Any success story always begins with the careful selection, proper identification of quality and purity of the materials required in the project. Research envisages the derivatization of drugs for the enhancement of half-life. Therefore, it is necessary to have a look for eligible candidates in selection of the drugs.

For the selection of drugs main criteria was that drug should have short biological half-life, its frequency of administration is higher and drug should have functional group that can be bonded with polyethylene glycols, thus it shall have groups like –COOH, -SO₃H etc. So that further derivatization can take place.

Based on the above considerations the following drugs were selected for derivatization and half-life enhancement.

- **Cloxacillin**
- **Cephalexin**
- **Ciprofloxacin**

Only the selection of drugs, for derivatization, is not sufficient unless the complete characterization and proper methods of quantitative estimations are also selected. For such selection complete awareness about the profiles of drugs is required. Their characteristics reported in the literature were collected and described as the profile of individual drug; however the literature available on these drugs is very bulky. Therefore, only a part of it was selected on the basis of relevance. The drugs were tested for quality on the basis of their pharmacopoeial monographs. Hence, the characterization is described along with the profile. The analytical methods are required for the quantitative analyses of drugs in plasma. The methods selected for such purpose are described along with the profile of individual drugs.
2.1 CLOXACILLIN SODIUM

2.1.1 Drug Profile

2.1.1.1 Description¹

Name, Formula, Molecular Weight

Sodium Cloxacillin is found in Chemical Abstracts under 4-Thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid, 6-[[3-(2-chlorophenyl)-5-methyl-4-isoxazoyl] carbonyl] amino]-3, 3-dimethyl-7-oxo-monosodium salt. It is more commonly known as 3-o-chlorophenyl-5-methyl-4-isoxazolyl penicillin sodium salt.

\[
\text{C}_{19}\text{H}_{17}\text{ClN}_3\text{NaO}_5\text{S} \quad \text{Molecular Weight = 457.89}
\]

Appearance

Sodium cloxacillin is a white, odorless, crystalline powder.
2.1.1.2 Physical Properties

Infrared Spectrum

Infrared absorption frequencies were reported for oil suspension of cloxacinill and other penicillins by E. A. Rudzit et. al. A spectrogram of Bristol Laboratories Primary Reference Standard recorded as a potassium bromide disk using a Beckman Model IR9 Spectrometer is shown in Fig. 2.1. Characteristic absorption frequencies (cm\(^{-1}\)) are as follows:

- a. H\(_2\)O \hspace{1cm} 3519
- b. N-H stretching band \hspace{1cm} 3370
- c. beta-lactam carbonyl \hspace{1cm} 1770
- d. secondary amide carbonyl \hspace{1cm} 1669
- e. aromatic ring \hspace{1cm} 1619
- f. carboxylate carbonyl \hspace{1cm} 1604

Within the carbonyl stretching region, the beta-lactam frequency is most characteristic of penicillins. Opening of the beta-lactam can be indicated by changes in this part of the spectrum.

![Figure 2.1 Infrared absorption spectrum of sodium cloxacillin monohydrate](image-url)
Nuclear Magnetic Resonance Spectra

Proton nuclear magnetic resonance spectra for a number of penicillins were reported by Pek and coworkers and the chemical shifts useful for identification were tabulated. An nmr spectrogram of Bristol Laboratories Primary Reference Standard sodium cloxacillin monohydrate as obtained on a Varian HA-100 spectrometer is shown in Figure 2.2. Protons resonance lines were measured in D$_2$O solution with Deuterated sodium trimethylsilyl propionate as the internal reference. Structural assignments are as follows:

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Chemical Shift, (Coupling Constant, Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic</td>
<td>7.50 m</td>
</tr>
<tr>
<td>6-H</td>
<td>5.62 d (J=4.0)</td>
</tr>
<tr>
<td>5-H</td>
<td>5.46 d (J=4.0)</td>
</tr>
<tr>
<td>3-H</td>
<td>4.81 s</td>
</tr>
<tr>
<td>5-CH$_3$ (isoxazole)</td>
<td>2.63 s</td>
</tr>
<tr>
<td>2-β-CH$_3$</td>
<td>1.43 s</td>
</tr>
<tr>
<td>2-α-CH$_3$</td>
<td>1.39 s</td>
</tr>
</tbody>
</table>

Figure 2.2 NMR spectrum of sodium cloxacillin monohydrate
Crystal Properties

Sodium cloxacillin is a microcrystalline powder exhibiting birefringence and extinction positions under a light polarizing microscope.

Melting Range

Sodium cloxacillin melts with decomposition at 170°C. Cloxacillin free acid melts with decomposition at 126-127°C.

Solubility

Sodium cloxacillin is very soluble in cold water. The ratio of sodium cloxacillin concentration in chloroform versus pH 6 buffer solution was determined to be 0.118. The solubility of penicillin salts in nonpolar solvents is significantly increased by the presence of a small amount of water, even water of hydration, also soluble in methanol, ethanol, pyridine and ethylene glycol.

Ionization Constant pKa

Budgaard and Ilver reported an apparent pKa of 2.68 ± 0.05 at 35°C., determined by measuring the pH of a partially neutralized 0.0025 M solution of sodium cloxacillin. Rapson and Bird obtained replicate apparent pK values of 2.73 ± 0.04 and 2.70 ± 0.03 at 25°C. by titrating 0.0025 M sodium cloxacillin solutions.
Optical Rotation

Cloxacillin has 3 asymmetric carbon atoms and is strongly dextrorotatory. Specific rotation values in the literature are shown below:

<table>
<thead>
<tr>
<th>Cloxacillin</th>
<th>[α]_D</th>
<th>Temp.</th>
<th>Conc. And Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium salt</td>
<td>+ 163°</td>
<td>20°C</td>
<td>1% in water</td>
</tr>
<tr>
<td>Free acid</td>
<td>+122°</td>
<td>20°C</td>
<td>1% in acetone</td>
</tr>
</tbody>
</table>

2.1.1.3 Methods of Analyses

Identification Tests

Sodium cloxacillin is identified by the infrared absorption spectrum and by the penicillin characteristic purple colour formed upon treatment with chromotropic acid in sulfuric acid at 150°C.

Quantitative Methods:

Volumetric Methods

Iodine is not consumed by penicillins but is consumed by the hydrolysis products. The difference in iodine consumption before and after alkaline hydrolysis is used as a standard method of determining cloxacillin content.

Hydrolysis with a measured excess of alkali generates an additional carboxyl group in the penicilloic acid. Back titration of the excess alkali with hydrochloric acid is used for determination of cloxacillin content.
Colorimetric Methods

Cloxacillin has been determined by measurement of the side chain absorbance at 275 nm. Penicillin have been determined by measurement of the absorption maximum near 340 nm produced by acid degradation to the corresponding penicillenic acids. Formation of the penicillanic acid is catalyzed by copper and other metals and by imidazole.

Penicillins have long been determined by reaction with hydroxylamine. The hydroxamic acids generated produce a red-colored chelate with iron (III). Details of the procedure are available in the Federal Register. Automated versions of the method have also been used.

Polarography

Penicillins in serum were determined by polarographic techniques. The analysis required about two hours. Cloxacillin was determined at levels of 5 μg/ml in sulfuric acid solution.

Gas Chromatography

Organic acid side chains produced by vigorous alkaline hydrolysis of penicillins were converted to methyl esters for gas chromatographic separation on a 3.5% SE-30 column. Intact penicillins were gas chromatographed as the corresponding methyl esters on a 2% fluoro-silicone phase by Martin and coworkers. Separation of the trimethylsilyl esters of several penicillins on a 2% OV-17 column was reported by Hishta, et. al. Cloxacillin was separated from several other penicillins and quantitation was indicated by reproducibility of response factors on reference samples.

Infrared Spectroscopy

The infrared absorption due to the beta-lactam has been used to quantitate penicillins after extraction into a suitable solvent. The cloxacillin beta-lactam band near 1760 cm\(^{-1}\) was used to measure cloxacillin inactivation by beta-lactamase as differentiated from
amidase. For this work solutions were lyophilized in the presence of potassium bromide and infrared absorption measurements were made from the solid disks.

**Optical Rotation**

The change in optical rotation upon treatment with penicillinases has been used to quantitate penicillins. Penicillins which are more susceptible to penicillinases can be determined in the presence of resistant penicillins. Ampicillin and cloxacillin were determined in combination.

**Biological Methods**

The cylinder plate agar diffusion method is the official microbiological method of determination. Staph aureus (ATCC 6538P) is the organism of choice. Cloxacillin has been microbiologically determined in the presence of Ampicillin using agar impregnated with penicillinase to destroy the Ampicillin activity or after ion exchange separation on a column of IRA-402. Cloxacillin is routinely measured by the turbidimetric method using Staph aureus FDA-209P (ATCC 6538P) or Staph aureus BL-A9596.

A microbiological paper disk procedure has been described for measuring cloxacillin and other antibiotics in as little as 10μl of plasma. Hooke and Ball used an agar plate method and Sarcina lutea NCIB 8553 to measure cloxacillin at levels below 10 μg/ml in milk.

In a test to detect trace levels in milk, penicillin inhibits growth of Streptococcus thermophilus B.C., which otherwise causes the dye 2, 3, 5-triphenyltetrazolium chloride, to turn from colorless to red. Other antibiotics interfere.

**Automated Methods**

Several chemical methods for penicillin determination have been automated, including the hydroxylamine methods, the iodometric method, the penicillenic acid method, and a
colorimetric method based on enzyme deacylation to 6-amino penicillanic acid and detection with p-dimethylamino benzaldehyde. Cloxacillin, specifically, is mentioned in the hydroxylamine and penicillanic acid automated procedures.

**Thin-layer Chromatography**

Reviews on chromatography and analysis of antibiotics are available. Cloxacillin has been chromatographed on paper, kieselguhr G, cellulose, commercial silica gel plates and silica gel G predeveloped with silicone (5% DC 200 in ether).

Cloxacillin has been separated from other penicillins, and from impurities, from cephalosporins, from other antibiotics, and from constituents of body fluids. Chromatography has been used to study solvent partitioning and structure activity relationships.

Visualization spray reagents used include iodine-azide solution followed by aqueous starch to give white spots on blue purple; 10% acetic acid in acetone followed by starch-iodine to give white spots on blue; ammoniated copper sulfate; 0.5% bromine solution; 0.25% fluorescein; ferric chloride and potassium ferricyanide with sulfuric acid to give blue spots on green; alkaline potassium permanganate and heat to give yellow spots on pink; chloroplatinic acid with potassium iodide in acetone to give white spots on red purple; and alkaline silver nitrate. Bacillus subtilis ATCC 6633 impregnated in agar has been used to detect penicillins by observation of the zone of inhibition of the zone of inhibition after contact with the plates.

**High Performance Liquid Chromatography**\(^2\) (H.P.L.C.)

HPLC method of assay reported in Indian Pharmacopeia 2007 is as following-

Buffer solution - Prepare by 0.02 M monobasic potassium phosphate solution and adjust the pH to 6.6 with 2 M sodium hydroxide.

Test solution - Weigh accurately about 55 mg of the substance under examination and dilute to 100.0 ml with the buffer solution.
Reference solution - Weigh accurately a suitable quantity of cloxacillin sodium RS, dissolve in the buffer solution and dilute to obtain a solution containing a known concentration of about 0.55 mg per ml.

Chromatographic system
A stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel 3 to 10 μm, mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of acetonitrile, flow rate. 1 ml per minute, spectrophotometer set at 225 nm, a 20 μl loop injector. Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent. Inject alternately the test solution and the reference solution. Calculate the content of C_{19}H_{18}ClN_{3}O_{5}S.

Validated HPLC Method for determining cloxacillin concentration in plasma

Composition of mobile phase was a mixture of 74 volumes of 100 mM potassium hydrogen phosphate solution and 26 volumes of acetonitrile. Flow rate was 1ml/minute. UV-Visible detector was set at 220 nm and a 20 μl loop injector was used. The lower limit of quantification was 0.12 μg/mL of plasma. Chromatographic system Hypersil ODS-2 column; 5 μ 250×4.6 mm.

The cloxacillin was extracted from their plasma samples by mixing the samples with precipitating agent (1% formic acid in acetonitrile) in a ratio of 1:3 v/v. The precipitated proteins were than removed by centrifugation at 3000 rpm for 10 minutes, and upper organic layer was transferred to a clean tube and dried under nitrogen flow at room temperature. The residue was reconstituted with 100 μL of mobile phase and 20 μL of aliquot was injected into chromatograph.

There are various methods reported for determining cloxacillin concentration in plasma in literature. Out of the available methods, the method described by F.W. Teare et al. was found most suitable because it was practical in nature, which can be easily performed in the laboratory provided for the study.
2.1.1.4 Antimicrobial Action

Cloxacillin is bactericidal with a mode of action similar to that of benzylpenicillin, but is resistant to staphylococcal penicillinase. It is active therefore against penicillinase-producing and non-penicillinase-producing staphylococci. Its activity against streptococci such as *Streptococcus pneumoniae* and *Str. pyogenes* is less than that of benzylpenicillin, but sufficient to be useful when these organisms are present with penicillin-resistant staphylococci. Cloxacillin is virtually ineffective against *Enterococcus faecalis*.

2.1.1.5 Pharmacokinetics

Cloxacillin is incompletely absorbed from the gastrointestinal tract, and absorption is reduced by the presence of food in the stomach. After an oral dose of 500 mg, a peak plasma concentration of 7 to 15 micrograms/mL is attained in fasting subjects in 1 to 2 hours. Absorption is more complete when given by intramuscular injection and peak plasma concentrations of about 15 micrograms/mL have been observed 30 minutes after a dose of 500 mg. Doubling the dose can double the plasma concentration. About 94% of cloxacillin in the circulation is bound to plasma proteins. Cloxacillin has been reported to have a plasma half-life of 0.5 to 1 hour. The half-life is prolonged in neonates. Cloxacillin crosses the placenta and is distributed into breast milk. There is little diffusion into the CSF except when the meninges are inflamed. Therapeutic concentrations can be achieved in pleural and synovial fluids and in bone.

Cloxacillin is metabolised to a limited extent, and the unchanged drug and metabolites are excreted in the urine by glomerular filtration and renal tubular secretion. About 35% of an oral dose is excreted in the urine and up to 10% in the bile. Cloxacillin is not removed by haemodialysis. Plasma concentrations are enhanced by probenecid. Reduced concentrations in patients with cystic fibrosis have been attributed to both enhanced tubular secretion and enhanced nonrenal clearance of cloxacillin.
2.1.6 Uses and Administration

Cloxacillin is an isoxazolyl penicillin used primarily for the treatment of infections due to staphylococci resistant to benzylpenicillin. These include bone and joint infections, endocarditis, pneumonia, skin infections (including soft-tissue infections), and toxic shock syndrome.

Cloxacillin is given by mouth as the sodium salt and doses are expressed in terms of the equivalent amount of cloxacillin. 1.09 g of cloxacillin sodium is approximately equivalent to 1 g of cloxacillin. It should be given at least 30 minutes before meals as the presence of food in the stomach reduces absorption.

Usual oral doses are 250 to 500 mg four times daily. Children may be given 50 to 100 mg/kg daily in divided doses every 6 hours.

Cloxacillin sodium has also been given by intramuscular or slow intravenous injection or infusion. Other routes of administration have included intra-articular or intrapleural injection, and inhalation.

Cloxacillin may be used with other antibacterials, including ampicillin, to produce a wider spectrum of activity. Cloxacillin benzathine is used in veterinary medicine.

2.1.2 Characterization of Cloxacillin Sodium

Cloxacillin Sodium is one of the well documented drugs; therefore, its characterization can be done with the help of experimental based on its official monograph. The procured cloxacillin sodium was characterized on the basis of pharmacopoeial monograph in I.P.

Experimental

2.1.2.1 Procurement and Identification of Cloxacillin Sodium:

Cloxacillin Sodium was obtained as gift sample from Biochem Pharmaceutical Industries ltd, Mumbai. The sample was a white crystalline powder. It was identified with the help of melting point, chemical test and its IR spectral fingerprint.
Melting Point: Melting point was determined by open capillary method in Mvtex Melting Point Apparatus. It was found 170°C with decomposition which complies with official data.

Chemical Test:
Sodium cloxacillin gave positive test for sodium salts as described in Indian Pharmacopeia. The procedure was as following-
0.1 g of the substance under examination was dissolved in 2 ml of water. 2 ml of a 15 per cent w/v solution of potassium carbonate was added and heated to boiling; no precipitate was produced. 4 ml of a freshly prepared potassium antimonate solution was added and heated to boiling. Allowed to cool in ice, a dense, white precipitate was formed.

Spectral Identification: Cloxacillin Sodium was identified by FTIR spectrophotometer. FTIR Spectrum of the Cloxacillin Sodium was recorded by FTIR spectrophotometer (Shimadzu FTIR 8400S). The recorded spectrum is shown in Figure 2.3. These spectra were matched with those shown in Figure 2.1 taken from official texts.

Figure 2.3: FTIR Spectrum of Cloxacillin Sodium.
2.2 CEPHALEXIN

2.2.1 Drug Profile

2.2.1.1 Description

Name, Formula, Molecular Weight

Chemical Abstract designates cephalexin as 5-thia-1-azabicyclo [4, 2, 0] oct-2-ene-2-carboxylic acid, 7-(2-amino-2-phenyl-acetamido)-3-methyl-8-oxo.

Cephalexin monohydrate is also known as 5-thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl) amino]-3-methyl-8-oxo, monohydrate, 7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid monohydrate, and 7-(D-α-aminophenylacetamido)-3-methyl-3-cephem-4-carboxylic acid monohydrate.

\[
\begin{align*}
\text{C}_{16}\text{H}_{17}\text{N}_{3}\text{O}_{4}\text{S}\cdot\text{H}_{2}\text{O} & \\
\text{Mol. Wt. } 365.41
\end{align*}
\]

Isomers

The synthesis of the L epimer of cephalexin has been reported. The D-isomer exhibits considerably more biological activity than the L-isomer. Penicillins derived from D-α-amino acids also show more biological activity than their L-epimers.
Hydrates
Pfeiffer et. al. provided x-ray powder diffraction data for the monohydrate and dehydrate of cephalexin. Cephalexin was found to crystallize from aqueous solutions at room temperature as the dehydrate but converted to the mono-hydrate when the relative humidity was below 70%.

Appearance
Cephalexin is a white to cream-colored crystalline powder, having a characteristic odor.

2.2.1.2 Physical Properties

Infrared Spectrum
The infrared spectrum of cephalexin monohydrate recorded as a potassium bromide disc is presented in Figure 2.4. Changes in the β-lactam carbonyl stretching region (1760 cm\(^{-1}\)) can indicate opening of the β-lactam ring. Interpretation of spectrum is as follows:

<table>
<thead>
<tr>
<th>Wavelength (cm(^{-1}))</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3500-3000 (series of broad bands)</td>
<td>OH from H(_2)O and amide NH stretch</td>
</tr>
<tr>
<td>2600 (broad)</td>
<td>NH(_3^+)</td>
</tr>
<tr>
<td>1760</td>
<td>β-lactam carbonyl stretch</td>
</tr>
<tr>
<td>1690</td>
<td>Amide carbonyl stretch</td>
</tr>
<tr>
<td>1600 (very broad)</td>
<td>Carboxylate stretch</td>
</tr>
<tr>
<td>820-690</td>
<td>Mainly skeletal vibrations including out-of-plane aromatic hydrogen bending</td>
</tr>
</tbody>
</table>
Figure 2.4 Infrared Spectrum of cephalaxin monohydrate (potassium bromide disc)

Nuclear Magnetic Resonance Spectrum

Figure 2.5 shows the proton magnetic resonance spectrum of cephalaxin monohydrate. The solvent used was deuterium oxide containing a small amount of trifluoroacetic acid to enhance solubility. 3-(Trimethylsilyl)-propane-sulphonic acid, sodium salt was added as the internal reference. The spectrum was recorded on a Varian T60-A instrument. The assignment of spectrum is shown as following:

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Chemical Shift, (Coupling Constant, Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃ (3)</td>
<td>2.07 s</td>
</tr>
<tr>
<td>CH₂ (2)</td>
<td>3.30 s</td>
</tr>
<tr>
<td>HOD (solvent)</td>
<td>4.85 s</td>
</tr>
<tr>
<td>H (6)</td>
<td>4.97 d (J=4.0)</td>
</tr>
<tr>
<td>H (benzyl)</td>
<td>5.34 s</td>
</tr>
<tr>
<td>H (7)</td>
<td>5.67 d (J=4.0)</td>
</tr>
<tr>
<td>C₆H₅</td>
<td>7.60 s</td>
</tr>
</tbody>
</table>
Crystal Properties

X-Ray Powder Diffraction
Cephalexin was found to occur in several solvated crystal forms, and often in widely varying mixtures of these forms. Some of the solvated crystals prepared were the dihydrate, monohydrate, diacetonitrilate, formamidate, methanolate, and acetonitrile hydrate.

Differential Thermal Analysis

Differential thermal analysis of cephalexin monohydrate was conducted on a DuPont Model 950 thermal analyzer in a nitrogen atmosphere. A heating rate of 20°C per minute was utilized. An endotherm was noted at 123°C indicating the loss of water, and an exotherm of 203°C indicating decomposition.
Solubility

The solubility of cephalexin monohydrate in the following solvents has been reported:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>mg. Cephalexin Monohydrate per ml. solvent, 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>13.5</td>
</tr>
<tr>
<td>Methanol</td>
<td>3.4</td>
</tr>
<tr>
<td>n-Octanol</td>
<td>0.03</td>
</tr>
<tr>
<td>Chloroform</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ether</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Dissociation Constant (pKa)

The following dissociation constants were reported:

<table>
<thead>
<tr>
<th>pKa</th>
<th>Solvent</th>
<th>Carboxyl group</th>
<th>Amino group</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>66% DMF</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>66% DMF</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>H₂O</td>
<td>7.1</td>
<td></td>
</tr>
</tbody>
</table>

Optical Rotatory Dispersion

Optical rotation has been used as an auxiliary method for the quantitation of cephalexin. The specific rotation [α]D reported for cephalexin, calculated on an anhydrous basis, was +153° (C = 1.0 in H₂O).

2.2.1.3 Cephalexin Stability

The stability of cephalexin in solution is dependent on pH, degrading rapidly in basic media and remaining stable under mild acidic conditions. No loss in cephalexin activity
occurred in 72 hours at 25° C in the pH range from 3 to 5. The rate of degradation found at pH 6 and pH 7 (25° C) was approximately 3% and 18% per day respectively. With refrigeration, no appreciable loss occurred between pH 3 and pH 7 after 72 hours. In U.S.P. hydrochloric acid buffer (pH 1.2), cephalexin lost 5% activity in 24 hours at 37°C as compared to a 45% loss in phosphate buffer at pH 6.5. The antibiotic retains activity well in serum and urine as no loss in activity was noted after storage at -20° C for 14 days. Cephalexin in serum was found to lose 10%, 50% and 75% activity, respectively, after storage at 5° C, 25° C, and 37° C for 48 hours. Some organisms have been found to produce a β-lactamase (cephalosporinase) which can rapidly degrade cephalexin. Degradation of cephalexin also results from heat, strong alkali, strong acids and ultraviolet light (260 nm).

2.2.1.4 Methods of Analysis

Identification Tests

Cephalexin may be identified by infrared spectroscopy. British Pharmacopoeia utilizes two characteristic color reactions for identity. Thin layer, paper, and column chromatography have been utilized for identity purposes.

Quantitative Methods:

Titration

The iodometric titration procedure has been used for the determination of cephalexin. The method is based on the fact that the intact cephalexin molecule does not consume iodine, whereas the alkali-hydrolysis product of cephalexin does. Alkaline hydrolysis of cephalexin results in cleavage of the β-lactam ring. Variations in hydrolysis time, temperature, pH of the iodine solution and concentration of cephalexin present influence the consumption of iodine by the test solution. The method compares favorably to the
microbiological cylinder-plate method in accuracy, and is much more rapid. Possible
intermediates used in the synthesis such as 7-ADCA will also respond to the test.
An automated iodometric assay has been used recently for the assay of cephalexin and
formulations thereof. The procedure incorporates a sample hydrolysis step at 37°C for 10
minutes followed by a 5-minute iodine consumption step (pH 5.3-5.5, 37°C). Concentration of the sample is related to the decrease in iodine color measured at 350
nm. A reference standard is run concurrently through the analyzer for comparative
purposes. The auto-recommended concentration range of cephalexin (0-1.5 mg. per ml.
sample solution), with all standard curve plots passing through the origin. The
reproducibility of the method on the same sample or standard solution on a given day is
generally better than ±1% relative standard deviation (RSD). Cephalexin can be titrated
with perchloric acid in a glacial acetic acid medium. Crystal violet indicator (2% in
glacial acetic acid) may be used to determine the endpoint.
Moll and Doker have reported formal titration procedure for the determination of
cephalexin, Ampicillin and related compounds. In this procedure 4 ml. of dilute
formaldehyde solution (neutralized to the phenolphthalein end point) is added to 10 ml.
of an aqueous solution containing 15.0 mg. of cephalexin. After 2 minutes the solution is
titrated with 0.02N sodium hydroxide. A precision of ± 0.5% RSD would be achieved in
the titration of cephalexin monohydrate raw material samples. Acidic compounds as well
as amino acids must not be present as impurities in the sample. The formal titration takes
advantage of the reaction between an amino acid and formaldehyde as a mean of
suppressing the basicity of the amino group and thus making possible the titration of the
acid.

**Colorimetric Determination**

Reaction with hydroxylamine has been utilized for the colorimetric determination of
cephalexin. The method is based on the fact that hydroxylamine cleaves the β-lactam ring
(pH 7.0) to form a hydroxamic acid which forms a colored complex with ferric ion.
Degradation products or intermediates having an intact β-lactam ring react as well.
Kirschbaum has described a procedure for the colorimetric determination of the antibiotic cepharadine and related cephalosporins. An aqueous solution of the compound (1 to 30 mcg. Per ml.) is reacted with sodium hydroxide, partially neutralized, and then reacted with 5, 5'-dithiobis-(2-nitrobenzoic acid) resulting in the formation of a yellow chromophore (412 nm.). The molar absorptivity $E \times 10^{-4}$ reported for cephalexin when carried through this procedure was 1.29. The formation of the yellow chromophore was attributed to the presence of the $R$-CHNH$_2$-CO-cephalosporin nucleus, in which $R$ is a mono-, di-, or tri- enyl cyclohexyl ring.

A specific colorimetric test was developed for the determination of cephalosporin derivatives having the following intact side chain in the 7-position: $R$- CHNH$_2$-CO- cephalosporin nucleus, $R$ being a heterocyclic or aromatic ring. The D-phenylglycin derivatives of both 7-ADCA (cephalexin) and 7-ACA (cephaloglycin) respond well. These compounds (0.5 – 1.0 mg. per ml. in H$_2$O) react with acetone and sodium hydroxide at 100°C to form characteristic red chromophores (520nm). At the 1 mg. per ml. level, these tests will visually differentiate cephalexin from cephradine.

**Thin Layer Chromatography**

The following thin layer chromatographic systems have been reported

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Solvent System</th>
<th>$R_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica Gel</td>
<td>Acetonitrile/Water (3:1)</td>
<td>0.67</td>
</tr>
<tr>
<td>Silica Gel</td>
<td>Ethyl Acetate/Acetone/Acetic Acid/Water (5:2:2:1)</td>
<td>0.22</td>
</tr>
<tr>
<td>Cellulose</td>
<td>Butanol/Acetic Acid/Water (3:1:1)</td>
<td>-</td>
</tr>
<tr>
<td>Chromatogram</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheet</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Drug Profile

Sheet Ethyl Acetate/Acetic Acid/
Water (3:1:1)

Sheet Acetonitrile/Ethyl Acetate/
Water (3:1:1)

Cellulose Acetonitrile/Water (3:1) 0.50

Cellulose Butanol/Acetic Acid/
Water (3:1:1) 0.70

Cellulose is the preferred sorbent since it is inert toward cephalexin. Additional thin layer chromatography systems used for cephalexin and other cephalosporins have been tabulated. Cephalexin may be detected by ultraviolet absorbance and quenching, ninhydrin, iodoplatinate, alkaline permanganate, and phosphomolybdic acid sprays. Iodine detection and vanillin-phosphoric acid spray have also been utilized. Of the microorganisms used, Sarcina lutea is preferred over Bacillus subtilis or Staphylococcus aureus.

High Performance Liquid Chromatography\(^6\) (H.P.L.C.)

HPLC method of assay reported in Indian Pharmacopeia 2007 is as following-

Test solution. Dissolve 50 mg of the substance under examination in water and dilute to 100.0 ml with the same solvent.

Reference solution (a). Dissolve 50 mg of cephalexin monohydrate RS in water and dilute to 100.0 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of cephradine RS in 20 ml of reference solution (a) and dilute to 100 ml with water.
**Chromatographic system**

A stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 μm), mobile phase: a mixture of 2 volumes of methanol, 5 volumes of acetonitrile, 10 volumes of a 13.6 g per liter solution of potassium dihydrogen phosphate and 83 volumes of water, flow rate. 1.5 ml per minute, spectrophotometer set at 254 nm, a 20 μl loop injector. Inject reference solution (b). In the chromatogram obtained, the resolution between the peaks due to cephalexin and cephradine is not less than 4.0. Inject alternately the test solution and reference solution (a). Calculate the content of C₁₆H₁₇N₃O₄S.

**Validated HPLC Method for determination of cephalexin concentration in plasma**

Composition of mobile phase was a mixture of acetonitrile-methanol-acetate buffer of pH 4.2 (10: 10: 80 %). Flow rate was 1.4 mL/minute. UV-Visible detector was set at 254 nm and a 20 μl loop injector was used. The lower limit of quantification was 1 μg/mL of plasma. The drug was eluted from a 5 μm, C-18, reversed phase column at ambient temperature.

The cephalaxin was extracted from their plasma samples by mixing the samples with precipitating agent (1% zinc sulphate solution). The precipitated proteins were then removed by centrifugation at 3000 rpm for 10 minutes, and upper organic layer was transferred to a clean tube and dried under nitrogen flow at room temperature. The residue was reconstituted with 100 μL of mobile phase and 20 μL of aliquot was injected into chromatograph.

There are various methods reported for determining cephalaxin concentration in plasma in literature. Out of the available methods, the method described by N.W. Nazib et al. was found most suitable because it was practical in nature, which can be easily performed in the laboratory provided for the study.

**Electrophoresis**

Paper electrophoresis has been utilized by the British Pharmacopoeia as a test for impurities present in cephalaxin.
Microbiological Assays

Microbiological assays for cephalexin have been discussed by Marrelli, Wick, Mann, and Simmons and are listed in the Federal Register. Briefly, the two plate systems well suited for the determination of cephalexin in pharmaceutical formulations are the cylinder plate methods utilizing Staphylococcus aureus (ATCC 6535) and Bacillus subtilis (ATCC 6633). The assay range for both is approximately 2.5 to 20 mcg. of cephalexin per ml. The B. subtilis plate test has an advantage over the S. aureus plate test in that better defined zones of inhibition are obtained, thereby increasing the assay precision. Since degradation products of cephalexin possess practically no antimicrobial activity, rapid and precise photometric microbiological assays are possible with cephalexin. The test organism for the photometric assay is Staphylococcus aureus 9144, 3 to 3.5 hours being required for incubation. If the automated AUTOTURB system is used, precision in the order of 1-2% is possible. Cephalexin in biological fluids may be assayed by a Sarcina lutea plate system. The concentration range for assay is 0.2 to 3.5 mcg./mL.

2.2.1.5 Antimicrobial Action

Cefalexin is a beta-lactam antibacterial. It is bactericidal and acts similarly to benzylpenicillin by inhibiting synthesis of the bacterial cell wall. It is most active against Gram-positive cocci, and has moderate activity against some Gram-negative bacilli. Sensitive Gram-positive cocci include both penicillinase-and non-penicillinase-producing staphylococci, although meticillin-resistant staphylococci are resistant; most streptococci are also sensitive, but not penicillin-resistant Streptococcus pneumoniae; enterococci are usually resistant. Some Gram-positive anaerobes are also susceptible. Cefalexin is usually inactive against Listeria monocytogenes.

Among Gram-negative bacteria cefalexin has activity against some Enterobacteriaceae including strains of Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella, and Shigella spp., but not against Enterobacter, indole-positive Proteus, or
**Drug Profile**

*Serratia* spp. It is also active against *Moraxella catarrhalis* (*Branhamella catarrhalis*) and *Neisseria* spp., though *Haemophilus influenzae* is moderately resistant. *Bacteroides fragilis* and *Pseudomonas aeruginosa* are not sensitive and neither are mycobacteria, mycoplasma, and fungi.

Resistance of bacteria to cefalexin may be due to several mechanisms: the drug may be prevented from reaching its site of action, for example in some Gram-negative organisms the cell wall may be a potential barrier; the target penicillin-binding proteins may be altered so that cefalexin cannot bind with these proteins; or, most importantly, the organism may produce beta-lactamases (cephalosporinases). Cefalexin is relatively resistant to hydrolysis by staphylococcal beta-lactamase, but is inactivated by a variety of beta-lactamases produced by Gram-negative organisms; resistance of Gram-negative organisms often depends on more than one factor. Resistance can be chromosomally or plasmid-mediated and may sometimes be inducible by cephalosporins.

Certain strains of bacteria may be inhibited but not killed by cephalosporins or penicillins and in such cases the minimum bactericidal concentration is much greater than the minimum inhibitory concentration; this is known as tolerance.

As well as with other cephalosporins, some cross-resistance may occur between cefalexin and the penicillinase-resistant penicillins.

Although cefalexin is generally less potent than Cefalotin. Some strains of Gram-negative bacteria may be inhibited only by the high concentrations achievable in the urinary tract. *Haemophilus influenzae* is moderately resistant to cefalexin.

**2.2.1.6 Pharmacokinetics**

Cefalexin is almost completely absorbed from the gastrointestinal tract and produces a peak plasma concentration of about 18 micrograms/mL 1 hour after a 500-mg oral dose. If cefalexin is taken with food, absorption may be delayed, but the total amount absorbed is not appreciably altered. Up to 15% of a dose is bound to plasma proteins. The plasma half-life is about 1 hour; it increases with reduced renal function.

Cefalexin is widely distributed in the body but does not enter the CSF in significant quantities. It crosses the placenta and small quantities are found in breast milk. Cefalexin
is not metabolised. About 80% or more of a dose is excreted unchanged in the urine in the first 6 hours by glomerular filtration and tubular secretion; urinary concentrations greater than 1 mg/mL have been achieved after a dose of 500 mg. Probenecid delays urinary excretion. Therapeutically effective concentrations may be found in the bile and some may be excreted by this route. Cefalexin is removed by haemodialysis and peritoneal dialysis.

2.2.1.7 Uses and Administration

Cefalexin is a first-generation cephalosporin antibacterial. It is given by mouth for the treatment of susceptible infections including those of the respiratory and urinary tracts and of the skin. For severe infections, treatment with parenteral cephalosporins is to be preferred.

Cefalexin is usually given as the monohydrate although the hydrochloride is sometimes used. Doses are expressed in terms of the equivalent amount of anhydrous cefalexin. 1.05 g of cefalexin monohydrate and 1.16 g of cefalexin hydrochloride are each approximately equivalent to 1 g of anhydrous cefalexin.

The usual dose for adults is 1 to 2 g daily given in divided doses at 6-, 8-, or 12-hourly intervals; in severe or deep-seated infections the dose can be increased to up to 6 g daily but when high doses are required the use of a parenteral cephalosporin should be considered. Children may be given 25 to 100 mg/kg daily in divided doses to a maximum of 4 g daily.

For the prophylaxis of recurrent urinary-tract infection, cefalexin may be given in a dose of 125 mg at night. Cefalexin sodium or cefalexin lysine has been used parenterally.
2.2.2 Characterization of Cephalexin

Cephalexin is also a well documented drug; therefore, its characterization can be done with the help of experimental based on its official monograph. The procured cephalexin was characterized on the basis of pharmacopoeial monograph in I.P.

Experimental

Procurement and Identification of Cephalexin:

Cephalexin was purchased from Central Drug House, Delhi. The sample was a white to cream-colored crystalline powder, having a characteristic odor. It was identified with the help of melting point and its IR spectral fingerprints.

Melting Point: Melting point was determined by open capillary method in Mvtex Melting Point Apparatus. It was found 326-327°C, which complies with official data.

Spectral Identification: Cephalexin was identified by using FTIR spectrophotometer. FTIR Spectrum of the Cephalexin was recorded by FTIR spectrophotometer (Shimadzu FTIR 8400S). The recorded spectrum is shown in Figure 2.6. These spectra were matched with those shown in Figure 2.4 taken from official texts.

![Figure 2.6: FTIR Spectrum of Cephalexin.](image-url)
2.3 CIPROFLOXACIN

2.3.1 Drug Profile

2.3.1.1 Description

Name, Formula, Molecular Weight

1-Cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.

\[
C_{17}H_{18}F N_3 O_3 \quad \text{Mol. Wt.} = 331.35
\]

Appearance

Ciprofloxacin is obtained as a light yellow crystalline powder.

2.3.1.2 Physical Properties

Infrared spectrum

The infrared absorption spectrum of ciprofloxacin was obtained in a KBr pellet using Perkin Elmer infrared spectrophotometer. The infrared spectrum is shown in Figure 2.7 and the assignments for the major infrared absorption bands are as following:
<table>
<thead>
<tr>
<th>Frequency (cm$^{-1}$)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3490</td>
<td>O-H stretch</td>
</tr>
<tr>
<td>3320</td>
<td>N-H stretch of piperazinyl moiety</td>
</tr>
<tr>
<td>2930</td>
<td>Aliphatic C-H stretch</td>
</tr>
<tr>
<td>1696</td>
<td>C=O stretch of carboxyl group</td>
</tr>
<tr>
<td>1605</td>
<td>C=O stretch of quinoline carboxyl</td>
</tr>
</tbody>
</table>

**Figure 2.7 Infrared spectrum of ciprofloxacin**

**Nuclear Magnetic Resonance spectrum**

The proton NMR spectrum of ciprofloxacin was obtained using a Bruker Advance system, operating at 300, 400 and 500 MHz. The sample was dissolved in D$_2$O and tetramethylsilane (TMS) was used as the internal standard. The proton NMR spectrum is shown in Fig. 2.8 and assignments for the proton NMR bands of ciprofloxacin are found as follow:
**Assignment**

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Chemical Shift, (Coupling Constant, Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2</td>
<td>8.63 s</td>
</tr>
<tr>
<td>H-5</td>
<td>7.46 d</td>
</tr>
<tr>
<td>H-8</td>
<td>7.52 d</td>
</tr>
<tr>
<td>H-2’, 6’ or 3’, 5’</td>
<td>3.66 d</td>
</tr>
<tr>
<td>H-1a</td>
<td>1.22 s</td>
</tr>
<tr>
<td>H-1b, 1c</td>
<td>1.47 d</td>
</tr>
</tbody>
</table>

**Figure 2.8** H¹-NMR spectrum of ciprofloxacin in D₂O

**Solubility characteristics**

Ciprofloxacin is practically insoluble in water, very slightly soluble in dehydrated alcohol & dichloromethane, and soluble in dilute acetic acid.

**Optical activity**

Since ciprofloxacin has no centers of dissymmetry, it does not exhibit optical activity.
2.3.1.3 Stability and storage

Solutions of ciprofloxacin are light sensitive and should be protected from light and freezing. When the concentrate formulated for intravenous injection, or the 1.2 g pharmacy bulk package, is diluted with 5% dextrose injection or 0.9% sodium chloride injection to a final concentration of 0.5-2 mg/ml, the resultant solution is stable for up to 14 days when stored at room temperature or when refrigerated at 2-8°C. The commercially available injection for intravenous infusion that contains 2 mg/mL in 5% dextrose is provided in a plastic container fabricated from a specially formulated polyvinyl chloride (PVC). Tammilehto et al. used thin-layer chromatography to study the degradation of ciprofloxacin hydrochloride solutions after these were irradiated by a high pressure mercury lamp.

2.3.1.4 Reported Methods of Analysis

Identification

The European Pharmacopoeia recommends the use of infrared absorption spectrophotometry for the identification of the pure drug substance. The procedure entails an examination by infrared absorption spectrophotometry, and a comparison with the spectrum obtained with the ciprofloxacin reference standard.

Quantitative Methods

Titration Method

Four DNA topoisomerase inhibitors, including ciprofloxacin (alone or in mixture with other drugs), were determined by a titration method. Tablets containing ciprofloxacin were extracted three times with 30 mL of methanol. A portion of the resulting solution was mixed with 2 mL water, and that solution was titrated with aqueous 5mM NaOH,
5mM tetrabutyl-ammonium hydroxide, or 5 mM AgNO\textsubscript{3} by adding 0.1 mL increments at 2 min intervals. The end-point was detected using the solution conductance.

**Spectrophotometric method**

Rizk et al\textsuperscript{9} developed a sensitive and selective derivative UV-spectrophotometric method for the determination of three fluoroquinolone compounds, including ciprofloxacin, in formulations and spiked biological fluids. The method depends on the complexation of Cu (II) with the studied compounds in an aqueous medium. A linear correlation was established between the amplitude of the peak and the drug concentration over the range of 35-120 ng/mL. The detection limit was reported as 1.3 ng/mL. The method was used for the determination of the ciprofloxacin bulk drug substance and its tablet formulation, with an overall percentage recovery of 99.22±0.55 to 100.33±1.60.

**Chemiluminescence method**

Aly et al\textsuperscript{9} reported a rapid and sensitive chemiluminescence (CL) method for the determination of three fluoroquinolone derivatives, including ciprofloxacin, in both pharmaceutical dosage forms and in biological fluids. The method is based on the CL reaction of the drugs with tris (2,2’-bipyridyl)ruthenium(II) and cerium(IV) in sulfuric acid medium. The CL intensity was proportional to the concentration of ciprofloxacin in solution over the range 0.05-6 \(\mu\)g/mL, and the limit of detection was reported to be 26 nM.

**Electrochemical methods**

The voltammetric behavior of the Ni (II) complexes of three 4-quinolone antibacterial compounds in tablets, including ciprofloxacin, was studied using direct current (Dct), differential pulse (DPP) and alternating current polarography (Act). A well defined, cathodic wave was detected at pH 5.5 for ciprofloxacin. The current-concentration relationship was found to be linear over the range 2 - 6.4 \(\times\) 10\textsuperscript{-5} M and 0.8 - 5.6 \(\times\) 10\textsuperscript{-5} M.
for ciprofloxacin using DCt and DPP modes, respectively. Limits of detection (S/N=2) for these species were found to be about $2 \times 10^{-7}$ M. The average percent recovery for ciprofloxacin was 99.58±0.72 to 100.50±0.79, and 99.50±0.71 to 100.17±0.29, when using DCt and DPP, respectively.

Thin layer chromatography

Mixtures of antibacterial agents (including ciprofloxacin hydrochloride) together with p-aminophenol (internal standard) in 0.1 M HCl were analyzed by thin layer chromatography (TLC). The system used a silica gel GF254 plate and a mobile phase consisting of chloroform/methanol/concentrated ammonium hydroxide (15:10:3). The chromatogram was examined under a UV lamp at 254 nm, and it was found that all spots were cleanly separated.

High Performance liquid chromatographic method

HPLC method of assay reported in Indian Pharmacopeia (2007) is as following-

Test solution. Weigh accurately about 25 mg, add 0.2 ml of a solution containing 7 per cent v/v of phosphoric acid and add sufficient of the mobile phase to produce 50.0 ml.
Reference solution (a). Prepare in the same manner as the test solution using an accurately weighed quantity of ciprofloxacin RS in place of the substance under examination.
Reference solution (b). A 0.05 per cent w/v solution of ciprofloxacin ethylenediamine analog RS in reference solution (a).
Chromatographic system
A stainless steel column 25 cm × 4 mm, packed with octadecylsilyl silica gel (5 μm), mobile phase: a mixture of 87 volumes of 0.025 M phosphoric acid, previously adjusted with triethylamine to a pH of 3.0 ± 0.1, and 13 volumes of acetonitrile, flow rate. 1.5 ml per minute, column temperaure. 30° ± 1°, spectrophotometer set at 278 nm, a 10 μl loop injector.
Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6. The column efficiency, determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 4.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent. Inject alternately the test solution and reference solution (a). Calculate the content of $\text{C}_{17}\text{H}_{18}\text{FN}_3\text{O}_3$.

**Validated HPLC Method for determining ciprofloxacin concentration in plasma**

Composition of mobile phase was a mixture of 0.01 M phosphate buffer (pH = 2.6, adjusted with concentrated orthophosphoric acid) and methanol in a ratio of 82 and 18 v/v respectively. Flow rate was 1.5 mL/minute. UV-Visible detector was set at 277 nm and a 20 µl loop injector was used. The lower limit of quantification was 20 ng/mL of plasma. Separation was carried on a Novapak C18 column.

The ciprofloxacin was extracted from their plasma samples by mixing the samples with precipitating agent (1% formic acid in acetonitrile) in a ratio of 1:3 v/v. The precipitated proteins were than removed by centrifugation at 3000 rpm for 10 minutes, and upper organic layer was transferred to a clean tube and dried under nitrogen flow at room temperature. The residue was reconstituted with 100 µL of mobile phase and 20 µL of aliquot was injected into chromatograph.

There are various methods reported for determining ciprofloxacin concentration in plasma in literature, out of the available methods, the method described by M. Amini et al. was found most suitable because it was practical in nature, which can be easily performed in the laboratory provided for the study.
Gas chromatography

Gas chromatography was used to analyze the residual piperazine of ciprofloxacin and norfloxacin in pharmaceutical preparations. The analyte was dissolved in water, and the solution analyzed by GC on a fused silica column (30 m × 0.3 mm i.d.) coated with 5% cross linked ph-Me silicone (3 μm). The temperature Programme consisted of an increase to 50° C, holding for 5 min, ramping to 180° C (ramp = 15°C/min), and finally holding for 10 min at 180°C (5°C /min). Nitrogen was used as the carrier gas (flow rate of 1ml/min), detection was effected using flame ionization. Piperazine was determined in the two mixtures by mixing for 10 min with cyclohexane, filtering, partitioning the filtrate with water, heating the aqueous phase at 45° C for 10 min, and then analyzing as above. The calibration graph was linear for 0.4-10 ppm piperazine, with a RSD (n =10) of 2.1% for 2μg piperazine. The recovery from ciprofloxacin was 98.1-98.4%.

2.3.1.5 Antimicrobial Action

Ciprofloxacin is bactericidal and acts by inhibiting the A subunit of DNA gyrase (topoisomerase) which is essential in the reproduction of bacterial DNA. It has a broader spectrum of activity and is more potent in vitro than the non-fluorinated quinolone nalidixic acid. Activity may be reduced in acid media.

Spectrum of activity. Among Gram-negative aerobic bacteria, ciprofloxacin is active in vitro against Enterobacteriaceae, including Escherichia coli and Citrobacter, Enterobacter, Klebsiella, Proteus, Providencia, Salmonella, Serratia, Shigella, and Yersinia spp. It is also active against Pseudomonas aeruginosa, but less so against other Pseudomonas spp. Haemophilus ducreyi, H. influenzae, Moraxella catarrhalis (Branhamella catarrhalis), and Neisseria gonorrhoeae are all very sensitive, including beta-lactamase-producing strains; N. meningitidis is also susceptible. Other Gram-negative aerobic bacteria reported to be sensitive to ciprofloxacin have included Acinetobacter spp., Campylobacter spp., Gardnerella vaginalis, Helicobacter pylori,
Legionella spp., Pasteurella multocida, and Vibrio spp. Variable activity has been reported against Brucella melitensis.

Among Gram-positive aerobic bacteria, ciprofloxacin is active against staphylococci, including penicillinase-producing and penicillinase-nonproducing strains, and against some meticillin-resistant strains. Streptococci, in particular Streptococcus pneumoniae and enterococci, are less susceptible. Other Gram-positive bacteria sensitive to ciprofloxacin in vitro are Bacillus anthracis and Corynebacterium spp.

Most anaerobic bacteria, including Bacteroides fragilis and Clostridium difficile, are resistant to ciprofloxacin, although some other Clostridium spp. may be susceptible. Ciprofloxacin has some activity against mycobacteria, mycoplasmas, rickettsias, and the protozoan Plasmodium falciparum. Chlamydia trachomatis is moderately susceptible, and Nocardia asteroides and Ureaplasma urealyticum are usually considered to be resistant. The spirochaete Treponema pallidum and fungi are also resistant.

Activity with other antimicrobials. There have been some reports of enhanced activity in vitro when ciprofloxacin has been used with other antimicrobials, such as aminoglycosides or azlocillin against Staphylococcus aureus and Pseudomonas aeruginosa, imipenem against Ps. aeruginosa, and cefotaxime or clindamycin against anaerobic bacteria.

Acquired resistance. Resistant strains, particularly of Staph. aureus (including meticillin-resistant strains) and Ps. aeruginosa have emerged during treatment with ciprofloxacin. There is complete cross-resistance between ciprofloxacin and the other fluoroquinolones, but not between ciprofloxacin and nalidixic acid. Like nalidixic acid, resistance to ciprofloxacin appears, so far, to be chromosomally rather than plasmid-mediated.

2.3.1.6 Pharmacokinetics\textsuperscript{9}

Ciprofloxacin is rapidly and well absorbed from the gastrointestinal tract. Oral bioavailability is approximately 70\% and a peak plasma concentration of about 2.5 micrograms/mL is achieved 1 to 2 hours after a dose of 500 mg by mouth. Absorption
may be delayed by the presence of food, but is not substantially affected overall. The plasma half-life is about 3.5 to 4.5 hours and there is evidence of modest accumulation. Half-life may be prolonged in renal impairment—a value of 8 hours has been reported in end-stage renal disease—and to some extent in the elderly. There is limited information on the effect of hepatic impairment; the half-life of ciprofloxacin has been reported to be slightly prolonged in patients with severe cirrhosis of the liver. With one or two exceptions, most studies have shown the pharmacokinetics of ciprofloxacin to be not markedly affected by cystic fibrosis.

Plasma protein binding ranges from 20 to 40%. Ciprofloxacin is widely distributed in the body and tissue penetration is generally good. It appears in the CSF, but concentrations are only about 10% of those in plasma when the meninges are not inflamed. Ciprofloxacin crosses the placenta and is also distributed into breast milk. High concentrations are achieved in bile.

Ciprofloxacin is eliminated principally by urinary excretion, but non-renal clearance may account for about a third of elimination and includes hepatic metabolism, biliary excretion, and possibly transluminal secretion across the intestinal mucosa. At least 4 active metabolites have been identified. Oxociprofloxacin appears to be the major urinary metabolite and sulfociprofloxacin the primary faecal metabolite. Urinary excretion is by active tubular secretion as well as glomerular filtration and is reduced by probenecid; it is virtually complete within 24 hours. About 40 to 50% of an oral dose is excreted unchanged in the urine and about 15% as metabolites. Up to 70% of a parenteral dose may be excreted unchanged within 24 hours and 10% as metabolites. Faecal excretion over 5 days has accounted for 20 to 35% of an oral dose and 15% of an intravenous dose. Only small amounts of ciprofloxacin are removed by haemodialysis or peritoneal dialysis.
2.3.1.7 Uses and Administration

Ciprofloxacin is a fluorinated 4-quinolone or fluoroquinolone antibacterial with a wider spectrum of activity than nalidixic acid and more favourable pharmacokinetics allowing its use in systemic infections. It has been used in the treatment of a wide range of infections including anthrax, biliary-tract infections, infected bites and stings, bone and joint infections, cat scratch disease, chancroid, exacerbations of cystic fibrosis, gastro-enteritis (including travellers' diarrhoea and campylobacter enteritis, cholera, salmonella enteritis, and shigellosis), gonorrhoea, infections in immunocompromised patients (neutropenia), legionnaires' disease, otitis externa, otitis media, peritonitis, Q fever, lower respiratory-tract infections (including pseudomonal infections in cystic fibrosis, but excluding infections due to *Streptococcus pneumoniae* such as pneumococcal pneumonia), septicaemia, skin infections (including soft-tissue infections), spotted fevers, typhoid and paratyphoid fever, typhus, and urinary-tract infections. Ciprofloxacin is used for meningococcal meningitis prophylaxis. It is also used for surgical infection prophylaxis. Fluoroquinolones such as ciprofloxacin and ofloxacin have been tried in the treatment of opportunistic mycobacterial infections and tuberculosis; ofloxacin may be used in the treatment of leprosy. Fluoroquinolones such as ciprofloxacin and ofloxacin are used topically in the treatment of eye infections.

2.3.1.8 Administration and dosage

Ciprofloxacin is given by mouth as the hydrochloride or base, by intravenous infusion as the lactate, and in eye drops or eye ointment as the hydrochloride. Doses and strengths are expressed in terms of the equivalent amount of ciprofloxacin base. 291.1 mg of ciprofloxacin hydrochloride is approximately equivalent to 250 mg of ciprofloxacin. 127 mg of ciprofloxacin lactate is approximately equivalent to 100 mg of ciprofloxacin. The usual adult oral dose of ciprofloxacin ranges from 250 to 750 mg twice daily depending on the severity and nature of the infection. The usual adult intravenous dose is 100 to 400 mg twice daily, given over 30 to 60 minutes as a solution containing the
equivalent of 1 to 2 mg/mL. A dose of 100 mg twice daily by mouth is suitable in women with acute uncomplicated cystitis.

For acute exacerbations of cystic fibrosis associated with *Pseudomonas aeruginosa* infection, ciprofloxacin may be given to adolescents and children aged 5 years or more in a dose of 20 mg/kg by mouth twice daily, up to a maximum of 750 mg twice daily. Alternatively, a dose of 10 mg/kg may be given by intravenous infusion over 60 minutes three times daily, to a maximum of 400 mg three times daily.

For inhalation anthrax, ciprofloxacin may be given to children and adolescents for 60 days in a dose of 15 mg/kg twice daily by mouth, up to a maximum of 500 mg twice daily. Alternatively, a dose of 10 mg/kg twice daily may be given by intravenous infusion, up to a maximum of 400 mg twice daily.

Ciprofloxacin is not generally recommended for other uses in children and adolescents but, if considered essential, doses of 5 to 15 mg/kg twice daily by mouth or 4 to 8 mg/kg twice daily intravenously have been suggested.

Doses should be reduced in patients with severe renal impairment.

Single oral doses of 250 or 500 mg are used for the treatment of gonorrhoea, depending upon patterns of resistance. A single oral dose of 500 mg is suggested for meningococcal meningitis prophylaxis. A single oral dose of 750 mg is suggested for surgical infection prophylaxis, given 60 to 90 minutes before the procedure.

For corneal ulcers and superficial ocular infections caused by susceptible strains of bacteria ciprofloxacin is given as the hydrochloride in eye drops and eye ointment containing the equivalent of 0.3% of ciprofloxacin base.

### 2.3.2 Characterization of Ciprofloxacin

Ciprofloxacin is one of the best documented drugs; therefore, its characterization can be done with the help of experimental based on its official monograph. The procured ciprofloxacin was characterized on the basis of pharmacopeial monograph in I.P.
Experimental

Procurement and Identification of Ciprofloxacin:

Ciprofloxacin was purchased from Central Drug House, Delhi. The sample was a light yellow crystalline powder. It was identified with the help of melting point and its IR spectral fingerprints.

Melting Point: Melting point was determined by open capillary method in Mvetex Melting Point Apparatus. It was found 318-320°C, which complies with official data.

Spectral Identification: Ciprofloxacin was identified by using FTIR spectrophotometer. FTIR Spectrum of the Ciprofloxacin was recorded by FTIR spectrophotometer (Shimadzu FTIR 8400S). The recorded spectrum is shown in Figure 2.9. These spectra were matched with those shown in Figure 2.7 taken from official texts.

Figure 2.9: FTIR Spectrum of Ciprofloxacin.
REFERENCES


