CHAPTER – 1

INTRODUCTION
1 Introduction

1.1 Quinolones Antibacterial (1 - 5)

The first quinolone emerged in the early 1960's with the isolation of 6-chloro - 1 - ethyl - 1, 4 - dihydro - 4 - oxoquinoline - 3 - carboxylic acid, a by product of the commercial preparation of chloroquine. This compound was found to have antibacterial activity and was subsequently modified to produce Nalidixic acid (NA). The first drug of quinolones antibacterial is Nalidixic acid which was found by Hasher et al in 1962. It was mainly active against gram-negative rods, and not cross resistant with existing antibacterial agent. NA was used for urinary, biliary and intestinal tract infection in 1963. Oral absorption of a Nalidixic acid was low with peak serum concentration of < 0.5 µg/mL and it was therefore unstable for the treatment of systemic infection. The addition of fluoride to the original quinolones antibacterial compound yielded a new class of drugs the fluoroquinolone, which have a broader antibacterial spectrum and improved pharmacokinetic properties. These agents were introduced in the late 1960's.

Fluoroquinolones (6 - 14)

Fluoroquinolones (FQs) are synthetic broad spectrum antibacterial agents related to the other quinolones Nalidixic acid and cinoxacin. The fluoroquinolones are a heterogeneous group of synthetic compounds each having a bi-cyclic nucleus with various substituents contributing of the antimicrobial activity, pharmacokinetic properties and adverse effects. These agents contain 6-fluoro and 7-piperazine substituents which greatly enhance their antimicrobial activity compared to Nalidixic acid. The fluorine molecule provided increased potency against gram negative organism and broadens the spectrum
include gram-positive organism. The piperazine moiety is reasonably for antipseudomonal activity. These agents are bactericidal.

Norfloxacin (NFLX) having 6-flouro group and piperazinyl group was discovered in 1978. This was the start of new quinolones era. NFLX was about one order magnitude more potent than the old quinolones in antibacterial activity and its antibacterial spectrum was broadened to gram positive bacteria. Further more NFLX was metabolically stable and penetrated well into various tissues although its oral absorption was not very good and its antibacterial activity towards gram-positive bacteria was slightly week. It was successfully used for the treatment of urinary tract infections.

Enoxacin (ENX) and Pefloxacin (PFLX) reported in 1979 and Ofloxacin (OFLX) reported in 1981, had the same antibacterial activity as NFLX and better oral absorption. Ciprofloxacin (CPLX) discovered in 1982 showed more potent antibacterial activity than NFLX, FNX, PFLX and OFLX. All these compounds exhibit intrinsically similar antibacterial properties. After that many new quinolone derivatives have been synthesized e.g. Lomefloxacin, Fleroxacin, Temofloxacin, Tosufloxacin, Sparfloxacin, Gatifloxacin, Moxifloxacin, DR-3355, etc.

The older quinolones are characterized by an unfavorable pharmacokinetic profile (low serum concentrations, short half-life etc.). However the introduction of fluorine atom in the C₆ position and different side chain in the C₁ and C₇ position resulted in broadening of the antibacterial spectrum increased activity and much better pharmacokinetic properties with higher serum concentrations, long elimination half-time and excellent bio-availability after oral administration.
The newer fluoroquinolone antibacterials also have improved pharmacokinetic parameters compared to the original quinolones. They are rapidly and almost completely absorbed from the gastrointestinal tract. Peak serum concentrations obtained after oral administration are very near to those achieved with intravenous administration. Consequently, the oral route is generally preferred in most situations, and hospitalized patients should be switched from intravenous to oral formulations as soon as oral medications can be tolerated.

Absorption of orally administered fluoroquinolones is significantly decreased when these agents are co-administered with aluminum, magnesium, calcium, iron or zinc, because of the formation of insoluble drug cationic chelate complexes in the gastrointestinal tract. The problem can be overcome largely by administering products containing these metal ions at least four hours before or two hours after oral administration of a fluoroquinolone. Because sucralfate contains aluminum, it can also reduce absorption of the quinolones. Adequate spacing of administration times has not been determined, and co-administration of quinolones and sucralfate should be avoided.

Because the fluoroquinolones have a large volume of distribution, they concentrate in tissues at levels that often exceed serum drug concentrations. Penetration is particularly high in renal, lung, prostate, bronchial, nasal, gall bladder, bile and genital tract tissues. Urine drug concentrations of some fluoroquinolones, such as Ciprofloxacin and Ofloxacin, may be as much as 25 times higher than serum drug concentrations. Consequently, these agents are especially useful in treating urinary tract infections.
Distribution of the fluoroquinolones into respiratory tract tissues and fluids is of particular interest because of the activity of these agents against common respiratory pathogens. Trovafloxacin penetrates non inflamed meninges and may have a future role in the treatment of bacterial meningitis.

The long half-lives of the newer fluoroquinolones allow once or twice daily dosing. The quinolones vary with respect to the relative contribution of renal and nonrenal pathways for their elimination. Only Ofloxacin and Levofloxacin are exclusively eliminated by the kidney. Renal and nonrenal (gastrointestinal or hepatic) mechanisms are responsible for the elimination of Nalidixic acid, Cinoxacin, Norfloxacin, Ciprofloxacin, Enoxacin, Lomefloxacin, Gatifloxacin, Moxifloxacin and Sparfloxacin. Dosage adjustments based on estimated creatinine clearance values must be made for the agents with significant renal elimination. In most instances, administering the usual dose at an extended interval is recommended.

Trovafloxacin is eliminated primarily by hepatic mechanisms. Approximately 50 percent of a Trovafloxacin dose is conjugated in the liver; 43 percent is excreted unchanged in the feces. Significant hepatic disease may increase the elimination half-life of Trovafloxacin. Dosage adjustments are required in patients with mild to moderate cirrhosis. No data are available on patients with severe liver disease.

With some exceptions, agents in the four fluoroquinolone classes can also be grouped by their clinical indications. The drugs can be further differentiated based on available formulations, required dosage adjustments in renal or hepatic disease, significant adverse effects and significant drug interactions.
Table 1.1 Classification of Quinolones Antibacterial (15)

<table>
<thead>
<tr>
<th>Generation</th>
<th>Agents</th>
<th>Antimicrobial spectrum</th>
<th>General clinical indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Nalidixic acid</td>
<td>Gram-negative organisms (but not Pseudomonas species)</td>
<td>Uncomplicated urinary tract infections</td>
</tr>
<tr>
<td></td>
<td>Cinoxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>Norfloxacin</td>
<td>Gram-negative organisms (including Pseudomonas species), some gram-positive organisms</td>
<td>Uncomplicated and complicated urinary tract infections and pyelonephritis, sexually transmitted diseases, prostatitis, skin and soft tissue infections</td>
</tr>
<tr>
<td></td>
<td>Lomefloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enoxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>Levofloxacin</td>
<td>Same as for second-generation agents plus expanded gram-positive coverage (penicillin-sensitive and penicillin-resistant S. pneumoniae ) and expanded activity against atypical pathogens</td>
<td>Acute exacerbations of chronic bronchitis, community-acquired pneumonia</td>
</tr>
<tr>
<td></td>
<td>Sparfloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gatifloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moxifloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fourth</td>
<td>Trovafloxacin</td>
<td>Same as for third-generation agents plus broad anaerobic coverage</td>
<td>Same as for first-, second- and third-generation agents plus intra-abdominal infections, nosocomial pneumonia, pelvic infections</td>
</tr>
</tbody>
</table>


### Table 1.2  Distinguishing characteristics of quinolone antibacterials (11, 15)

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>HALF-LIFE (HRS)</th>
<th>ROUTE OF ADMINISTRATION</th>
<th>DOSAGE ADJUSTMENT REQUIRED</th>
<th>SIGNIFICANT ADVERSE EFFECTS</th>
<th>SIGNIFICANT DRUG INTERACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalidixic acid</td>
<td>1.0-1.5</td>
<td>Oral</td>
<td>Renal impairment</td>
<td>Photo toxicity, abdominal pain, nausea</td>
<td>Warfarin, Cyclosporine</td>
</tr>
<tr>
<td>Cinoxacin</td>
<td>1.1-2.7</td>
<td>Oral</td>
<td>Renal impairment</td>
<td>Photo toxicity (mild)</td>
<td>Warfarin, Ranitidine, Bismuth subsalicylate, Theophylline, Caffeine</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>2.3-5.5</td>
<td>Oral</td>
<td>Renal impairment</td>
<td>Insomnia</td>
<td>Warfarin</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>5.0-8.0</td>
<td>Oral, Intravenous</td>
<td>Renal or hepatic impairment</td>
<td>Nausea, vomiting, abdominal pain</td>
<td>Warfarin, Theophylline, Caffeine, cyclosporine, glyburide</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>3.0-5.4</td>
<td>Oral, Intravenous</td>
<td>Renal impairment</td>
<td>Headache, nausea, diarrhea</td>
<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>6.0</td>
<td>Oral, Intravenous</td>
<td>Renal impairment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>21.0</td>
<td>Oral</td>
<td>Renal impairment</td>
<td>Photo toxicity, QT interval prolongation,</td>
<td>Drugs that prolong the QT interval, including class I antiarrhythmics, tricyclic antidepressants, phenothiazines, disopyramide and erythromycin</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>7.0</td>
<td>Oral, Intravenous</td>
<td>Renal impairment</td>
<td></td>
<td>Same as for sparfloxacin</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>12.0</td>
<td>Oral</td>
<td>Hepatic impairment</td>
<td>QT-interval prolongation</td>
<td>Same as for sparfloxacin</td>
</tr>
<tr>
<td>Trovafoxacin</td>
<td>7.8</td>
<td>Oral</td>
<td>Hepatic impairment</td>
<td>Dizziness severe, hepatotoxicity, candidal vaginitis</td>
<td>Morphine, citric acid.</td>
</tr>
<tr>
<td>Alfloxacin</td>
<td></td>
<td>Intravenous</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Nitroimidazole** (13)

Amoebiasis is caused by protozoal parasite called Entamoeba histolytica. Amoebiasis occurs mainly in tropics, but can also occur in temperate areas with poor sanitation. The most common manifestation is dysentery, but the disease can also bring about fever, chills and intestinal burning. Blood born organisms have been reported to cause abscesses in the liver and lungs.

The drugs belonging to nitroimidazole group like Metronidazole, Tinidazole, Ornidazole are widely used in treatment of amoebiasis. The drugs belonging to this group have excellent activity against anaerobic microorganisms. The antimicrobial activity of nitroimidazole is due to the reduction of the nitro group to a more reactive amine that attacks microbial DNA, brings about loss of helical structure of DNA and subsequent DNA breakage thus inhibiting further synthesis and causing degradation of existing DNA.

As compared to Metronidazole and Tinidazole, Ornidazole exhibits the lowest MICs against anaerobes like B.fragilis, Clostridium perfringens, Gram-negative bacilli, Clostridium spp., anaerobic gram-positive cocci. It is less bitter as compared to Tinidazole. Also it shows less side effects compared to Metronidazole and Tinidazole as proved by a study involving 53 patients.

**Benefits of gatifloxacin and ornidazole**

- Both are pharmacokinetically compatible.
- Expanded spectrum of activity covering aerobic as well as anaerobic bacteria.
No drug-drug interactions.

Both the drugs are well tolerated.

**Gatifloxacin and Omidazole Combination: Place in Therapy**

- Respiratory tract infections like lung abscess, aspiration pneumonia, empyema and bronchiectasis.
- ENT infections like chronic sinusitis, chronic suppurative otitis media, cholesteatoma and mastoiditis.
- Dermatological infections like cellulitis, breast and other cutaneous abscesses, gangrene and decubitus ulcers.
- Orofacial and dental infections.
- Surgical wound infections.
- Gynaecological infections including PID.

### Table 1.3 Comparative activities of Nitroimidazole Against anaerobes

<table>
<thead>
<tr>
<th>Organism</th>
<th>Drug</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; mcg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. fragilis</td>
<td>Metronidazole</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Tinidazole</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Omidazole</td>
<td>1.6</td>
</tr>
<tr>
<td>Gram-negative bacilli</td>
<td>Metronidazole</td>
<td>≤ 0.1</td>
</tr>
<tr>
<td></td>
<td>Tinidazole</td>
<td>≤ 0.1</td>
</tr>
<tr>
<td></td>
<td>Omidazole</td>
<td>≤ 0.1</td>
</tr>
<tr>
<td>Clostridium perfingeries</td>
<td>Metronidazole</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Tinidazole</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Omidazole</td>
<td>0.8</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>Metronidazole</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Tinidazole</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Omidazole</td>
<td>0.4</td>
</tr>
<tr>
<td>Anaerobic gram-positive cocci</td>
<td>Metronidazole</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Tinidazole</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Omidazole</td>
<td>0.4</td>
</tr>
</tbody>
</table>
1.2 Drug Profile

1.2.1 Gatifloxacin (14 - 18)

- Nomenclature

*Chemical name:* \((\pm)-1\text{-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-}(3\text{-methyl-1-piperazinyl})\text{-4-oxo-3-quinolinecarboxylic acid sesquihydrate.}*

*Generic name:* Gatifloxacin

- Formula

*Empirical formula:* \(\text{C}_{19}\text{H}_{22}\text{FN}_{3}\text{O}_{4} \cdot 1.5\text{H}_{2}\text{O}\)

*Structural formula:*

![Structural formula of gatifloxacin]

- Molecular Weight

402.42

- Appearance

Gatifloxacin is white to pale yellow colored crystalline powder.

- Physical properties

*Melting range*: 182 - 185 °C

*Solubility:* The solubility of Gatifloxacin is pH dependent. The
maximum aqueous solubility (40 - 60 mg/mL) occurs at a pH range of 2 to 5. The solubility of Gatifloxacin in water and various organic solvents at 25 ± 0.5°C are listed in the following table

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility (mg / mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid (100%)</td>
<td>400</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>7.9</td>
</tr>
<tr>
<td>Methanol</td>
<td>2.4</td>
</tr>
<tr>
<td>Ethanol (99.5%)</td>
<td>2.1</td>
</tr>
<tr>
<td>1-Octanol</td>
<td>1.4</td>
</tr>
<tr>
<td>Diethyl Ether</td>
<td>0.33</td>
</tr>
<tr>
<td>Water</td>
<td>1.2</td>
</tr>
</tbody>
</table>

• **pKa(s)** pK$_{a1}$=5.94, pK$_{a2}$=9.21

• **Spectral analysis**

Gatifloxacin solutions were prepared in water, 0.1 N HCL, 0.1 N NaOH. U.V. absorption maxima of these solutions were as follows-

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{max}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>286</td>
</tr>
<tr>
<td>0.1 N HCl</td>
<td>292</td>
</tr>
<tr>
<td>0.1 N NaOH</td>
<td>289</td>
</tr>
</tbody>
</table>
• **I.R. Spectra**

<table>
<thead>
<tr>
<th>Wave length Cm$^{-1}$</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3400</td>
<td>O-H stretching</td>
</tr>
<tr>
<td>1720</td>
<td>C=O stretch of carboxylic acid</td>
</tr>
<tr>
<td>1640</td>
<td>C=O stretch of pyridine carbonyl</td>
</tr>
<tr>
<td>1210</td>
<td>Absorption of aryl fluorides</td>
</tr>
<tr>
<td>810</td>
<td>C-H bending of trisubstitution double bond</td>
</tr>
</tbody>
</table>

• **Therapeutic category**  Antibacterial

• **Mechanism of action** (11,19,20)

DNA gyrase and topoisomerase IV are thought to be essential for replication of DNA and partition of replicated chromosomal DNA. DNA gyrase, a tetrameric enzyme consisting of two A and two B subunits, is a primary target of fluoroquinolones in Escherichia coli and is the only enzyme capable of introducing negative super helical twists into bacterial DNA. The two subunits of gyrase are encoded by gyrA and gyrB, which are also potential sites of mutation and subsequent quinolone resistance. Topoisomerase IV, a recently characterized topoisomerase, seems to be a primary target of many fluoroquinolones in gram-positive bacteria such as Staphylococcus aureus and Streptococcus pneumoniae. Bacterial topoisomerase IV appears to be the principal enzyme that resolves or decatenates interlocked daughter DNA circles occurring at the completion of a round of DNA replication, allowing segregation of daughter chromosomes into daughter
cells. Topoisomerase IV, like DNA gyrase, is composed of four subunits, two each of the parC and parE gene products.

As part of the topoisomerase reaction mechanism, DNA gyrase and topoisomerase IV transiently break the DNA backbone and pass a double strand of DNA through those breaks, thus introducing a negative supercoil into the strand (Fig.1). Fluoroquinolone antibiotics target DNA gyrase and topoisomerase IV while these enzymes are functionally attached to the DNA strand, resulting in a drug-enzyme-DNA complex in which DNA probably remains broken. Cell death apparently results from release of double-stranded DNA breaks from numerous drug-enzyme-DNA complexes throughout the chromosome.

**Fig. 1.1 : Mechanism of action: Fluoroquinolone**

- **Structure activity relationship**

Like most fluoroquinolones, gatifloxacin has a piperazine group at the C7 position. The methyl substitute on the piperazine ring
contributes to its gram-positive activity, prolongs the half-life (allowing for once-daily dosing), provides metabolic stability (as evident by primarily renal elimination of unchanged drug), and may limit potentially adverse interactions with drug-metabolizing enzymes (17). Gatifloxacin has a cyclopropyl group at the N_1 position that enhances gram-negative activity and provides some gram-positive activity. Gatifloxacin lacks the 2,4-difluorophenyl group at the N_1 position that was postulated to induce hepatic and hematological toxicities associated with trovafloxacin and temafloxacin, respectively (15,21). Most notably, gatifloxacin is characterized by the presence of a methoxy group at the C_8 position. This substituent, also possessed by moxifloxacin, confers enhanced activity against both DNA gyrase and topoisomerase IV and may be important in limiting the potential for development of bacterial resistance (19,23). In addition, lack of a halide at the C_8 position reduces the potential for phototoxicity (22).

- **Clinical use** (23,24)

  Gatifloxacin is indicated in the treatment of:
  
  - Acute bacterial sinusitis
  - Community acquired pneumonia
  - Acute exacerbation of chronic bronchitis
  - Chronic Respiratory tract infection
  - Uncomplicated Urinary tract infection
  - Complicated Urinary tract infection
  - Uncomplicated Gonococcal infection
  - Skin and tissue infection.
- **Common side effects**

  Nausea, diarrhea, headache, dizziness

- **Pharmacokinetic properties**

  Gatifloxacin is administered as a racemate, with the disposition and antibacterial activity of the R and S enantiomers virtually identical. (25)

**Absorption:**

Gatifloxacin is well absorbed from the gastrointestinal tract after oral administration and can be given without regard to food (27,28). The absolute bioavailability of Gatifloxacin is 96%. Peak plasma concentration of Gatifloxacin usually occurs 1-2 hours after oral dose. The oral and intravenous formulations are considered to be bioequivalent.(26)

**Table 1.4 Pharmacokinetic properties of gatifloxacin following single dose of drug administered orally or intravenously (26,29,30)**

<table>
<thead>
<tr>
<th>DOSE</th>
<th>Oral</th>
<th>Intravenous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 mg</td>
<td>400 mg</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>(0.50, 2.50)</td>
<td>(0.50, 6.00)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (pg/mL)</td>
<td>2.0 ± 0.4</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>$AUC$ pg·h/mL)</td>
<td>14.2 ± 0.4</td>
<td>33.0 ± 6.2</td>
</tr>
<tr>
<td>$V_d$ (L/kg)</td>
<td>2.0 ± 0.1</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>$f_e$</td>
<td>80 - 83</td>
<td>73 - 90</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>-</td>
<td>7.8 ± 1.3</td>
</tr>
<tr>
<td>$C_{\text{IR}}$ (mL/min)</td>
<td>-</td>
<td>151 ± 46</td>
</tr>
<tr>
<td>$C_{\text{I}}$ (mL/min)</td>
<td>241 ± 40</td>
<td>210 ± 44</td>
</tr>
<tr>
<td>$U_R$ (%)</td>
<td>73.8 ± 10.9</td>
<td>72.4 ± 18.1</td>
</tr>
</tbody>
</table>
C\text{max}: Maximum serum concentration; \(T_{\text{max}}\): Time to \text{C}\text{max}; \text{AUC}: Area under concentration versus time curve; \(T_{1/2}\): Serum half-life; \(V_{\text{dss}}\): Volume of distribution; \text{Cl}: Total clearance; \text{Cl}_R: Renal clearance; \text{Ur}: Urinary recovery, \(f_u\)-fraction of total dose recovered in urine.

**Distribution**

Gatifloxacin is widely distributed throughout the body into many body tissues and fluids. Rapid distribution of the drug into tissues results in higher concentration in most target tissues than in serum. Table 1.5 shows gatifloxacin tissue-fluid to serum or plasma drug concentration ratios. The mean peak concentration of drug in urine is 675 mg/L after administration of a single 400 mg oral dose and occurred 4-6 h after oral administration.

**Table 1.5 Gatifloxacin : Tissue -- Fluid/Serum Ratio (31-35)**

<table>
<thead>
<tr>
<th>Fluid or Tissue</th>
<th>Tissue-Fluid/Serum Ratio (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiratory</strong></td>
<td></td>
</tr>
<tr>
<td>Alveolar macrophages</td>
<td>26.5 (10.9-61.1)</td>
</tr>
<tr>
<td>Bronchial mucosa</td>
<td>1.65 (1.12-2.22)</td>
</tr>
<tr>
<td>Lung epithelial lining fluid</td>
<td>1.67 (0.81-4.46)</td>
</tr>
<tr>
<td>Lung parenchyma</td>
<td>4.09 (0.50-9.22)</td>
</tr>
<tr>
<td>Sinus mucosa</td>
<td>1.78 (1.17-2.49)</td>
</tr>
<tr>
<td>Sputum (Multiple dose)</td>
<td>1.28 (0.49-2.38)</td>
</tr>
<tr>
<td><strong>Skin</strong></td>
<td></td>
</tr>
<tr>
<td>Skin blister fluid</td>
<td>1.00 (0.50-1.47)</td>
</tr>
<tr>
<td><strong>Reproductive</strong></td>
<td></td>
</tr>
<tr>
<td>Ejaculate</td>
<td>1.07 (0.86-1.32)</td>
</tr>
<tr>
<td>Seminal fluid</td>
<td>1.01 (0.81-1.21)</td>
</tr>
<tr>
<td>Vagina</td>
<td>1.22 (0.57-1.63)</td>
</tr>
<tr>
<td>Cervix</td>
<td>1.45 (0.56-2.64)</td>
</tr>
</tbody>
</table>

**Metabolism and Elimination**

Gatifloxacin undergoes minimal metabolism (< 1%) and is excreted primarily in urine as ethylenediamine and methylethylenediamine metabolites. Generally 65.0 - 90.2% of a
drug is excreted unchanged in the urine and about 5.6% excreted in the feces.

The mean elimination half-life of Gatifloxacin ranges from 7 to 14 h and is independent of dose and route of administration. Renal clearance is independent of dose with mean value ranging from 124 to 161 mL/min. The magnitude of this value, coupled with the significant decrease in the elimination of Gatifloxacin seen with concomitant Probenecid administration, indicates that Gatifloxacin undergoes both glomerular filtration and tubular secretion. Gatifloxacin may also undergo minimal biliary and/or intestinal elimination, since 5% of dose was recovered in the feces as unchanged drug.

- **Pharmacokinetics with respect to gender, age, race and disease**

There is no significant difference in pharmacokinetic data between males and females, between adult and elderly recipients and between recipients with normal and those with impaired hepatic function. (36) No adjustments in gatifloxacin dosage are recommended.

- **Pharmacokinetics in patients with renal dysfunction**

Gatifloxacin elimination decreases as a function of creatinine clearance. Thus, dosage adjustment of the drug is recommended for the warfarine, midazolam, glyburide, cimetidine and theophylline. Gatifloxacin concentration is increased by concomitant administration of probenecid treatment of patients with renal dysfunction. (37)
• Drug interaction

It does not interact with milk, calcium carbonate and digoxin, while antacids containing aluminum, magnesium or iron salts reduce its concentration. (38,39)

• Dosage and Administration

Gatifloxacin can be given with food. Oral doses of gatifloxacin should be given at least 4 h before administration of ferrous sulfate and dietary supplements containing magnesium, aluminum or iron. The recommended dosage of gatifloxacin for all types of indications is 400 mg once daily followed by 200 mg everyday subsequent.

No dosage adjustment is required in the elderly patients with mild hepatic impairment. In renal impairment, a dosage modification is recommended for patient with creatinine clearance of < 40 mL/min.
1.2.2 Ornidazole (40)

- **Nomenclature**

  *Chemical name:*
  
  1- (3-chloro-2-hydroxypropyl) - 2-methyl - 5-nitroimidazole.

  1- (Chloro-3-(2-methyl-5-nitroimidazole-1-yl) propan-2-ol.

  α-(Chloromethyl)-2-methyl-5-nitro-1H-imidazole-1-ethanol.

  *Generic name:* Ornidazole

- **Formula**

  *Empirical Formula:* $\text{C}_7\text{H}_{10}\text{ClN}_3\text{O}_3$

  *Structural Formula:*

  ![Structural Formula of Ornidazole]

- **Molecular weight**

  219.63

- **Elemental analysis**

  The calculated elemental composition is:

<table>
<thead>
<tr>
<th>Element</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>carbon:</td>
<td>38.28</td>
</tr>
<tr>
<td>Hydrogen:</td>
<td>04.57</td>
</tr>
<tr>
<td>chlorine:</td>
<td>16.14</td>
</tr>
<tr>
<td>nitrogen:</td>
<td>19.13</td>
</tr>
<tr>
<td>oxygen:</td>
<td>21.85</td>
</tr>
</tbody>
</table>
### Appearance

Ornidazole is white to pale yellow colored, practically odorless, crystalline powder.

### Physical properties

**Melting range:** 89 - 91°C and the substance appeared to melt without decomposition. Other reported values in the literature for the melting range are 77 - 78°C and 90 - 92°C.

**Ionization constant:** pKa is approximately 2.4.

**Partition coefficient:** The octanol - water partition coefficient: 4.8 corresponding to log P value of 0.618124. This indicates it's some hydrophilic character at neutral pH values.

**Solubility:**

Ornidazole is practically insoluble in nonpolar solvents but its solubility is very high in moderately polar and highly polar solvents. Solubility data is provided in the table below.

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>0.015</td>
</tr>
<tr>
<td>Toluene</td>
<td>9.85</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>431.99</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>296.14</td>
</tr>
<tr>
<td>Acetone</td>
<td>610.40</td>
</tr>
<tr>
<td>Ethanol (95%)</td>
<td>708.82</td>
</tr>
<tr>
<td>Methanol</td>
<td>627.58</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>662.50</td>
</tr>
<tr>
<td>Water</td>
<td>17.31</td>
</tr>
</tbody>
</table>
Hygroscopicity:

Nonhygroscopic

Optical activity:

(R)-form: \([\alpha]_{20}^D = +65.5^\circ\) (C = 1.0, CH\(_2\)Cl\(_2\)), 98% ee (1H-NMR),

(S)-form: \([\alpha]_{20}^D = -67.8^\circ\) (C = 0.99, CH\(_2\)Cl\(_2\)), greater than 98% ee (1H-NMR).

Crystallographic properties:

Single crystal structure: Crystallographic data for racemic Ornidazole have been reported, with the compound crystallizing in the triclinic space group P1. The reported unit cell parameters were:

\[
\begin{align*}
a &= 13.605 (2) \\
b &= 14.054 (1) \\
c &= 8.913 (5) \\
\alpha &= 71.590 (2) \\
\beta &= 78.730 (2) \\
\gamma &= 64.860 (1) \\
Z &= 6.000
\end{align*}
\]

Calculated density = 1.499 g/mL

Measured density = 1.497 g/mL

Spectral analysis

A. UV Spectra

Ornidazole solution was prepared in isopropanol. UV absorption maxima of this solution was as follows.
Drug Profile of Ornidazole

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$</th>
<th>Molar absorptivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu g/mL$</td>
<td></td>
<td>nm</td>
<td>L $\cdot$ cm/mol</td>
</tr>
<tr>
<td>20</td>
<td>isopropanol</td>
<td>230</td>
<td>5292</td>
</tr>
<tr>
<td></td>
<td></td>
<td>311</td>
<td>11447</td>
</tr>
</tbody>
</table>

B. I.R. Spectra peaks

Wave length Cm $^{-1}$

- 3174.1 O-H stretching mode
- 3223.5 and 3085 C-H stretching mode
- 1536.9 Asymmetric NO$_2$ stretching mode
- 1361 and 1269.5 Symmetric NO$_2$ stretching mode
- 1149 C-O stretching mode
- 828 C-N, NO$_2$ stretching mode

C. $^1$H- NMR spectrum

The following table provides a summary of assignments for the observed resonance bands.

<table>
<thead>
<tr>
<th>Chemical shift (ppm)</th>
<th>Number of protons</th>
<th>Multiplicity</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.47</td>
<td>3</td>
<td>Singlet</td>
<td>2-CH$_3$</td>
</tr>
<tr>
<td>3.65-3.75</td>
<td>2</td>
<td>Multiplet</td>
<td>3'-CH$_2$Cl</td>
</tr>
<tr>
<td>4.14-4.21</td>
<td>2</td>
<td>Triplet</td>
<td>1'-CH$_2$N</td>
</tr>
<tr>
<td>4.60-4.68</td>
<td>1</td>
<td>Multiplet</td>
<td>2'-CHOH</td>
</tr>
<tr>
<td>5.04</td>
<td>1</td>
<td>Singlet</td>
<td>2'-CHOH</td>
</tr>
<tr>
<td>7.79</td>
<td>1</td>
<td>Singlet</td>
<td>4</td>
</tr>
</tbody>
</table>
D. $^{13}$C-NMR spectrum

Spectra were used to develop the $^{13}$C chemical shift assignments that are summarized in the following table-

\[
\begin{array}{ccc}
\text{Chemical shift (ppm)} & \text{Carbon number} \\
14.51 & 6 \\
46.935 & 3 \\
49.66 & 1 \\
69.98 & 2 \\
132.480 & 4 \\
138.267 & 5 \\
151.842 & 2 \\
\end{array}
\]

E. Mass spectrometry.

The molecular ion peak was found at m/z = 219 (6.63%), and the other observed characteristic peaks are 202, 184, 173, 124, 112, 95, 81 and 53 (base Peak C$_4$H$_5$+).

- Identification of Ornidazole

Infrared spectrum

The infrared spectrum of Ornidazole obtained in a potassium bromide pellet, can be used for identification of the drug.
substance. The compound will exhibit peaks at 3174.1, 1536.9, 1361 and 1269.5 cm\(^{-1}\).

**Ultraviolet absorption**

A 20 μg/mL solution of Ornidazole dissolved in Isopropanol will exhibit absorption maxima at 230 and 311 nm.

**High performance liquid chromatography**

The retention time of the Ornidazole peak in the HPLC procedure is same as that of authentic Ornidazole reference standard.

- **Therapeutic category**
  
  Antibacterial, Antiprotozoal

- **Mechanism of action**

  Ornidazole belongs to the nitroimidazole group. The drugs belonging to this group has excellent activity against anaerobic microorganisms. The antimicrobial activity of Ornidazole is due to the reduction of the nitro group to a more reactive amine that attacks microbial DNA, brings about loss of helical structure of DNA and subsequent DNA breakage thus inhibiting further synthesis and causing degradation of existing DNA.

- **Clinical use**

  Ornidazole is also used in the treatment of:
  
  o Severe hepatic and intestinal amoebiasis, giardiasis and trichomoniasis of the uro-genital tract.
  
  o Bacterial vaginosis.
  
  o Treatment and prophylaxis of susceptible anaerobic infections in dental and gastrointestinal surgery.
  
  o In the management of Helicobacter pylori duodenal ulcers. (Combination with other drugs).
- **Common side effects**

  Omidazole is well-tolerated with a mild and transient side effect profile. The most frequently encountered side effect of Omidazole is dizziness, alone or in combination with other adverse effects.

  The other side effects occurring to a less extent are nausea, pyrosis, intestinal spasms and metallic taste.

  Vertigo, fatigue and other discomforts such as loose stools, insomnia, skin rash and headache have also been reported.

- **Interactions**

  In contrast to other nitroimidazole derivatives, Omidazole does not inhibit aldehyde dehydrogenase and is therefore not incompatible with alcohol. However, omidazole potentiates the effect of coumarin-type oral anticoagulants. The dosage of the anticoagulant has to be adjusted accordingly. Omidazole prolongs the muscle-relaxant effect of vecuronium bromide.

- **Contraindications**

  Omidazole is contraindicated in patients with known hypersensitivity to the medicine or to other nitroimidazole derivatives. Caution should be exercised in patients with diseases of the CNS, e.g., epilepsy or multiple sclerosis. The effect of other medicines can be intensified or impaired.

- **Pharmacokinetic properties**

  Omidazole is administered as a racemate, with the disposition and antibacterial activity of the R- and S-enantiomers virtually identical.

  - Omidazole is readily absorbed from gastrointestinal tract and vaginal mucosa.

  - A peak plasma concentration of about 30 μg/mL has been
achieved within 2 hr of a single oral dose.

- Less than 15% of the drug is bound to plasma proteins.
- It is widely distributed in body tissues and fluids, including cerebrospinal fluid.
- Omidazole is metabolized in liver.
- It is excreted in urine mainly as conjugates and metabolites and to a lesser extent in the faeces.
- The two major metabolites of Omidazole are:
  1. 3- (2 -methyl - 5 - nitroimidazole - 1- yl) 1, 2-propanediol (hydrolysis product) and
  2. α - (chloromethyl) - 2 - hydroxymethyl-5- nitroimidazole -1- ethanol (oxidation product).
- The plasma elimination half-life is 12 - 14 hours.

Fig. 1.2 : Metabolism of Omidazole
**Dosage**

Dosage required for various conditions is summarized in the following table.

<table>
<thead>
<tr>
<th>Infection</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adults</strong></td>
<td></td>
</tr>
<tr>
<td>Amoebiasis</td>
<td>500 mg bid for 5-7 days.</td>
</tr>
<tr>
<td>Amoebic dysentery</td>
<td>1.5 g od for 3 days.</td>
</tr>
<tr>
<td>Giardiasis</td>
<td>1.5 g od for 1-2 days.</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>1.5 g once or 500 mg bd for 5 days. Sexual partner should be</td>
</tr>
<tr>
<td></td>
<td>simultaneously treated.</td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>1.5 g once or 500 mg od for 5-7 days.</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td></td>
</tr>
<tr>
<td>Amoebiasis</td>
<td>10-25 mg/kg od for 3 days.</td>
</tr>
<tr>
<td>Amoebic dysentery</td>
<td>40 mg/kg od for 3 days.</td>
</tr>
<tr>
<td>Giardiasis</td>
<td>40 mg/kg for 2 days.</td>
</tr>
</tbody>
</table>
1.3 **Analytical Validation** (41 - 45)

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

The differences in the USP and ICH terminology is for most part one of semantics, however, with one notable exception. ICH treats system suitability as a part of method validation, whereas the USP treats in a separate chapter.

As per ICH, validation of analytical procedures is directed to the four most common types of analytical procedures:

- **Identification tests** - are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g. spectrum, chromatographic behavior, chemical reactivity, etc) to that of a reference standard.

- **Quantitative tests for impurities content** - is intended to accurately reflect the purity characteristics of the sample.

- **Limit tests for the control of impurities** - is intended to accurately reflect the purity characteristics of the sample.

- **Assay** - These are quantitative tests of the active moiety in sample of drug substance or drug product or other selected component(s) in the drug product.

The USP divides analytical methods into three separate categories:

- Quantitation of major components or active ingredients
- Determination of impurities or degradation products
- Determination of performance characteristics
The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated.

1.3.1 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. It is also termed trueness.

Accuracy should be established across the specified range of the analytical procedure.

Assay

A. Drug Substance

Several methods of determining accuracy are available:

- Applications of an analytical procedure to an analyte of known purity (e.g., reference material).

- Comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined.

- Accuracy may be inferred once precision, linearity and specificity have been established.

B. Drug Product

Several methods for determining accuracy are available:

- Application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analyzed
have been added.

- In cases where it is impossible to obtain samples of all drug product components, it may be acceptable either to add known quantities of the analyte to the drug product, the accuracy of which is stated and/or defined.

- Accuracy may be inferred once precision, linearity and specificity have been established.

**Impurities (Quantitation)**

Accuracy should be assessed on sample (drug substance/drug product) spiked with known amounts of impurities.

In cases where it is impossible to obtain sample of certain impurities and/or degradation products, it is considered acceptable to compare results obtained by an independent procedure. The response factor of the drug substance can be used. It should be clear how the individual or total impurities are to be determined e.g., weight / weight or area percent, in all cases with respect to the major analyte.

**RECOMMENDED DATA**

Accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g. three concentrations/ three replicated each of the total analytical procedure).

Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.
1.3.2 PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate and reproducibility.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Repeatability should be assessed using:

- A minimum of 9 determinations covering the specified range for the procedure (e.g. 3 concentrations/3 replicates each).
- A minimum of 6 determinations at 100% of the test concentration.

Intermediate Precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, different reagents etc.

The extent to which the procedure is intended to be used, the applicant should establish the effect of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment etc.
**Reproducibility / Ruggedness**

Reproducibility expresses the precision *between laboratories* and is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias. These data are not part of the marking authorization dossier.

**RECOMMENDED DATA**

The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated.

**1.3.3 SPECIFICITY**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

**Identification**

Suitable identification tests should be able to discriminate between compounds of closely related structures which are likely to be present, the discrimination being confirmed by obtaining positive results (by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte.

**Assay and Impurity Tests**

Representative chromatograms should be used to demonstrate specificity and individual components should be appropriately
labeled.

A. Impurities are available

This is done by spiking pure substances (drug substance or drug product) with appropriate levels of impurities and/or excipients and demonstrating that the assay results are unaffected by the presence of these materials (by comparison with the assay result obtained in unspiked samples).

B. Impurities are not available

If impurity or degradation product standards are unavailable, specificity may be demonstrated products to a second well-characterized procedure e.g.: pharmacopoeial method or other validated analytical procedure (independent procedure). As appropriate this should include samples stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis and oxidation.

- For the assay, the two results should be compared.

- For the impurity tests, the impurity profiles should be compared.

Peak purity tests may be useful to show that the analyte chromatographic peak is not attributable to more than one component (e.g. diode array, mass spectrometry).

1.3.4 DETECTION LIMIT

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-
instrumental or instrumental.

a. **Based on Visual Evaluation**

Visual evaluation may be sued for non-instrumental methods but may also be used with instrumental methods.

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

b. **Based on Signal-to-Noise**

This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.

c. **Based on the Standard Deviation of the Response and the Slope**

The detection limit (DL) may be expressed as:

$$DL = 3.3 \frac{\sigma}{S}$$

Where, \(\sigma\) – standard deviation of the response, \(S\) – slope of the calibration curve

The slope \(S\) may be estimated from the calibration curve of the analyte. The estimate of \(\sigma\) may be carried out in a variety of ways, for example:
**Based on the Standard Deviation of the Blank**

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

**Based of the Calibration Curve**

A specific calibration curve should be studied using samples containing an analyte in the range DL. The residual standard deviation of a regression line of the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

**RECOMMENDED DATA**

The detection limit and the method used for determining the detection limit should be presented. If DL is determined based on visual evaluation or based on signal to noise ratio, the presentation of the relevant chromatograms is considered acceptable for justification.

In case where an estimated value for the detection limit is obtained by calculation of extrapolation this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be bear or prepared at the detection limit.

**1.3.5 QUANTITATION LIMIT**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used
particular for the determination of impurities and/or degradation products.

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

**Based on Visual Evaluation**

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

**Based on Signal-to-Noise Approach**

This approach can only be applied to analytical procedures that exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing signals from samples with known low concentration of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.

**Based on the Standard Deviation of the Response and the Slope**

The quantitation limit ($Q_L$) may be expressed as:

$$Q_L = 10 \sigma / S$$

Where,

$\sigma$ – Standard deviation of the response,

$S$ – Slope of the calibration curve

The slope $S$ may be estimated from the calibration curve of the
analyte. The estimate of \( \sigma \) may be carried out in a variety of ways, for example:

**Based on Standard Deviation of the Blank**

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

**Based of the Calibration Curve**

A specific calibration curve should be studied using samples, containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

**RECOMMENDED DATA**

The quantitation limit and the method used for determining the quantitation limit should be presented.

The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit.

**1.3.6 LINEARITY**

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighing of synthetic mixtures of the drug product components, using
the proposed procedure.

**RECOMMENDED DATA**

For the establishment of linearity, a minimum of 5 concentrations is recommended.

The correlation coefficient, y-intercept, slope of the regression line and residual sum of square should be submitted. A plot of the data (signal area vs. analyte concentration) should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity.

**1.3.7 RANGE**

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including this concentration) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. It is normally derived from linearity.

The following minimum specified ranges should be considered:

- For **assay** of a drug substance or a finished (drug) product: from 80 to 120% of the test concentration;

- For **content uniformity**: covering a minimum of 70 to 130% of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g. metered dose inhalers), is justified.

- For the determination of an **impurity**: from the reposting level of an impurity to 120% of the specification. (For toxic or more potent impurities, the range should be commensurate with the
-For **dissolution testing**: +/-20% over the specified range of the test, e.g. if the specifications for a controlled released product cover a region from 20%, after 1 hour, up to 90% after 24 hours, the validated range would be 0-110% of the label claim.

### 1.3.8 ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. If measurements are susceptible to variations in analytical conditions, they should be suitably controlled or a precautionary statement should be included in the procedure. Its evaluation should be considered during the development phase.

Examples of typical variations are:

- Influence of variations of pH in a mobile phase.
- Influence of variations in mobile phase composition.
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate

### 1.3.9 SYSTEM SUITABILITY TESTING

System suitability testing is an integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept
that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole.

System suitability test parameters to be established for a particular procedure depend in the type of procedure being validated.

### Table 1.6 Recommended SST acceptance criteria

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity factor k'</td>
<td>k' = 1 - 10</td>
</tr>
<tr>
<td>Injection precision</td>
<td>CV &lt; 2 % for n ≥ 5</td>
</tr>
<tr>
<td>Resolution Rs</td>
<td>Rs &gt; 2</td>
</tr>
<tr>
<td>Railing factor T</td>
<td>T ≤ 2</td>
</tr>
<tr>
<td>Theoretical plate N</td>
<td>N &gt; 2000</td>
</tr>
</tbody>
</table>

### 1.3.10 Data Elements Required For Assay Validation

Both the USP and the ICH recognize that it is not always necessary to evaluate every analytical performance parameter. The type of method and its intended use dictates which parameters need to be investigated, as illustrated in Table 1.7.

The USP divides analytical methods into three separate categories:

- Quantitation of major components or active ingredients.
- Determination of impurities or degradation products.
- Determination of performance characteristics.

For assays in category 1, LOD and LOQ evaluations are not necessary because the major component or active ingredient to
be measured is normally present at high levels. However, since quantitative information is desired, all of the remaining analytical performance parameters are pertinent. Assays in category 2 are divided into two sub-categories: Quantitative and Limit Tests. If quantitative information is desired, a determination of LOD is not necessary, but the remaining parameters are required. The situation reverses itself for a Limit Test. Since quantitation is not required, it is sufficient to measure the LOD and demonstrate specificity and ruggedness.

Table 1.7 USP Data Elements Required For Assay Validation

<table>
<thead>
<tr>
<th>Analytical Performance Parameter</th>
<th>Assay Category 1</th>
<th>Assay Category 2</th>
<th>Assay Category 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>Yes</td>
<td>Yes</td>
<td>*</td>
</tr>
<tr>
<td>Precision</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Specificity</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>LOD</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>LOQ</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
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<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Range</td>
<td>Yes</td>
<td>Yes</td>
<td>*</td>
</tr>
<tr>
<td>Ruggedness</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* May be required, depending on the nature of the specific test.

The parameters that must be documented for methods in USP assay category 3 are dependent upon the nature of the test. Dissolution testing, for example, falls into this category. The ICH treats analytical methods in much the same manner, as shown in Table 1.8.

USP categories 1 and 2 match the ICH categories of Assay and Impurity Testing, respectively, and the corresponding discussion above still applies.
Table 1.8 ICH Validation Characteristics versus type of Analytical Procedure

<table>
<thead>
<tr>
<th>Type of Analytical Procedure</th>
<th>Identification</th>
<th>Impurity Testing</th>
<th>Assay</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Quantitative</td>
<td>Limit Tests</td>
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<tr>
<td>Accuracy</td>
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<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Repeatability</td>
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</tr>
<tr>
<td>Interm. Prec.</td>
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<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Specificity</td>
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<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>LOD</td>
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</tr>
<tr>
<td>Range</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

The ICH has not yet chosen to specifically address methods for performance characteristics (USP category 3), however, but has suggested analytical methods for compound identification. In ICH category, it is only necessary to prove that the method is specific for the compound being identified.
1.4 *In vitro evaluation of the tablets* (46 - 51)

Nearly all drug substances dispensed in the oral dosage forms are stable under ordinary conditions. The pharmaceutical tablets are evaluated for their chemical, physical, and biological (bioavailability and drug performance) properties. These properties in contrast, describe the total quality of any given tablet or formulation, according to its particular method of manufacture, in its package/container, and under fixed storage or a range of storage conditions (including possible and reasonable environmental exposures under conditions of use).

In the case of physical and chemical properties, a series of tests are generally required to fully identify the particular property. All three property classes – chemical, physical, biological are interrelated. For example chemical breakdown of or interactions between tablets components may alter physical tablet properties in such a way that the biological properties are significantly changed. Even without chemical breakdown, various physical properties of the tablets can undergo change under environmental or artificial stressed conditions, and these changes may be of more significance and concern in some tablet systems than chemical stability. Evaluation of particular formulation or establishment of an expiry date for a product requires stability of all three classes of properties.

The development of tablet formulation goes through several phases, with an increasing number of tablet evaluations performed as each new phase is entered. The essential qualities of a good compressed tablet are characterized by a number of specifications. These include appearance, size, shape, thickness, weight, homogeneity, stability, hardness, dissolution time, and disintegration time. The appearance, size, shape, and thickness of the tablet are generally used to distinguish and identify the active ingredients which they contain. The
remaining specifications assure the manufacturer that the tablets do not vary from allowable limits within the same lot or from one production lot to another. All such qualities are designed to ensure a safe, therapeutically effective oral solid dosage form. A description of those tablet properties considered critical by formulator and evaluated for tablets is given as follows.

1.4.1 Size and Shape

The most significant factors contribute to overall size and shapes of the tablet are drug dosage, density of the final granulation mix, and over all tablet weight. It can also influence the particle size of the granulation used, the type of the tablet press required, production lot sizes, the type of the tablet processing, packaging operations, and overall production cost. The maintenance of high degree control over shape and size is essential from the perspective of consumer acceptance, tablet to tablet uniformity, lot to lot uniformity, and manufacturability. This requires the tablet tooling be uniform and within established specifications.

1.4.2 Tablet Thickness

Once the tablet size (weight) and shape have been established, tablet thickness remains the overall dimension variable. Although thickness specifications may be set on an individual product basis, as a general rule, the thickness should be controlled to with in 5 % or less of an established standard value. Tablet thickness is often used as one of the several in-process tests and is monitoring at regular intervals during the compressing stage of the tablet manufacture. The thickness of the tablet is generally measured with the micrometer, which permits very accurate measurement and provide information on
the tablet to tablet variation. Digital read out calipers have recently been introduced which provide accurate single tablet thickness measurements to one hundredth of a millimeter.

1.4.3 Hardness

Once ejected from the compressing machine, a tablet requires a certain amount of mechanical strength to withstand the shocks of handling in its manufacture, packing, shipping and dispensing. The tablets may also be able to withstand a reasonable amount of abuse imparted by the customer.

Hardness and friability are the most common measures to evaluate tablet strength. Tablet hardness can have a significant influence on tablet parameters such as disintegration and dissolution. Tablet hardness can be defined as the force required breaking a tablet in a diametric compression test. This test consists of placing tablet between two anvils and applying pressure to the anvils until a tablet breaks. The crushing strength that just causes the tablet to break is recorded. Hardness is thus sometimes referred to as "tablet crushing strength".

Hardness is function of applied compression force and is therefore a function of those factors that causes the forces to vary. As additional force is applied to compress a tablet, the tablet hardness will increase. This relationship will hold up to a maximum value beyond which increases in pressure will not cause an increase in hardness, but will cause the tablet to laminate or cap, thus destroying its integrity. The optimum hardness for the given tablet formulation will depend to some degree on the intended use of the tablet. For tablets intended to be directly packaged and eventually swallowed by a consumer, disintegration and dissolution properties may be prime
considerations for tablet hardness, so long as the tablets can withstand packaging. An appropriate balance between a minimally acceptable tablet hardness to produce an adequate friability value and a maximally accepted hardness to achieve an adequate dissolution may be required.

Tablet size, shape, and orientation in the tester will also affect the measured hardness values for a given formulation. Large tablets require a greater force to cause fracture and are often considered "harder" than small tablets. In present study, the crushing strength of the tablets (P, Kg) is converted into the tensile strength using the relationship simplified by Gohel et al. (55), using following equation.

\[
T_s = \frac{0.624 \times P}{D \times T}
\]

Where D = Diameter in cm and T = Thickness in cm.

Several instruments have been developed for measuring tablet hardness. These include the Stokes (Monsanto) tester, the Strong -Cobb tester, The Pfizer tester, the Erweka tester, and the Vander Kamp tester. In the present study, the tablet hardness was measured by Monsanto tester. It consists of a barrel containing a compressible spring held between the plungers. The lower plunger was placed in contact with the tablet and the zero reading was taken. The upper plunger was then forced against the spring by turning a threaded bolt until the tablet fractured. As the spring was compressed, a pointer rod along a gauge in the barrel moved to indicate pressure. The pressure of fracture was recorded and the zero pressure was deducted from it. The zero pressure was the gauge reading at which the lower plunger barely contacted the tablet when it was initially placed in the instrument.
1.4.4 Friability

Another measure (in addition to hardness) of a tablet's strength is its friability. Friability is a measure of a tablet's ability to withstand both shock and abrasion without crumbling during the handling of manufacturing, packaging, shipping, and consumer use. As a measure of controlling, and even quantization, the measurement of friability, a laboratory device, known as Roche Friabilator, was developed. This device subjects a number of the tablets to combined effect of shock and abrasion by utilizing a plastic chamber which revolves at 25 rpm, dropping the tablets at a distance of 6 inches with each revolution. Normally, a pre weighed tablet sample is placed in friabilator chamber which is then rotated at 100 revolutions. The tablets are removed from the chamber, dusted, and reweighed. Conventional tablets that lose less than 0.5 to 1.0 % in weight is generally consider acceptable.

1.4.5 Uniformity of the tablet dosage units

The uniformity of tablet dosage units can be demonstrated either weight variation or content uniformity test. The weight variation test is required when tablets to be tested are uncoated and contains 50 mg or more of single active ingredient comprising 50 % or more, by weight of tablet dosage form unit. Content uniformity test is required for coated tablets, and all other cases except for special tablets.

Weight variation

Tablet dosage form uniformity by weight variation is determined by selecting not less that 30 tablets form each production batch and weighing accurately 10 tablets individually and calculating the average weight. From the results of the assay obtained as directed in the individual compendia monograph, the content of
active ingredient in each of the individual 10 tablets is calculated. The USP/NF weight variation requirements are met if the amount of the active ingredient in the each of the 10 tablets under test lies within the range of 85 - 115% of the label claim and the relative standard deviation is less than or equal to 6%. If one tablet is out side the above mentioned range and no other tablet is out side the range of 75 - 125% of the label claim and/or if the relative standard deviation is greater than 6%, the extra 20 tablets should be tested. USP/NF tablet dosage form uniformity test requirements are met if not more than one tablet of 30 individual tablets tested are out side the range of 85 - 115% of the label claim and no tablet is out side the range of 75 - 125% of the label claim and the relative standard deviation of the 30 tablets tested does not exceed 7.8%.

Content Uniformity

Tablet dosage form content uniformity is determined by selecting not less than 30 tablets from each production batch and assaying 10 tablets individually as directed in the assay of the individual compendia monograph. If the amount of active ingredient in the individual tablet is less than required in the assay make necessary dilution of the solutions and/or volume of aliquots so that the concentration of the active ingredients in the final solution is the same as that in the assay procedure. The USP/NF content uniformity requirements are met if the amount of active ingredient in each of 10 the tablets under test lies within the range of 85 - 115% of the label claim and the relative standard deviation is less than or equal to 6%. If one tablet is out side the above mentioned range and no other tablet is out side the range of 75 - 125% of the label claim and/or if the relative standard deviation is greater than 6%, the extra 20 tablets should be tested. USP/NF tablet dosage form uniformity
test requirements are met if not more than one tablet of the 30 individual tablets tested is outside the range 85 - 115% of the label claim and no tablet is outside the range of 75 - 125% of the label claim and the relative standard deviation of the 30 tablets tested does not exceed 7.8 %. Label claim average limits specified in the potency definition on the individual tablet product compendial monograph should be approximately adjusted if greater than 100 %.

1.4.6 Disintegration Testing (46-55)

It is generally accepted that in order for a drug to be available to the body, it must first be in solution.

Fig. 1.3 : Processes involved when a tablet is exposed to fluid (water) under in vitro or in vivo conditions

Figure 1.3 illustrates a scheme of the ways in which drugs formulated into a tablet become available to the systemic circulation. For most conventional tablets, the first important
step in the sequence is the breakdown of the tablet into smaller particles or granules. This process is known as disintegration. The time it takes for a tablet to disintegrate in aqueous medium is measured using a device described in USP. The USP XXIV and NF XIX disintegration apparatus consist of a basket - rack holding six open glass tubes, each 7.75 ± 0.25 cm long and having an inside diameter and wall thickness of approximately 21.5 and 2 mm, respectively and attached by screws to the underside of the lower plate holding the tubes in 10 mesh stainless steel wire cloth. The basket rack is immersed in a 1L beaker containing an appropriate fluid at 37 ± 2°C. The basket rack is raised and lowered through a distance of 5 to 6 cm at the rate of 28 - 32 cps. The volume of fluid used is such that during the operation, the basket rack is never less than 2.5 cm below the surface of the fluid or above the bottom of the beaker. Each tube is provided with a slotted and perforated cylinder, transparent plastic disk which is placed on the top of the table.

**Uncoated Tablets**

One uncoated tablet is placed in each glass tube and a disk is added to each tube. The basket rack is immersed and moved in water as the immersion fluid, unless another fluid is specified in the individual monograph. At the end of the time limit specified in the monograph, the basket rack is lifted; and all tablets should have disintegrated completely. If one or two tablets fail to disintegrate completely, the test is repeated with 12 additional tablets. Not less than 16 of the total 18 tablets tested must disintegrate completely.

**Plain - Coated Tablets**

Plain-coated tablets are tested by first placing a tablet in each glass tube and immersing the basket rack at room temperature
for 5 min; then a disk is added to each tube, and the apparatus is operated for 30 min using simulated gastric fluid at 37 ± 2°C, as the immersion fluid. If the tablets have not disintegrated completely, they are immersed in simulated intestinal fluid, at 37 ± 2°C, and the test is continued for the time specified in the monograph plus 30 min. If one or two tablets fail to disintegrate completely, the test is repeated with 12 additional tablets. Not less than 16 out of 18 tablets tested must disintegrate completely.

1.4.7 Dissolution Testing for Immediate Release Drug Products

Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological condition, and the permeability across the gastro-intestinal tract. Because of the critical nature of the first two of these steps, in vitro dissolution may be relevant to the predictions of in vivo performance. Based on this general consideration, in vitro dissolution test for immediate release solid oral dosage forms, such as tablets and capsules, are used to -

- Assess the lot to lot quality of a drug product;

- Guide development of new formulations; and ensure continuing product quality and performance after certain changes, such as changes in the formulation, the manufacturing process, the site of manufacture, and the scale - up to the manufacturing process.

Based on drug solubility and permeability, the following Biopharmaceutical Classification System (BCS) is recommended:
Class 1: High Solubility – High Permeability Drugs

Class 2: Low Solubility – High Permeability Drugs

Class 3: High Solubility – Low Permeability Drugs

Class 4: Low Solubility – Low Permeability Drugs

This classification can be used as a basis for setting in vitro dissolution specifications and can also provide a basis for predicting the likelihood of achieving a successful in vivo - in vitro correlation (IVIVC).

The BCS suggests that for high solubility, high permeability (case 1) drugs and some instances for high solubility, low permeability (case 3) drugs, 85% dissolution in 0.1 N HCl in 15 min can ensure that bioavailability of the drug is not limited by solubility. In this case, the rate limiting step for drug absorption is gastric emptying.

In vitro dissolution specifications are established to ensure batch to batch consistency and to signal potential problems with in vivo bioavailability.

- **Single-point specifications**
  
  As a routine quality test (For high soluble and rapidly dissolving drug products)

- **Two-point specifications**
  
  o For characterizing the quality of the drug product

  o As a routine quality control test for certain types of drug products (e.g. slow dissolving or poorly water soluble drug products like Carbamazepine
• Dissolution profile comparison
  o For accepting product sameness under SUPAC – related changes
  o To waive bioequivalence requirements for lower strengths of a dosage form.
  o To support waivers for other bioequivalence requirements.
  o Dissolution testing should be carried out under mild test conditions, basket method at 50/100 rpm or paddle method at 50/75 rpm, at 15 min intervals, to generate a dissolution profile.

For highly soluble and rapidly dissolving drug products (BCS classes 1 and 3), a single-point dissolution test specification of NLT 85% in 60 min or less is sufficient as routine quality control test for batch to batch uniformity.

Special Case

• Two-Point Dissolution Test

For poorly water soluble drug products, dissolution testing at more than one time point for routine quality control is recommended to ensure in vivo product performance; a dissolution profile may be used for purpose of quality control.

Dissolution Testing Conditions

Apparatus (USP)

The most commonly employed dissolution test methods are

o the basket method (Apparatus 1)
the paddle method (Apparatus 2)

The official in vitro dissolution methods described in U.S. Pharmacopoeia (USP), Apparatus 1 and Apparatus 2 should be used unless shown to be unsatisfactory.

**Dissolution medium**

Dissolution testing should be carried out under physiological conditions, if possible. This allows interpretation of dissolution data with regard to in vivo performance of the product. However strict adherence to the gastrointestinal environment need not be used in routine dissolution testing. The volume of the dissolution medium is generally 500, 900 or 1000 mL. Sink conditions are desirable but not mandatory. As aqueous medium with pH range 1.2 to 6.8 (ionic strength of buffer, the same as in USP) should be used. Use of water as a dissolution medium also is discouraged because test conditions such as pH and surface tension can vary depending on the source of water and may change during the dissolution test itself, due to the influence of the active and inactive ingredients. All dissolution tests for IR dosage forms should be conducted at 37 ± 0.5 °C.

**Agitation**

In general, mild agitation conditions should be maintained during dissolution testing to allow maximum discriminating power and to detect products with poor in vivo performance. Using the basket method the common agitation is 50 - 100 rpm; with the paddle method, it is 50 – 75 rpm. Apparatus 3 and 4 are seldom used to assess the dissolution of immediate release drug products.