1. RIFAMPICIN (RIF)

RIF is the first line drug for treatment of tuberculosis. It belongs to a new class of antibiotics obtained from macrocyclic ring bridged across two nonadjacent positions of an aromatic nucleus called ansamycine. RIF has broad spectrum of antimicrobial gram-positive bacteria and particularly against *Mtb*.

1.1. Structure:

1.2. **IUPAC Name:** 2,7- (epoxypentadeca[1,11,13]trienimino)naphtho [2,1-b]-furan-1,11(2H)-dione,5,6,9,17,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-1-8-[N-(4-methyl-1-piperazinyl)-formimidoy-21-acetate

1.3. **Molecular Formula:** C_{43}H_{58}N_{4}O_{12}

1.4. **Molecular Weight:** 822.96

1.5. **Category:** Antibacterial

1.6. **Physical Characteristics:** Brick red to reddish, odorless, crystalline powder

1.7. **Melting Point:** 183-188°C, melts with decomposition

1.8. **pH (1% solution in water):** 4.5-6.5
1.9. **Solubility:**
Soluble in methanol, DMSO, chloroform, ethylacetate; slightly soluble in water, ethanol, acetone, carbon tetrachloride; practically insoluble in butanol, cyclohexane, glycerol and propylene glycol.

1.10. **Mechanism of Action:**
RIF inhibits DNA dependent RNA polymerase (DDRP) of mycobacteria and other microorganisms leading to suppression of initiation of chain formation in RNA synthesis. The mammalian DDRP is resistant even to high concentrations of RIF. The inhibition of bacterial RNA polymerase is due to formation of a rather stable, noncovalent complex between RIF and the enzyme with binding constant of $10^{-9}$ M at 37°C. One molecule of RIF (MW 823) is bound with one molecule of enzyme (MW 455,000). RIF is bactericidal for both intracellular and extracellular microorganism (Wolff, 1996).

1.11. **Antibacterial Activity:**
RIF inhibits the growth of most gram positive bacteria, as well as many gram negative microorganisms, such as *E. coli*, *pseudomonas*, indole positive and indole negative *proteus*. RIF increases the *in vitro* activity of streptomycin and isoniazid but not of ethambutol against *Mtb*.

1.12. **Pharmacokinetics:**
RIF is well absorbed following oral administration. Its absorption from the gut is almost complete, but is impaired or delayed by food. A peak plasma level of about 10 μg/ml is reached 3 h after a single oral dose of 600 mg and provides effective blood level for 8 h or more. The $t_{1/2}$ is 2 to 5 h, and renal insufficiency does not significantly raise the plasma levels. It is widely distributed in the body, about 85-90% of the drug is protein bound in plasma. It is metabolized in urine to an active deacetylated metabolite (deacetyl RIF), which is excreted mainly in bile, feaces, and to some extent in urine also (making it orange colored). RIF and it’s deacetyl derivative undergo Enterohepatic circulation. It
should be administered on an empty stomach. It is 70-80% bound to protein in the plasma and its half-life is varied from 2 to 5 h. After oral administration, RIF is well absorbed and produces maximum plasma levels at 1.5-3 h over a wide range of single dose, i.e., 0.1 to 1.2 g. RIF elimination which must be considered slow, occurs mainly through the bile but also through the urine. The total amount of RIF eliminated in the bile is not proportional to the dose administered, while the urinary elimination increases with the dose.

1.13. Adverse Effects/Uncommon Effects:

   **Nervous system reactions:** headache, drowsiness, fatigue, ataxia, dizziness, inability to concentrate, mental confusion, visual disturbances, muscular weakness, pain in extremities and generalized numbness.

   **Gastrointestinal disturbances:** in some patients heartburn, epigastric distress, anorexia, nausea, vomiting, gas, cramps, and diarrhoea.

   **Hepatic reactions:** transient abnormalities in liver function tests e.g., elevations in serum bilirubin, BSP, alkaline phosphatase, serum transaminases have been observed. Rarely, hepatitis or a shock like syndrome with hepatic involvement and abnormal liver function tests.

   **Renal reactions:** elevations in BUN and serum uric acid have been reported. Rarely, haemolysis, haemoglobinuria, hematuria, interstitial nephritis, renal insufficiency, and acute renal failure have been noted. These are generally considered to be hypersensitivity reactions. They usually occur during intermittent therapy or when treatment is resumed following intentional or accidental interruption of a daily dosage regimen, and are reversible when RIF is discontinued and appropriate therapy instituted.

   **Hematologic reactions:** thrombocytopenia, transient leukopenia, hemolytic anemia, eosinophilia, and decreased hemoglobin have been observed. Thrombocytopenia has
occurred when rifampin and ethambutol were administered concomitantly according to an intermittent dose schedule twice weekly and in high doses.

**Allergic and immunological reactions:** occasionally pruritus, urticaria, rash, pemphigoid reaction, eosinophilia, sore mouth, sore tongue, and exudative conjunctivitis. Although RIF has been reported to have an immunosuppressive effect in some animal experiments, available human data indicate that this has no clinical significance.

**Metabolic reactions:** elevations in BUN and serum uric acid have occurred.

**Miscellaneous reactions:** fever and menstrual disturbances have been noted.

### 1.14. Contraindications:

RIF is contraindicated in known cases of hypersensitivity to the drug. It may be contraindicated in pregnancy (because of teratogenicity noted in animal studies and since the effect of drugs on fetus has not been established) except in the presence of a disease such as severe TB. It is contraindicated in alcoholics with severely impaired liver function and with jaundice.

### 1.15. Route of Administration and Dosage Forms:

- **Oral**
  - RIF capsules of 150 mg, 300 mg, 450 mg, and 600 mg.
  - RIF Syrup 100 mg/5ml
- **Ophthalmic**
  - RIF 1% ophthalmic ointment
- **Intravenous**
  - Powder for reconstitution, as RIF 300 mg, supplied with solvent.

### 1.16. Dose:

- **TB**
  - **Adult**-10 mg/kg bodyweight single daily dose (maximum 600 mg) in combination with other antimycobacterial agents. 15 mg/kg bodyweight (maximum 900 mg) 2 or 3 times weekly in combination with other antimycobacterial agents. Child – 10-15 mg/kg/day; Adult – 450 mg for < 50 kg; 600 mg for > 50 kg.
Biweekly dose – 600 mg.

**Leprosy**

600 mg once monthly or once daily in combination with other anti-leprotic drugs.

**Haemophilus influenzae type B infection**

20 mg/kg body weight once a day for 4 days (maximum daily dose 600 mg).

Meningococcal carriers 600 mg twice daily for two days.

**Eye**

Trachoma (i.e., hyperendemic trachoma or transmitted trachoma-inclusion conjunctivitis) 1% ophthalmic ointment applied three times daily for six weeks.

**1.17. Indication:**

RIF belongs to the rifamycin group of antibiotics and is used in the treatment of various infections due to mycobacteria and other susceptible organisms. The primary indications for RIF are for treatment of TB (pulmonary and extrapulmonary lesions) and for leprosy. It is also useful for elimination of *Neisseria meningococci* in carriers (but not recommended for active meningococcal infection) and for Gram positive (*Staphylococcus aureus* and *epidermidis*, *Streptococcus pyogenes*, *viridans* and *pneumoniae*) and gram negative bacteria (*Haemophilus influenzae* type B). It has some anti-chlamydial activity and *in-vitro* activity against some viruses (poxvirus and adenovirus) at high doses (Van Scy and Wilkowske, 1987). It has recently been used for brucellosis.

RIF is used, notably in combination with isoniazid and pyrazinamide, as a component of multidrug regimens for the treatment of TB. In the meningeal infection with *Haemophilus influenzae* type b and bacterial meningitis caused by *Neisseria meningitides*. In leprosy regimens, RIF is usually given with dapsone for paucibacillary leprosy and with dapsone and clofazimine for multibacillary leprosy.
2. ISONIAZID (INH)

2.1. Structure:

![ISONIAZID Structure](image)

2.2. IUPAC Name: Pyridine-4-carboxyhydrazide

2.3. Molecular Formula: C$_6$H$_7$N$_3$O

2.4. Molecular Weight: 137.139

2.5. Category: Antibacterial

2.6. Physical Characteristics: White crystalline powder

2.7. Melting Point: 172°C

2.8. pH (1% solution in water): 6.6-8.0

2.9. Solubility:

Freely soluble in water, sparingly soluble in alcohol and very slightly soluble in ether and other organic solvents.

2.10. Indications:

INH is an antimycobacterial agent which is bactericidal for both extracellular and intracellular microorganisms. It is the primary drug for the treatment of TB when the disease is caused by INH-sensitive strains of Mtb. However, active TB must be treated with multiple concomitant anti-TB medications to prevent the emergence of drug resistance. Single-drug treatment of active tuberculosis with INH, or any other medication, is inadequate for therapy. INH is recommended as preventive therapy for the following groups, regardless of age:-

Persons with AIDS and persons with risk factors for HIV infection, whose infection status is unknown.
Preventive therapy may be considered for HIV infected persons who are tuberculin-negative. For preventive therapy candidates, who have HIV infection, should have minimum of 12 months therapy.

2.11. Mechanism of Action:

The primary action of INH is to inhibit the biosynthesis of mycolic acid, the important constituent of the mycobacterial cell wall. Exposure to INH leads to a loss of acid fastness and a decrease in the quantity of methanol-extractable lipid of microorganism (Vilcheze et al., 2000; Takamaya et al., 1975).

2.12. Pharmacokinetics:

INH is rapidly and almost completely (90-95%) absorbed from the gastrointestinal tract. Peak plasma concentrations are reached within 1 to 2 h after ingestion. Bioavailability is reduced when INH is administered with food. It diffuses readily 2 h after ingestion. Bioavailability is reduced when INH is administered with food. It diffuses readily into all body fluids (including serebrospinal, pleural and ascitic), tissues, organs and excreta (saliva, sputum and feces). The drug also passes through the placental barrier and into milk in concentrations comparable to those in plasma. INH is <10% bound to plasma proteins.

The liver metabolizes INH mainly by acetylation and dehydration. The N-acetylhydrazine metabolite is believed to be responsible for the hepatotoxic effect seen in patients treated with INH. The rate of acetylation is genetically determined. The half life in slow acetylators is 2 to 5 h while in fast acetylators it is 1 to 2 h. Elimination is largely independent of renal function; however the half life may be prolonged in liver diseases. The rate of acetylation has not been shown to significantly alter the effectiveness of INH. However, slow acetylation may lead to higher blood concentrations with chronic administration of the drug, with an increased risk of toxicity. INH and its metabolites are excreted in urine with 75 to 90% of the dose excreted in 24 h. Small amount is also
excreted in saliva, sputum and feces. INH is removed by hemodialysis and peritoneal dialysis.

2.13. **Adverse Effects:**

The most frequent reactions are those affecting the nervous system and the liver.

**Nervous system reactions:** Peripheral neuropathy is the most common toxic effect. It is dose–related, occurs most often in the malnourished and in those predisposed to neuritis (eg., alcoholics and diabetics), and is usually preceded by parathesias of the feet and hands. The incidence is higher in “slow acetylators”. Other neurotoxic effects, which are uncommon with conventional doses, are convulsions, toxic encephalopathy, optic neuritis and atropy, memory impairment and toxic psychosis.

**Gastrointestinal reactions:** Nausea, vomiting and epigastric distress.

**Hepatic interactions:** Elevated serum transaminases (SGOT, SGPT), bilirubinemia, bilirubinuria, jaundice, and occasionally severe and sometimes fatal hepatitis. The common symptoms are anorexia, nausea, vomiting, fatigue, malaise, and weakness. Mild and transient elevation of serum transaminases level occurs in 10 to 20% of persons taking INH. The abnormality usually occurs in the first 4 to 6 months of treatment but can occur at any time during therapy. In most instances, enzyme levels return to normal with no necessity to discontinue medication. In occasional instances, progressive liver damage occurs, with accompanying symptoms.

**Hematological reactions:** Agranulocytosis, hemolytic sideroblastic or aplastic anemia, thrombocytopenia, and eosinophilia.

**Hypersensitivity reactions:** Fever, skin eruptions, lymphadenopathy, and vasculitis.

2.14. **Dose**

Adult: 15 mg/ Kg

Child: 5-10 mg/ Kg
Neonate: The recommended intravenous and intramuscular dose for neonate is 3-5 mg/Kg with a maximum of 10 mg/Kg daily.

Adults and children: the usual intramuscular or intravenous dose for adults is 200 to 300 mg as a single daily dose, for children 100 to 200 mg daily (10-20 mg/Kg), but some time much larger doses are given, especially in conditions such as an undiluted bolus injection, although other methods may be employed.

3. RIF AND INH COMBINATION PRODUCTS:

Many fixed dose combination of these two first line anti-TB drugs are available in the market. The combination therapy not only improves the therapy, it also decreases the chances of drug resistance by the microorganism. These drugs possess the different modes of actions. The toxicity, contraindication, adverse reactions, interaction and dosage are same as of the parent drugs.

Table. 2.1: Marketed products (Combination) (Physicians’ Desk Reference, 2002)

<table>
<thead>
<tr>
<th>Product</th>
<th>Dosage form</th>
<th>Strength (RIF+INH)</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIPNEX</td>
<td>Tab</td>
<td>450+300 mg</td>
<td>Ind-swift</td>
</tr>
<tr>
<td>ZUCOX PLUS</td>
<td>Cap</td>
<td>450+300 mg</td>
<td>GSK</td>
</tr>
<tr>
<td>CAPTAB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIMPAZID-450</td>
<td>Tab</td>
<td>450+300 mg</td>
<td>Zydus Cadila</td>
</tr>
<tr>
<td>XEED 2</td>
<td>Tab(SR)</td>
<td>150+75 mg</td>
<td>Panacea</td>
</tr>
<tr>
<td>COXINEX</td>
<td>Cap</td>
<td>450+300 mg</td>
<td>Aristo</td>
</tr>
<tr>
<td>ANTICOX-II</td>
<td>Tab</td>
<td>450+300 mg</td>
<td>Unichem</td>
</tr>
<tr>
<td>AKT-2</td>
<td>Cap</td>
<td>600+300 mg</td>
<td>Lupin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>450+300 mg</td>
<td></td>
</tr>
<tr>
<td>IPCACIN</td>
<td>Tab</td>
<td>450+300 mg</td>
<td>Ipca</td>
</tr>
<tr>
<td>RIFAKEM INH</td>
<td>Cap</td>
<td>450+300 mg</td>
<td>Alkem</td>
</tr>
</tbody>
</table>
4. ANALYTICAL METHODS FOR DETERMINATION OF RIF AND INH

Numbers of methods are available for the estimation of RIF and INH in various working solutions and biological fluids. Some of them are summarized here:

**High Performance Liquid Chromatography (HPLC)**

Glass *et al.*, 2007 developed and optimized simple and reliable HPLC method for the simultaneous determination of RIF and INH in a fixed dose combination. The best separation and reasonably short retention times were produced on the micro-Bondapak C18, 4.6 x 250-mm column, 10 micron/125 A using ACN-tBAH (42.5:57.5, v/v)(0.0002 M) as the mobile phase.

A solution containing 15 µg/ml of RIF and INH in methanol was analyzed by HPLC using a column (Bondapak C18) with aqueous 85% methanol as mobile phase (1ml/min) and detected at 254 nm (Mandal *et al.*, 1986). The mixture of drugs in methanolic solution was analyzed using Radialpak C18 (10 µm) cartridge (10 cm x 8 mm) with aqueous 16.67% K$_2$HPO$_4$-methanol (1:3) as mobile phase at 2 ml/min flow rate. The UV detection was implicated at wavelength 254 nm (Mohammad and Aila, 1998).

A reversed phase HPLC method has been described for the simultaneous estimation of RIF and INH and its major metabolite desacetyl RIF, in the presence of INH in human plasma and urine. The assay involved simple liquid extraction of drug, metabolite and internal standard from biological specimens and their subsequent separation on a C18 reversed phase column and UV spectroscopic detection. Using methanol-sodium phosphate buffer (pH 5.2; 0.01 M) (65:35 v/v) as a mobile phase under isocratic conditions, it was established that INH, PZA and ascorbic acid (added to prevent oxidative degradation of analytes) did not interfere with the analyte peaks. Extraction efficiencies for the drug were greater than 90% in both plasma and urine, whereas for
metabolite the values were 79% and 86% in plasma and urine, respectively (Panchagnula et al., 1999).

Ruben et al., 2007 reported a novel, simple, precise, and accurate method for the quantification of RIF in both cells and plasma. After protein precipitation, aliquots of supernatant were injected into the HPLC-MS system for chromatographic separation and detection. RIF calibration curves using standard concentrations from 100 -12800 ng/ml were prepared. The assay was successfully utilized to determine the pharmacokinetic profile of RIF in plasma and peripheral blood mononuclear cells (PBMC).

Khuhawar and Rind, 2002 developed a simultaneous estimation method for INH, PZA and RIF in biological samples using HPLC method. The drugs were separated on YMC-ODS column. INH was derivatized with 2-fluorene-carboxaldehyde (FA). The separation was affected using ethanol-chloroform-acetonitrile-water by isocratic elution and detected at 337 nm. The detection limits were 0.11 ng, 0.2 ng, and 13 ng/injection (5µL) for INH, PZA, and RIF respectively. The method of analysis was used to analyze the pharmaceutical preparations and the blood samples of the patients suffering from TB after have received chemotherapy.

**Thin layer Chromatographic Method (TLC)**

TLC method has been used by Moffat, 1986 for identification of INH using silica gel plate and ethyl acetate, methanol and ammonia as a mobile phase.

TLC method was developed by Schmid et al., 1963 for the separation of INH from other drugs using chloroform: methanol (8:2) as mobile phase and folin ciocaltau reagent as detecting agent. The method was selective and highly reproducible for separation of drugs.

Ali et al., 2007 developed a simple, selective, precise, and stability indicating HPTLC method. The method was validated for analysis of RIF and INH both as the bulk drugs and in formulations. The compounds were separated on aluminum-backed silica gel
60-F\textsubscript{254} plates with n-hexane-2-propanol-acetone-ammonia-formic acid, 3:3.8:2.8:0.3:0.1 (v/v) as mobile phase. This system was found to give discrete and compact spots for INH and RIF. Densiometric analysis of RIF and INH was conducted at 254 nm. Regression analysis data for the calibration plots were indicative of good linear relationships between response and concentration over the range 100-700 ng per spot. The correlation coefficients were 0.994 and 0.997 for INH and RIF respectively.

Jindal et al., 1994 described a TLC assay for the determination of RIF and its degradation components in drug excipient interaction studies. The chromatography was performed on thin layer plates (silica gel) with a mobile phase consisting of chloroform-methanol-water (80:20:2.5, v/v/v). The peaks were quantified by densiometric evaluation of the chromatograms. The method showed a limit of detection of 10 ng per band and good precision and linearity in the range 50-3000 ng per band for RIF, 3-formylrifamycin, RIF N-oxide and 25-desacetyl RIF and 100-350 ng per band for RIF quinine.

**UV-Visible Spectroscopy**

Various spectrophotometric methods used for determining RIF in biological fluids have been reported in the literature, but due to their low sensitivity they could be used only for the determination of relatively high level of concentration (more than 5µg/mL). These are based on the extraction of RIF and its metabolites using organic solvents and measurement of visible absorbance of the organic extract (Abbamonte et al., 1969; Maggi et al., 1969; Sunahara and Nakagawa, 1972).

Sferruzza and Rangone, 1964 reported a spectrophotometric method for the determination of RIF in various pharmaceutical preparations, which was subsequently modified by Pasqualucci et al., (1970).

Manna et al., (2000) developed a simple spectrophotometric method for simultaneous estimation of RIF and INH in their combined pharmaceutical dosage forms.
The method did not require any extraction or isolation procedure. The method is simple, rapid, specific and reproducible and having satisfactory recovery.

**Other Methods**

RIF was determined fluorometrically by using transformed hydrogen peroxide as a fluorescent product. The maximum fluorescence develops in an aqueous carbonate-bicarbonate buffer, pH 9.2 at 480 nm, when the excitation wavelength is 370 nm. The relative fluorescence intensity is linear with concentration of RIF in the range of 0.1 to 10 µg/ml (Sensi et al., 1960; Finkel et al., 1971)

Simoncini et al., 1968 developed a turbidimetric microbiological assay for RIF using automatic analyzer. The method is mainly based on the measurement of the optical density of the bacterial suspension (Escherichia coli ATCC 10536 as test microorganism) in Difco medium containing RIF, after incubation at 37°C for 3.5 h.

A number of titrimetric methods have been employed for the determination of INH in bulk and in the formulations. USP recommends a nitrite titration method. According to BP, the INH is reacted with bromine and an excess bromine is titrated with thiosulfate after liberation of iodine by addition of potassium iodide (BP, 1973).

A.C. polarography has been used by Vallon et al., 1975 in the assay of INH. In this assay INH was reacted with 1, 2- naphthoquinone-4-sulfonic acid and the by-product was determined by polarographic method.

Akiyama et al., 1956 precipitated INH as Cu (II) or Hg (II) salts. The salts were redissolved in hydrochloric acid and the metal then subsequently reprecipitated as the sulfide, which was finally estimated gravimetrically.

Several agar diffusion microbiological assays utilizing strains of *Mtb* have been used to measure INH content (Tabekin et al., 1952; Carotti and Atti, 1953; Ifrim & Coniver, 1958).
Dickinson et al., 1974 described two microbiological methods for the determination of RIF in serum. The first is a conventional plate diffusion method, which could measure down the concentrations up to 0.02 µg/ml, and the second a chemical extraction followed by measurement of inhibition of uptake of 14C-uridine by *Staphylococcus aureus*, which estimates RIF in the range of 0.02 to 0.001 µg/ml. The methods were used to measure serum concentrations in human blood plasma following doses of about 105.0 mg and 75 mg of RIF.