducing fever (pyrexia/paresis)
2. Analgesics: reducing pain (painkillers)
3. Antimalarial drugs: treating malaria
4. Antibiotics: inhibiting germ growth
5. Antiseptics: prevention of germ growth near burns, cuts and wounds

6. Mood stabilizers: lithium and valpromide
7. Hormone replacements: premiering
8. Oral contraceptives: Envois, "biphasic" pill, and "diphasic" pill
9. Stimulants: methylphenidate, amphetamine
10. , diazepam, and alprazolam
11. Statins: lovastatin, pravastatin, and simvastatin

Pharmaceuticals may also be described as "specialty", independent of other classifications, which is an ill defined class of drugs that might be difficult to administer, require special handling during administration, require patient monitoring during and immediately after administration, have particular regulatory requirements restricting their use, and are generally expensive relative to other drugs.(14)

1.1.1 Antibiotics:

Antibiotics, also called **antibacterial**, are a type of antimicrobial drug used in the treatment and prevention of bacterial infections They may either kill or inhibit the growth of bacteria. A limited number of antibiotics also possess antiprotozoal activity.

Antibiotics are not effective against viruses such as the common cold or influenza, and their inappropriate use allows the emergence of resistant organisms. In 1928, Alexander
Fleming identified penicillin, the first chemical compound with antibiotic properties. Fleming was working on a culture of disease bacteria when he noticed the spores of a little

green mold (*Penicilliumchrysogenum*), in one of his culture plates. He observed that the presence of the mold killed or prevented the growth of the bacteria. [15][16][17][18][19][20]

Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. Most target bacterial functions or growth processes. Those that target the bacterial cell wall (penicillin's and cephalosporin's) or the cell membrane (polymyxins), or interfere with essential bacterial enzymes (rifamycins, lipiarmycins, quinolones, and sulfonamides) have bactericidal activities. Those that target protein synthesis(macrolides, lincosamides and tetracycline) are usually bacteriostatic (with the exception of bactericidal aminoglycosides). Further categorization is based on their target specificity. "Narrow-spectrum" antibiotics target specific types of bacteria, such as gram-negative or gram-positive, whereas broad-spectrum antibiotics affect a wide range of bacteria. Following a 40-year break in discovering new classes of antibacterial compounds, four new classes of antibiotics have been brought into clinical use in the late 2000s and early 2010s: cyclic lipopeptides (such as daptomycin),glycylcyclines (such as tigecycline), oxazolidinones (such as linezolid), and lipiarmycins (such as fidaxomicin). [21][22][23][24]

The quinolones and fluoroquinolones are synthetic antimicrobials considered bactericidal, effective in the treatment of various infections, particularly bacterial infections of the urinary tract. The

mechanism of action is the inhibition of DNA replication conferred, by their chemical structures. Nalidixic acid became the most significant treatment for urinary tract infections caused by Gram-negative bacteria. However, its indication for systemic infection was later restricted as many microorganisms acquired resistance to the antibiotic.[25]

Due to the development of bacterial resistance, many other analogs of nalidixic acid began to be developed. In 1980, it was noticed that the insertion of a fluorine atom in the ring quinolone , increased antimicrobial activity, facilitating the entry of the drug into the bacterial cell, which broadened the spectrum of action of the drug to Gram positive bacteria and potentiated the action against Gram-negative bacteria.

The changes made in the structure of the molecules in the ‘80s led to the synthesis of compounds of the second-generation flourquinolones or quinolones.[26][27]

1.1.2 Ciprofloxacin:

Ciprofloxacin, an antibacterial of the quinolone group, was developed from these molecular changes. It is the antibiotic most active against Gram-negative bacteria of the class and is widely used in urinary and respiratory tract infections, as well as against skin, bone, and joint infections. 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.[28]

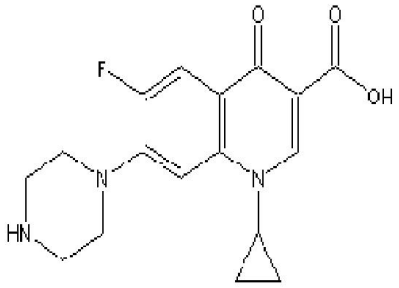


Fig. 1: Chemical structure of ciprofloxacin

1.1.2.1 Medical use:

Ciprofloxacin is used to treat a wide variety of infections, including infections of bones and joints, endocarditis, gastroenteritis,malignant otitis externa, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, and chancroid.[29]

Ciprofloxacin only treats bacterial infections; it does not treat viral infections such as the common cold. For certain uses including acute sinusitis, lower respiratory tract infections and uncomplicated gonorrhea, ciprofloxacin is not considered a first-line agent.

Ciprofloxacin occupies an important role in treatment guidelines issued by major medical societies for the treatment of serious infections, especially those likely to be caused by Gram-negative bacteria, including *Pseudomonas aeruginosa*. For example, ciprofloxacin in combination with metronidazole is one of several first-line antibiotic regimens recommended by the Infectious Diseases

Society of America for the treatment of community-acquired abdominal infections in adults. It also features prominently in treatment guidelines for acute pyelonephritis, complicated or hospital-acquired urinary tract infection, acute or chronic prostitutes, certain types of endocarditic, certain skin infections, and prosthetic joint infections. [30][31][32][33][34]

In other cases, treatment guidelines are more restrictive, recommending in most cases that older, narrower-spectrum drugs be used as first-line therapy for less severe infections to minimize fluoroquinolone-resistance development. For example, the Infectious Diseases Society of America recommends the use of ciprofloxacin and other fluoroquinolones in urinary tract infections be reserved to cases of proven or expected resistance to narrower-spectrum drugs such as nitrofurantoin or trimethoprim/sulfamethoxazole. The European Association of Urology recommends ciprofloxacin as an alternative regimen for the treatment of uncomplicated urinary tract infections, but cautions that the potential for "adverse events have to be considered". [35]

Pregnancy

In the United States ciprofloxacin is pregnancy category C. This category includes drugs for which no adequate and well-controlled studies in human pregnancy exist, and for which animal studies have suggested the potential for harm to the fetus, but potential benefits may warrant use of the drug in pregnant women despite potential

risks. An expert review of published data on experiences with ciprofloxacin use during pregnancy by the Teratogen Information System concluded therapeutic doses during pregnancy are unlikely to pose a substantial teratogenic risk (quantity and quality of data=fair), but the data are insufficient to state no risk exists. [36][37]

Two small post-marketing epidemiology studies of mostly short-term, first-trimester exposure found that fluoroquinolones did not increase risk of major malformations, spontaneous abortions, premature birth, or low birth weight. The label notes, however, that these studies are insufficient to reliably evaluate the definitive safety or risk of less common defects by ciprofloxacin in pregnant women and their developing fetuses.[38][39]

Breastfeeding

Fluoroquinolones have been reported as present in a mother's milk and thus passed on to the nursing child.The U.S. FDA recommends that because of the risk of serious adverse reactions (including articular damage) in infants nursing from mothers taking ciprofloxacin, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.[40][41]

Children

Oral and intravenous ciprofloxacin are approved by the FDA for use in children for only two indications due to the risk of permanent injury to the musculoskeletal system:

1) Inhalational anthrax (postexposure)

2) Complicated urinary tract infections and pyelonephritis due to *Escherichia coli*, but never as first-line agents. Current recommendations by the American Academy of Pediatrics note the systemic use of ciprofloxacin in children should be restricted to infections caused by multidrug-resistant pathogens or when no safe or effective alternatives are available. [42][43][44]

1.1.2.2 Spectrum of activity

Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including Gram-negative(*Escherichia coli*, *Haemophilusinfluenzae*, *Klebsiellapneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*), and Gram-positive (methicillin-sensitive, but not methicillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and*Streptococcuspyogenes*) bacterial pathogens.

1.1.2.3 Bacterial resistance

As a result of its widespread use to treat minor infections readily treatable with older, narrower spectrum antibiotics, many bacteria have developed resistance to this drug in recent years, leaving it significantly less effective than it would have been otherwise.[45][46]

Resistance to ciprofloxacin and other fluoroquinolones may evolve rapidly, even during a course of treatment. Numerous pathogens, including enterococci, *Streptococcus pyogenes* and *Klebsiellapneumoniae* (quinolone-resistant) now exhibit resistance. Widespread veterinary usage of the fluoroquinolones, particularly in Europe, has been implicated. Meanwhile, some *Burkholderiacepacia*, *Clostridium innocuous* and *Enterococcus faecium* strains have developed resistance to ciprofloxacin to varying degrees. [47][48][49]

Fluoroquinolones had become the class of antibiotics most commonly prescribed to adults in 2002. Nearly half (42%) of those prescriptions in the U.S. were for conditions not approved by the FDA, such as acute bronchitis, otitis media, and acute upper respiratory tract infection, according to a study supported in part by the Agency for Healthcare Research and Quality. Additionally, they were commonly prescribed for medical conditions that were not even bacterial to begin with, such as viral infections, or those to which no proven benefit existed.[50]

1.1.2.4 Side Effects:

Rates of side effects appear to be higher than with some groups of antibiotics such ciprofloxacin but lower than with others such as clindamycin. Compared to other antibiotics some studies find a higher rate of side effectswhile others find no difference. [51][52][53]

In pre-approval clinical trials of ciprofloxacin most of the adverse events reported were described as mild or moderate in severity, abated soon after the drug was discontinued, and required no treatment. Ciprofloxacin was discontinued because of an adverse event in 1% of people treated with the medication by mouth. The most frequently reported drug-related events, from trials of all formulations, all dosages, all drug-therapy durations, and for all indications, were nausea (2.5%), diarrhea (1.6%), abnormal liver function tests (1.3%), vomiting (1%), and rash (1%). Other adverse events occurred at rates of <1%.

A wide range of rare but potentially fatal side effects spontaneously reported to the U.S. FDA or the subject of case reports published in medical journals includes, but is not limited to, toxic epidermal necrolysis, Stevens-Johnson syndrome, heart arrhythmias (*torsades de pointes* or QT prolongation), allergic pneumonitis, bone marrow suppression, hepatitis or liver failure, and sensitivity to light. The drug should be discontinued if a rash, jaundice, or other sign of hypersensitivity occur.

Children and the elderly are at a much greater risk of experiencing adverse reactions.[54]

It is very important to determine flour quinolones for the purpose of pharmaceutical quality control. Most of the analytical methods for the determination of ciprofloxacin employ HPLC; it is also the official method in United States Pharmacopoeia, British Pharmacopoeia and

Brazilian Pharmacopoeia. Numerous others methods have been reported for the determination of ciprofloxacin using techniques such as visible spectrophotometry. Some methods for analysis of quinolones using UV spectrophotometric method were described in the literature, meantime, for determination of ciprofloxacin are relatively few ones and no method for direct quantitation of ciprofloxacin hydrochloride was found. Assay of CPF in pharmaceuticals has previously been achieved by several analytical techniques such as: HPLC, HPTLC, capillary electrophoresis, high performance capillary electrophoresis, spectrophotometry, fluorimetry, spectrophotometry, chemiluminometry, ISE-based potentiometer and voltammetry.(55)

1.2 Spectrophotometry:

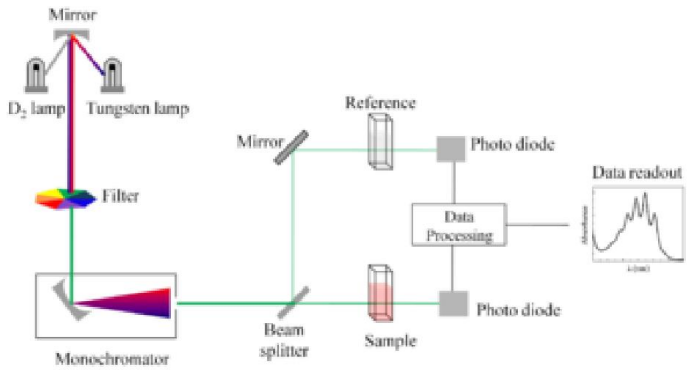
Spectrophotometeric analysis continues to be one of the most widely used analytical techniques available. The greatest use of UV-Vis absorption spectroscopy lies in its application to quantitative measurements. The reasons for this stem from the ease with which most spectrophotometeric measurements can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

The instrument used in ultraviolet-visible spectroscopy is called a ***UV/Vis spectrophotometer***. It measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (I_o). The ratio I/I_o is called the

transmittance, and is usually expressed as a percentage (%T). The ***absorbance***, A,is based on the transmittance:

$$A = -log(\%T/100\%)$$

The basic parts of a spectrophotometer are a light source, a holder for the sample, a diffraction grating in a monochromator or a prism to separate the different wavelengths of light, and a detector. The radiation source is often a *Tungsten filament* (300-2500 nm), a *deuterium arc lamp*, which is continuous over the ultraviolet region (190-400 nm), *Xenon arc lamp*, which is continuous from 160-2,000 nm; or more recently, *light emitting diodes(LED)* for the visible wavelengths. The detector is typically a *photomultiplier tube*, a *photodiode*, a photodiode array or a *charge-coupled device* (CCD). Single photodiode detectors and photomultiplier tubes are used with scanning monochromators, which filter the light so that only light of a single wavelength reaches the detector at one time. Fixed monochromators are used with CCDs and photodiode arrays. As both of these devices consist of many detectors grouped into one or two dimensional arrays, they are able to collect light of different wavelengths on different pixels or groups of pixels simultaneously.



UV- Visible spectrophotometer

A spectrophotometer can be either *single beam* or *double beam*. In a single beam instrument all of the light passes through the sample cell. $I_o$must be measured by removing the sample. This was the earliest design and is still in common use in both teaching and industrial labs.In a double-beam instrument, the light is split into two beams before it reaches the sample. One beam is used as the reference; the other beam passes through the sample. The reference beam intensity is taken as 100% Transmission (or 0 Absorbance), and the measurement displayed is the ratio of the two beam intensities. Some double-beam instruments have two detectors(photodiodes), and the sample and reference beam are measured at the same time. In other instruments, the two beams pass through a beam chopper, which blocks one beam at a time. The detector alternates between measuring the sample beam and the reference beam in synchronism with the chopper. Samples for UV/Vis spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also

be measured. Samples are typically placed in a transparent cell, known as a *cuvette*. Cuvettes are typically rectangular in shape, commonly with an internal width of 1 cm. (This width becomes the path length, L, in the Beer-Lambert law). The type of sample container used must allow radiation to pass over the spectral region of interest. The most widely applicable cuvettes are made of high quality fused silica or quartz glass because these are transparent throughout the UV, visible and near infrared regions. Glass and plastic cuvettes are also common, although glass and most plastics absorb in the UV, which limits their usefulness to visible wavelengths. A complete spectrum of the absorption at all wavelengths of interest can often be produced directly by a more sophisticated spectrophotometer.The method is most often used in a quantitative way to determine concentrations of an absorbing species in solution, using the Beer-Lambert law:

$$A = \log_{10}(I_0/I) = \epsilon \cdot c \cdot L,$$

where A is the measured *absorbance*, in **A**bsorbance **U**nits (**AU**), I_0 is the *intensity of the incident light* at a given wavelength, I is the *transmitted intensity*, L the *path length* through the sample, and c the *concentration* of the absorbing species. For each species and wavelength, ε is a constant known as the *molar absorptivity*or*extinction coefficient*. By removing the concentration dependence, the extinction coefficient (ε) can be determined as a function of wavelength.[56]

1.3 Chromatography:

1.3.2 High-performance liquid chromatography (HPLC)

Is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column.

Medical use of HPLC can include drug analysis, but falls more closely under the category of nutrient analysis. While urine is the most common medium for analyzing drug concentrations, blood serum is the sample collected for most medical analyses with HPLC. Other methods of detection of molecules that are useful for clinical studies have been tested against HPLC, namely immunoassays. In one example of this, competitive protein binding assays (CPBA) and HPLC were compared for sensitivity in detection of vitamin D. Useful for diagnosing vitamin D deficiencies in children, it was found that sensitivity and specificity of this CPBA reached only 40% and 60%, respectively, of the capacity of HPLC. While an expensive tool, the accuracy of HPLC is nearly unparalleled.[57]

1.3 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.[58]

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.[59]

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 .[60]

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. Accordingly, the following performance characteristics are recommended for assay method validation: specificity, linearity, precision (repeatability, intermediate precision, and reproducibility),
accuracy, range and robustness . Limit of detection (LOD) and limit of quantification (LOQ) may also be done for quantitative tests like testing for impurities .[62]

1.3.1 Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present .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 .

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).

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. [62]

1.3.2 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 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 + 20% of the target or nominal concentration.

Under normal circumstances, linearity is achieved when the coefficient of determination (*r*2*)* is ≥ 0.997. A minimum of five concentrations is recommended. ([63])

1.3.3 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 (coefficient of variation)[(64)].

Precision should be considered at different levels as follows:

1.3.3.1 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. [62][63]

1.3.3.2 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.[61]

1.3.3.4 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.[60]

1.3.4 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.

1.3.5 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 analytical within or at the extremes of the specified range.[62][63]

1.4 Objectives:

Validation of an analytical proceed methods for Ciprofloxacin HCL using ultra-violet spectrophotometer.

2.0 Experimental:

2.1 material:

- Ciprofloxacin HCl tablet (Standard, sample 1, sample 2, sample3).

2.2 Chemicals:

- Phosphoric acid (0.025M).
- Acetonitrite.
- Tri ethylene amine.
- Hydrochloric acid (0.01M).

2.3 Apparatus:

- Pipette.
- Volumetric flask.
- Measuring cylinder.
- Mortar.
- Beaker.
- Silica plate.

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2.4 Instruments:

- UV/Visible spectroscopy.
- High-performance liquid chromatography (HPLC).
- Dissolution.
- Sensitive balance

2.5 Procedure:

2.5.1 Preparation of ciprofloxacin tablets for dissolution (USP):

Hydrochloric acid solution - Dissolution media - (0.01M) in 900ml of distilled water was prepared, amount of ciprofloxacin(sample1, sample2, sample3,) was dissolved 30 minute by employing UV absorption at the wavelength of maximum absorbance at about 276 nm on filtered portion of the solution, solutions were diluted with Dissolution media.

2.5.2 Validation method:

2.5.2.1 Standard solutions:

An accurately weighed quantity of 20 mg of ciprofloxacin was transferred into a 100 ml volumetric flask. The solvent consisting of a mixture of acetonitrile: Phosphoric acid in the ratio (13:87) previously adjusted (with tri ethylamine) to a pH of 2.0 + 0.1 solvent was added to mark to produce a solution having a concentration of 200 µg/ml of ciprofloxacin.

2.5.2.2 Preparation of working standard:

From standard stock solution 5 ml was pipetted out into 50 ml of volumetric flask and made up the volume with solvent to produce a solution having a concentration of 20 µg/ml of ciprofloxacin.

2.5.2.3 Preparation of assay working solution:

2 ml of ciprofloxacin plus (ciprofloxacin 10 mg) equivalent to 20 mg of ciprofloxacin was transferred into volumetric flask. Methanol was added to the mark and ultra-solicited for 5 minutes to produce a solution having a concentration of 200 µg/ml of ciprofloxacin.

5 ml of this solution was diluted to 50 ml with the same solvent to produce a solution having a concentration of 20 µg/ml of ciprofloxacin.

2.5.2.4 System suitability test:

From the standard working solution, the concentration was prepared having 20 µg/ml of ciprofloxacin. The system suitability test was performed from five replicate injections of standard working solution

2.5.2.5 Linearity:

In order to study linearity of the response, a series of seven concentrating levels from 80%- 120% of assay analytic concentrations were prepared from stock solutions. The linearity performed used for the determination of limits of quantification and detection.

From standard solution having the concentration 200 µg/ml of ciprofloxacin. Transfer accurately the specified volume into each of 100 ml volumetric flask to obtain the required working standard concentrations explained as follows:

Ciprofloxacin linearity:

I. 80 µg/ml : 2 ml was transferred from 200 µg/ml standard stock solution to 200 ml volumetric flask, then made up to volume with solvent, 0.8 ml was transferred from this solution to 50 ml volumetric flask, then made up to volume with solvent (80%).

II. 900 µg/ml : 2 ml was transferred from 200 µg/ml standard stock solution to 200 ml volumetric flask, then made up to volume with solvent, 0.9 ml was transferred from this solution to 50 ml volumetric flask, then made up to volume with solvent (90%).

III. 100 µg/ml : 2 ml was transferred from 200 µg/ml standard stock solution to 200 ml volumetric flask, then made up to volume with solvent, 1 ml was transferred from this solution to 50 ml volumetric flask, then made up to volume with solvent (100%).

IV. 110µg/ml : 2 ml was transferred from 200 µg/ml standard stock solution to 200 ml volumetric flask, then made up to volume with solvent, 1.1 ml was transferred from this solution to 50 ml volumetric flask, then made up to volume with solvent (110%).

V. 120 µg/ml : 2 ml was transferred from 200 µg/ml standard stock solution to 200 ml volumetric flask, then made up to volume with solvent, 1.2 ml was transferred from this solution to 50 ml volumetric flask, then made up to volume with solvent (120%).

2.4.2.6 Statistical analysis and calculation formula used:

Linearity data should be evaluated using appropriate statistical methods. A simple regression line of the detector response versus the

sample concentration is the most common means of evaluation. Regulatory agencies require the submission of the correlation coefficient, y-intercept, slope of regression line, and the residual sum of squares for linearity evaluation.

For the current study statistics data analysis is used.

Formula:

$$\text{Assay\%} = \frac{At}{As} X \frac{Cs}{Ct} X100$$

Where: At = Area of the test.

As = Area of the standard.

Cs = Concentration of the standard.

Ct = Concentration of the test.

2.4.2.7 Limit of detection:

The LOD is the smallest amount of analytic than can be detected, but not necessarily quantities using a given method.

There are several means of calculating LOD for HLC methods. The most common approach is to determine the sample amount that provides a signal-to-noise ratio of 3:1. Many chromatographic data acquisition systems provide integrated functions to determine the signal-to-noise ratio that are easily employed by analyst.

An alternative to the signal-to-noise approach is to estimate LOD based on the standard deviation of response. For this calculation, LOD = 3.3 (SD/S), where SD is the standard deviation of response based on the standard deviation of the blank, the residual standard deviation of the regression line, and the standard deviation of the y-intercepts of the regression line, and S is the slope of the calibration curve.

2.4.2.8 Limit of quantification:

The LOQ is the lowest level that an analyst be quantitated with any degree of certainty.

The LOQ can be determined by a signal-to-noise ratio of 10:1, or approximated by multiplying the LOD by 3.3. As with LOD, this function is easily obtained from current data-acquisition software. Similarly, LOQ can be estimated by the LOQ= 10 (SD/S).

2.4.2.9 Specificity:

Specificity is the ability of a method to discriminate between the analyst (s) of interest and other components that are present in the sample

The sample weight was taken and solution prepared similarly to the sample solution. The solution was analyzed as per the proposed method. Sample solution was also analyzed as per the proposed method. No interference from placebo was observed at the retentiontime of the drugs peaks. The absence of a peak at the

retention time of the active ingredient is sufficient to demonstrate specificity for excipients.

2.4.2.10 Accuracy:

Accuracy is the closeness in a agreement between the accepted true value or a reference value and the measured result obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyst into the matrix of the sample to be analyzed.

The accuracy of the assay method was evaluated with the recovery of standards from excipients. Three different quantities (low, medium, and high i.e. 80%, 100%, and 120%) of the authentic standards were added to the placebo. Sample solutions are prepared in triplicate for each spike level as described in the test preparation.

2.4.2.11 Precision:

Precision is a measure of the ability of the method to generated reproducible results. For the precision study, precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) in triplicate. Repeatability refers to the use of analytical procedure over a short period of time that was evaluated by assaying of six determinations at 100% of the test concentrations during the same day prepared in the manner described above in the Assay working solution. Compared the assay of six determinations at 100% of the test concentrations no different days or time.

2.4.3 Liquid chromatography conditions:

Chromatography conditions were obtained using a stainless column (Thermo BDS 18,150 mm X 4.6 mµ), which was maintained at ambient temperature . The analytical wavelength was set at 245 nm and samples of 10 ml were injected to HPLC system. The mobile phase consisting of a mixture of acetonitrile: Phosphoric acid in the ratio (13:87) previously adjusted(with tri ethylamine) to a pH of 2.0 + 0.1 , at flow rate of 1 ml/min. The mobile phase was filtered through 0.45 mµ filter and degassed for 10 minutes by sanitation

2.4.4 Preparation of ciprofloxacin tablets for HPLC:

0.025M of phosphoric acid (mobile phase) was prepared ,filtered and degassed, previously adjusted(with tri ethylamine) to a pH of 2.0 + 0.1, and acetonitrile (87:13) The amount of ciprofloxacin(sample1, sample 2 ,sample 3,) was crushed and dissolved with mobile phase and then the High-performance liquid chromatography (HPLC) was recorded the result.

The result of obtained from the UV in the device were compared by HPLC technique with smarter and found to be within the allowable range of the assay, which proves that this method is steady and precise and not affected by change minor.

3. Result

3.1.1 Uniformity of weight

Average Weighted for sample 1	
1	0.7492
2	0.8186
3	0.8295
4	0.8163
5	0.8131
6	0.8022
7	0.805
8	0.8107
9	0.8084
10	0.8055
11	0.8142
12	0.8082
13	0.7992
14	0.8082
15	0.7959
16	0.8338
17	0.8068
18	0.8014
19	0.8085
20	0.7807
*Avg	0.80564
*Min	0.7492
*Max	0.8338
Rsd%	1.32

.

3.1.2 Uniformity of weight

3.1.3 Uniformity of weight

Average Weight for sample 3	
1	0.7677 g
2	0.7684 g
3	0.7612
4	0.7829
5	0.7569
6	0.7606
7	0.7375
8	0.7505
9	0.7681
10	0.7508
11	0.7813
12	0.7754
13	0.7721
14	0.7467
15	0.7649
16	0.7592
17	0.7662
18	0.7617
19	0.7739
20	0.773
Avg	0.76349
Min	0.7375
Max	0.7829
Rsd%	1.59

3.2.1 Dissolution test

Sample 1 USP						
*P		*W.C	Average	Claim		*F
99.88		6.35	805.64	500		0.905396
STD	T1	T2	T3	T4	T5	T6
56	795.3	833.7	806.8	814.3	799.1	805.6
0.597	0.722	0.757	0.733	0.739	0.727	0.74
0.597	0.722	0.757	0.733	0.739	0.727	0.74
0.597	0.722	0.757	0.733	0.739	0.727	0.74
0.597	0,722	0.757	0.733	0.739	0.727	0.740
Q %	104.58	104.60	104.66	104.55	104.81	105.82
	Min	104.55		Max	105.82	

*P: potency or Assay% of working standard.

*W.C. : water contend of working standard.

*F: factor for correction =

$$\frac{\text{Molecular weight of Ciprofloxacin.}}{\text{Molecular weight of Ciprofloxacin HCL}}$$

*Avg.: Average weight of tablet.

Label claim (Dose).

3.2.2 Dissolution test

Sample2 USP						
P		**W.C**	**Average**	**Claim**		**F**
99.88		**6.35**	686.10	**500**		**0.905396**
STD	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
56	688.3	682.2	678.8	678.4	686.2	693.9
0.597	0.706	0.699	0.709	0.705	0.711	0.716
0.597	0.706	0.699	0.709	0.705	0.712	0.716
0.597	0.706	0.699	0.71	0.706	0.711	0.716
0.597	0,706	0.699	0.709	0.705	0.711	0.716
Q %	100.63	100.52	102.52	102.00	101.70	101.23
	Min	**100.52**		**Max**	**102.52**	

3.2.3 Dissolution test

sample 3 USP						
P		**W.C**	**Average**	**Claim**		**F**
99.88		**6.35**	763.49	**500**		**0.905396**
STD	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
56	752.9	771.8	767.1	767.4	776.1	751.5
0.597	0.682	0.702	0.702	0.708	0.717	0.716
0.597	0.683	0.702	0.703	0.708	0.716	0.716
0.597	0.683	0.702	0.702	0.709	0.716	0.716
0.597	0.683	0.602	0.702	0.708	0.716	0.716
Q %	98.99	99.30	99.96	100.77	100.77	98.98
	Min	**98.98**		**Max**	**100.77**	

Not less than 80%

$$\frac{absorbance\ sampel}{absorbance\ standard}\text{X}\frac{weight\ of\ std}{volume\ of\ std}\text{X}dilution\ \text{X}\frac{purity\ of\ std}{100}\text{X}\frac{(100-water\ content}{100}$$

$$\text{X}\frac{volume}{wt\ of\ sample}\text{X}factor\ \text{X}100$$

3.3 Result of validation:

3.3.1 System Stability:

Result of System Suitability Test

No	Abs
1	0.540
2	0.539
3	0.539
4	0.542
5	0.541
6	0.541

Average: 0.540

Stander deviation (Std. D.) = 0.0012

R-Squire Deviation (RSD)

$$\textbf{RSD} = \frac{ST.d}{AV} \times 100$$

$$= \frac{0.0012}{0.541} \times 100$$

RSD = 2.218

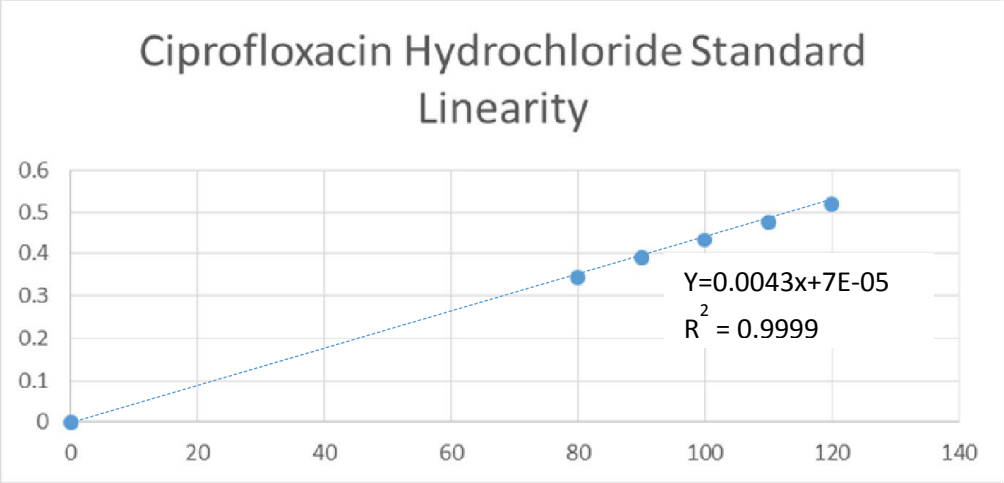
3.3.2 Method of Analyses

Ciprofloxacin Hydrochloride Standard Linearity

No	Theoretical Cone µg / ml	Response (Area)
1	80.0	0.343
2	80.0	0.344
3	80.0	0.343
4	90.0	0.391
5	90.0	0.391
6	90.0	0.392
7	100.0	0.433
8	100.0	0.433
9	100.0	0.434
10	110.0	0.437
11	110.0	0.477
12	110.0	0.476
13	120.0	0.517
14	120.0	0.518
15	120.0	0.516

Average= 0.4333

Concentration %	Mean Area
0	0
80	0.343
90	0.391
100	0.433
110	0.475
120	0.517



S. T.D:

*** Limit of Quantification from Linearity (LAQ)**

$$\mathbf{LAQ} = \frac{10 \times S.T.D}{SLOP} = \frac{10 \times 0.1865}{0.0043}$$

LAQ = 433.72

*** Limit of Detection from Linearity (LAD)**

$$\mathbf{LAD} = \frac{3{,}3 \times S.T.D}{SLOP} = \frac{3.3 \times 0.1865}{0.0043}$$

LAD = 143.13

3.3.3 Accuracy:

No	Concentration	Absorbance of standard	Absorbance of sample
1	80%	0.433	0.343
2	100%	0.433	0.433
3	120%	0.433	0.517

$$\frac{\textbf{Absorbance } \boldsymbol{smple}}{\textbf{Absorbance } \boldsymbol{Stander}} \times \mathbf{20}$$

$$= \frac{\mathbf{0.343}}{\mathbf{0.433}} \times \mathbf{20} = \mathbf{15.48\ mg/\ ml}$$

$$= \frac{\mathbf{0.433}}{\mathbf{0.433}} \times \mathbf{20} = \mathbf{20\ mg/\ ml}$$

$$= \frac{\mathbf{0.517}}{\mathbf{0.433}} \times \mathbf{20} = \mathbf{23.89\ mg/\ ml}$$

Recover:

Concentration	Found content	actual content	Recover
80%	15.84	16	99%
100%	20	20	100%
120%	23.89	24	99.5%

$$\text{Recover} = \frac{found\ content}{actual\ content} \text{ X } 10$$

3.3.4 Result of Precision test (repeatability):

3.3.4.1 Description: Assay Method Spectrophotometric validation Repeatability Ciprofloxacin Hydrochloride

Sample Std	WL276.0
1	0.434
2	0.4335
3	0.436
4	0.434
5	0.434
6	0.435

3.3.4.2 Description: Assay Method Spectrophotometric validation Repeatability (sample 1)

Sample 1	WL276.0
1	0.569
2	0.571
3	0.574
4	0.575
5	0.575
6	0.573

3.3.4.3 Description: Assay Method Spectrophotometric validation Repeatability (sample 2)

Sample 2	WL276.0
1	0.540
2	0.539
3	0.539
4	0.542
5	0.541
6	0.541

3.3.4.4 Description: Assay Method Spectrophotometric validation Repeatability (sample 3)

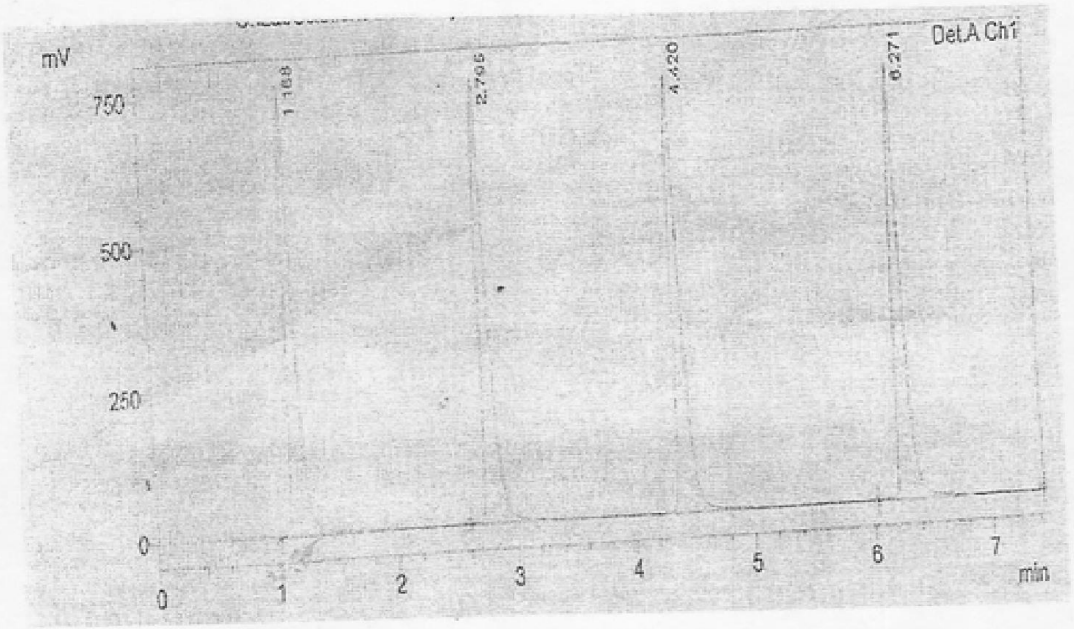
Sample 3	WL276.0
1	0.518
2	0.517
3	0.520
4	0.519
5	0.519
6	0.520

Sample Name: Ciprofloxacin HCI 500 mg std. 1cm

Injection Volume: 20 ul

Data File Name: Ciprofloxacin HCI 500 mg std. 1cm

(Chromatogram)



Peak Table

1Det.Ach1 /278n

Detector A cin 1 278nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.168	3818914	766005	24.813	25.379
2	2.795	3860184	758886	25.081	25.143
3	4.420	3860985	747896	25.086	24.779
4	6.271	3850982	745488	25.021	24.699
Total		15391065	3018276	100.00	100.00

Average of Area = 3857383.67

Shimadzu

Model: 2004

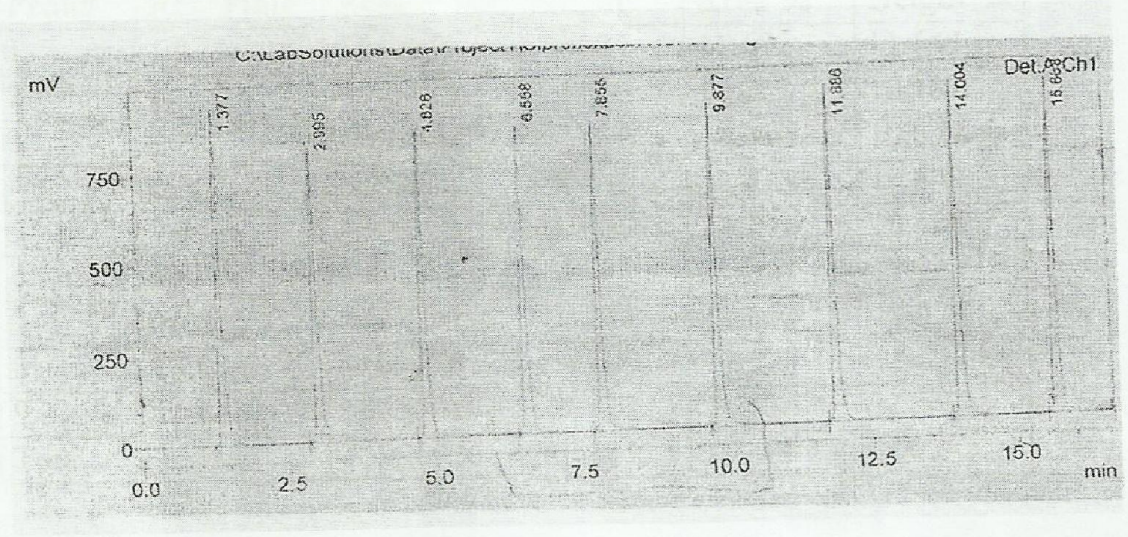
Serial number: 20134403614

Sample Name: Ciprofloxacin HCl sample1, sample 2

Injection Volume: 20 ul

Data File Name: Ciprofloxacin HCl sample 1, sample 2

(Chromatogram

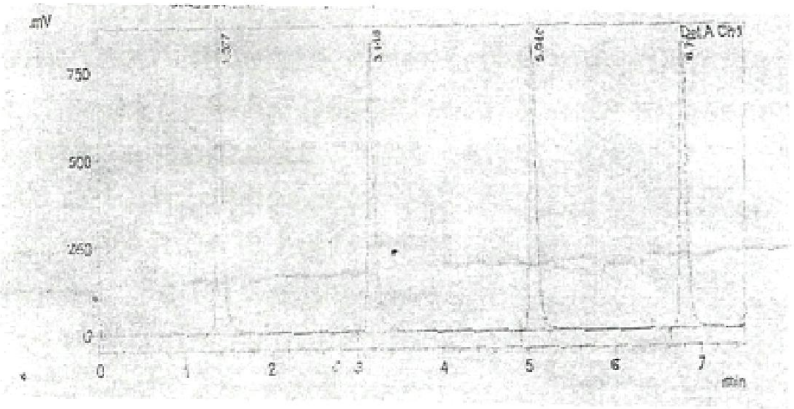


Sample Name: Ciprofloxacin HCl sample 3

Injection Volume: 20 ul

Data File Name: Ciprofloxacin HCl sample 3

(Chromatogram)



Peak Table

1Det.Ach1 /278n

Detector A ch 1 278nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.377	4529319	822477	25.041	24.924
2	3.148	4560651	826519	25.214	25.047
3	5.040	4510491	822083	24.937	24.912
4	6.781	4487035	828829	24.807	25.117
Total		18087496	3299908	100.00	100.00

Average of Area = 4533487

Peak Table

IDet. Ach 1/278 nm

Dector A ch 1 278 nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.377	5135440	933077	11.672	11.643
2	2.995	4514835	816346	10.262	10.187
3	4.828	4698669	855629	10.680	10.677
4	6.558	4695838	853381	10.673	10.649
5	7.855	4708176	856500	10.701	10.688
6	9.877	4938108	898566	11.224	11.213
7	11.886	5121194	935235	11.640	11.670
8	14.004	5109176	936301	11.613	11.638
9	15.668	5075501	928921	11.536	11.591
Total		43996936	8013955	100.00	100.00

Average from sample **1= 47700894.3333**

Average from sample **2= 5101957**

$$\frac{\text{area of sample}}{\text{area of standard}} \times \frac{\text{concentration of Standared}}{\text{concentration of sample}} \times \text{purity} \times \text{avearge weight} \times \text{doese} \times \text{fuctor}$$

Purity = 100.2

$$\text{Factor} = \frac{331.34}{385.82}$$