ABSTRACT:

Rifampicin is a first line medication used as an anti-tubercular agent. Rifampicin acts by binding and inhibiting DNA dependent RNA polymerase. It is active against gram positive and negative both types of bacteria. The clinical and pharmaceutical analysis of this drug requires effective analytical procedures for quality control and pharmacodynamic and pharmacokinetic studies as well as stability study. An extensive survey of the literature published in various analytical and pharmaceutical chemistry related journals has been conducted and the instrumental analytical methods which were developed and used for determination of Rifampicin as single or combination with other drugs in bulk drugs, formulations and biological fluids have been reviewed. This review covers the time period from 1997 to 2014 during which 33 analytical methods including spectrophotometric methods like UV and derivative; visible which is based on formation of metal complexation, spectrofluorometric methods and chromatographic method including HPLC, HPTLC, and miscellaneous method like HPLC-MS were reported. The application of these methods for the determination of Rifampicin in pharmaceutical dosage form and biological samples has also been discussed.

KEY WORDS: Rifampicin, UV spectroscopy, HPLC, HPLC-MS, HPTLC Cobalt complexes

INTRODUCTION:

Mycobacteria are intrinsically resistant to most antibiotics. Because they grow slowly compared with other bacteria, antibiotics that are most active against growing cells are relatively ineffective. Mycobacterial cells can also be dormant and thus completely resistant to many drugs or get killed very slowly. The lipid-rich mycobacterial cell wall is impermeable to many agents. Mycobacterial species are intracellular pathogens, and organisms residing within macrophages are inaccessible to drugs that penetrate these cells poorly. Finally, mycobacteria are notorious for their ability to develop resistance. Combinations of two or more drugs are required to overcome these obstacles and to prevent emergence of resistance during the course of therapy. The response of mycobacterial infections to chemotherapy is slow, and treatment must be administered for months to years, depending on which drugs are used.1

Tuberculosis is the most important communicable disease. The one-third of the world’s population is infected by Mycobacterium tuberculosis according to the World Health organization (WHO) estimation. HIV-infected persons, immigrants from countries with high rates of tuberculosis, the homeless, health care professionals, intravenous drug users, persons taking immunosuppressive agents, and those in institutional settings, such as nursing homes and correctional facilities groups are at high risks for tuberculosis infection. There is a progressive increase in multidrug-resistant (MDR) tuberculosis.2
Classification of tuberculosis:

I. **First line drugs** - rifampicin, isoniazid, pyrazinamide, ethambutol and streptomycin (superior in efficacy and possess an acceptable degree of toxicity)

II. **Second line drugs** - cycloserine, ethionamide, aminosalicylic acid, rifabutin, quinolones, capreomycin, viomycin, and thiacetazone (more toxic and less effective, and they are indicated only when the *M. tuberculosis* organisms are resistant to the first-line agents)

To decrease the possibility of the emergence of resistant organisms, compound drug therapy is employed, involving the following:

- a first initial phase of about 2 months consisting of three drugs used concomitantly: isoniazid, rifampicin, pyrazinamide (plus ethambutol if the organism is suspected to be resistant)
- a second, continuation phase, of 4 months, consisting of two drugs: isoniazid and rifampicin; longer-term treatment is needed for patients with meningitis, bone/joint involvement or drug-resistant infection.

<table>
<thead>
<tr>
<th>Table 1 Antimicrobials Used in the Treatment of Tuberculosis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
</tr>
<tr>
<td>------</td>
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<tr>
<td><strong>First-line agents</strong> (in approximate order of preference)</td>
</tr>
<tr>
<td>Isoniazid</td>
</tr>
<tr>
<td>Rifampin</td>
</tr>
<tr>
<td>Pyrazinamide</td>
</tr>
<tr>
<td>Ethambutol</td>
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<tr>
<td>Streptomycin</td>
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<tr>
<td><strong>Second-line agents</strong></td>
</tr>
<tr>
<td>Amikacin</td>
</tr>
<tr>
<td>Aminosalicylic acid</td>
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<tr>
<td>Capreomycin</td>
</tr>
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<td>Ciprofloxacin</td>
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<tr>
<td>Clofazimine</td>
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<tr>
<td>Cycloserine</td>
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<td>Ethionamide</td>
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<tr>
<td>Levofloxacin</td>
</tr>
<tr>
<td>Rifabutin</td>
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<tr>
<td>Rifapentine</td>
</tr>
</tbody>
</table>

1 Assuming normal renal function.

2 150 mg/d if used concurrently with a protease inhibitor.

### Table 2 Recommended Duration of Therapy for Tuberculosis

<table>
<thead>
<tr>
<th>Regimen (in approximate order of preference)</th>
<th>Duration in Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid, rifampin, pyrazinamide</td>
<td>6</td>
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<tr>
<td>Isoniazid, rifampin</td>
<td>9</td>
</tr>
<tr>
<td>Rifampin, ethambutol, pyrazinamide</td>
<td>6</td>
</tr>
<tr>
<td>Rifampin, ethambutol</td>
<td>12</td>
</tr>
<tr>
<td>Isoniazid, ethambutol</td>
<td>18</td>
</tr>
<tr>
<td>All others</td>
<td>24</td>
</tr>
</tbody>
</table>

**Rifampicin (Rifampin):**

**Mechanism of Action:**
Rifampicin acts by inhibiting and binding to DNA-dependent RNA polymerase in prokaryotic but not in eukaryotic cells. It is one of the well known most active antituberculosis agents. It is also active against most Gram-positive bacteria as well as many Gram-negative species. It enters phagocytic cells and can kill intracellular microorganisms including the tubercle bacillus. Resistance can develop rapidly in a one-step process and due to chromosomal mutation is thought to be caused by chemical modification of microbial DNA-dependent RNA polymerase.

**Pharmacokinetic:**

Rifampicin is readily absorbed from the gastrointestinal tract. The biological half-life of rifampicin in serum averages about 3 hours after a 600mg dose and increases to 5.1 hours after a 900 mg dose in normal subjects. After absorption, rifampicin (oral or iv) is rapidly eliminated in the bile, and an enterohepatic circulation ensues. Intestinal reabsorption is reduced by deacetylation, and elimination is
facilitated. With about half of this being unchanged rifampicin, up to 30% of a dose is excreted in the urine.

On food ingestion, absorption of rifampicin is reduced. Rifampicin is widely distributed throughout the body. It is also present in many organs and body fluids, and also in cerebrospinal fluid. Rifampicin is about 80% protein bound. Most of the unbound fraction is not ionized and therefore is diffused freely in tissues.

Pharmacodynamics:

Rifampicin has high activity against many organisms, Mycobacterium tuberculosis and M.leprae, including Staphylococcus aureus, coagulase-negative staphylococi, Listeria monocytogenes, Neisseria meningitidis, Haemophilus influenzae, Legionella spp., Brucella, some strains of E. coli, Proteus mirabilis, anaerobic cocci, Clostridium spp., and Bacteroides. It is also reported that rifampicin exhibits an immunosuppressive effect which has been seen in some animal experiments, but this may not be clinically significant in humans. Depending on the concentration of drug attained at site of infection, rifampicin may be bacteriostatic or bactericidal. The bactericidal actions are secondary to interfering with the synthesis of nucleic acids by inhibiting bacterial DNAdependent RNA polymers at the B-subunit thus preventing initiation of RNA transcription.

Indications:

Clinical Uses

Rifampicin, a first-line antitubercular drug is used in the treatment of all forms of pulmonary and extrapulmonary tuberculosis. It is an alternative to isoniazid in the treatment of latent tuberculosis infection. Rifampicin may also be combined with an antileprosy agent for the treatment of leprosy and to protect those in close contact with patients having H. influenza type B and N. meningitidis infection; and is also used inmethicillin-resistant staphylococcal infections, such as osteomyelitis and prosthetic valve endocarditis.

Adverse Reactions

The most commonly observed side effects are GI disturbances and nervous system symptoms, such as nausea, vomiting, headache, dizziness, and fatigue. A major adverse effect is Hepatitis, and the risk is highest in patients with underlying liver diseases and in slow isoniazid acetylators; if isoniazid and rifampin are combined, the rate of hepatotoxicity is increased. Rifabutin is commonly substituted for rifampin in the treatment of tuberculosis in HIV-infected patients. Hypersensitivity reactions, such as pruritus, cutaneous vasculitis, and thrombocytopenia, are seen in some patients, and an immune-mediated systemic flulike syndrome with thrombocytopenia also has been described.

Rifampicin imparts a harmless red-orange color to urine, feces, saliva, sweat, tears, and contact lenses. Patients should be advised of such discoloration of body fluids.

Different analytical methods for Rifampicin and its combination:

Development of new methods, provide determinations with maximum accuracy, which are responsible to increase the interest in analytical methods as such. They should enable to simultaneously determine the individual components in multicomponent preparations and in Dosage form as well as biological material. These developed validated Methods confirms them the appropriate quality of the product and of the analytical method used. These are different parameters that validate reliability of the results and enable comparing efficiency of the methods used. Validation parameters were done according to the ICH guideline.

Several techniques like HPLC [10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33], HPTLC [11,32], Spectroscopic methods [1, 2, 4, 5, 6, 7, 8, 9], Colorimetric method [3], have been used for the determination of Rifampicin. Chromatographic methods have been extensively used and recommended. Since, these methods require complex and expensive equipment, provision for use and disposal of solvents, labour-intensive sample preparation procedures and personal skills in such techniques.

There was no review published covering all different analytical method used for the determination of Rifampicin. The high importance of Rifampicin in Tuberculosis prompted us to review the most important recent analytical methods for their analysis in pure forms, in different pharmaceutical dosage forms and in biological fluids reported so far in the literature. The present review comprises references covering the period from 1997 to 2014. The official method of determination of rifampicin was HPLC method in Indian Pharmacopoeia and spectroscopy and HPLC in British Pharmacopoeia.
Different analytical methods for Rifampicin and its combination:

<table>
<thead>
<tr>
<th>SR NO.</th>
<th>DRUG</th>
<th>METHOD</th>
<th>DESCRIPTION</th>
<th>DETECTION AT</th>
<th>SPECIFICATION</th>
<th>REF NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Rifapentine</td>
<td>UV</td>
<td>Chemito UV 2600 spectrophotometer with 1 cm matched quartz cells Y = 0.004x+0.024</td>
<td>478nm (0.1N HCl)</td>
<td>[ R^2 \text{- } 0.999 \ Linearity range: 5-50µg/ml \ LOD: 3.28 µg/ml(2.15) \ LOQ: 9.96 µg/ml(6.25) \ %RSD: 0.003985 ]</td>
<td>7.</td>
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<tr>
<td>2.</td>
<td>Rifampicin &amp; Piperine</td>
<td>2nd dvt.</td>
<td>No interference from the capsule excipients</td>
<td>341nm for rifampicin (0 cross point for piperine) 241nm for piperine (0 cross point for rifampicin)</td>
<td>[ R^2 - 0.999 \ Linearity range: 10-60 µg/ml for rifampicin &amp; 2-20 µg/ml for piperine \ LOD: RIFA-1.65, PIPE-0.57, LOQ: RIFA-5.03, PIPE-1.75 \ Repeatability(%RSD): RIFA-0.31, PIPE-0.37 ]</td>
<td>8.</td>
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<tr>
<td>3.</td>
<td>Rifampicin</td>
<td>Visible</td>
<td>Shimadzu double beam spectrophotometer UV-140 A=0.0009+0.0119C Buffer solution (pH=7.0)</td>
<td>400-650nm (max at 510nm)</td>
<td>[ R^2 - 0.9999 \ Linearity range: Conc range:5-50µg/ml \ Molar absorptivity(L/mole cm): 9.83x10³ \ Sandell’s sensitivity(µg/cm² / 0.001 absorbance unit): 0.084 \ Optimum photometric range: 7.9-39.1µg/ml \ %RSD=1.90 ]</td>
<td>9.</td>
</tr>
<tr>
<td>4.</td>
<td>Rifampicin and Isoniazid</td>
<td>Simultaneous equation method)</td>
<td>Non interference of the excipients Solvent-ethanol</td>
<td>RIF-337nm INH-263nm</td>
<td>[ R^2 - RIF- 0.9991, INH- 0.9998 \ Linearity range: RIF- 5-35 µg/ml, INH- 5-25 µg/ml \ Molar absorptivity(L/molcm) RIF-25349, INH-9341 ]</td>
<td>10.</td>
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<tr>
<td>5.</td>
<td>Rifampicin &amp; Piperine</td>
<td>Q- Absorbance ratio</td>
<td>A Shimadzu model 1700 double beam UV/Visible spectral width - 2 nm, wavelength accuracy-0.5 nm &amp; pair of 10 mm matched</td>
<td>In methanol RIF-337nm PIPE-337nm RIFA+PIPE-387nm</td>
<td>[ R^2 - 0.999 \ Linearity range: RIF- 5-40 µg/ml, PIPE-2-20 µg/ml ]</td>
<td>11.</td>
</tr>
<tr>
<td>No.</td>
<td>Method</td>
<td>Solvents/Reagents</td>
<td>Results</td>
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<tr>
<td>6</td>
<td>Visible &amp; 1st derivative</td>
<td>Buffer solution pH 7.4 as solvent</td>
<td>RIF+PIPE 0.80 and 0.32 µg/ml</td>
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<td>257nm(1st derivative, HCl 0.012M)</td>
<td>[LOQ]= RIF- 4.6 µg/ml, PIP- 0.86 µg/ml, RIFA+PIPE- 2.45 and 0.98 µg/ml</td>
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<td></td>
<td>220-360nm with interval of 10nm</td>
<td>%RSD= RIF-0.54, PIP-0.091, RIF+PIPE-0.68</td>
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<td>Precision: RIFA-0.14-0.71, PIP-0.11-1.50, RIFA and PIP-0.17-1.31</td>
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<tr>
<td>7</td>
<td>UV &amp; RPLC-PDA Methods</td>
<td>2 chemometric methods applied-ILS and CLS</td>
<td>Linearity range: INH- 30-270 µg/ml RIF- 20-180 µg/ml, PIP- 1-9 µg/ml</td>
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<tr>
<td></td>
<td>UV, RP-HPLC</td>
<td>Reversed phase: phenomenex Luna C18 column-</td>
<td>Linearity range: INH- 30-270 µg/ml RIF- 20-180 µg/ml, PIP- 1-9 µg/ml</td>
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<td>gradient elution with mobile phase 0.05M Na&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt; buffer pH 7 and acetonitrile</td>
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<td>Total run time: 12min</td>
<td>[RSD]= INH- 0.3, RIF- 0.05, PIP- 0.08, RIFA+PIPE- 0.06-0.14, PIP- 0.05-0.1</td>
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<tr>
<td>8</td>
<td>Simultaneous spectrophotometric by 1st derivative</td>
<td>RIF at ZCP for INH and PYZ(262.2 and 268.8)</td>
<td>Correlation coefficient-0.995</td>
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<td>PYZ at ZCP for RIF and PYZ (254 and 268.8nm)</td>
<td>Linearity range: INH- 12-34.5 µg/ml RIFA- 8-23 µg/ml, PIP-0.4-1.15 µg/ml</td>
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<td>PYZ at ZCP for INH and RIF (262.2 and 254 nm)</td>
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<tr>
<td>9</td>
<td>UV, RP-HPLC</td>
<td>Methanol and distilled water was used as</td>
<td>INH-262nm RIFA-477nm</td>
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<tr>
<td></td>
<td></td>
<td>diluents 2nd RP-HPLC- acetonitrile as diluents</td>
<td>Linearity range: INH- 12-34.5 µg/ml RIFA- 8-23 µg/ml, PIP-0.4-1.15 µg/ml</td>
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<td>Flow rate of 0.9mL/min</td>
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<tr>
<td>10</td>
<td>SP-C18 monolithic</td>
<td>with detection at 254 nm</td>
<td>Linearity range: RQ: 1.5-60 µg/ml SV- 1-40 µg/ml, RNO-1-40 µg/ml, RIF- 5-200 µg/ml</td>
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<tr>
<td></td>
<td>HPLC</td>
<td>UV detection at 254 nm</td>
<td>LOD, LOQ 0.2 µg/ml, 1 µg/ml, %RSD 2.5%</td>
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<tr>
<td>11</td>
<td>SP- Al backed silica gel</td>
<td>254nm</td>
<td>R&lt;sup&gt;2&lt;/sup&gt;-INH 0.994</td>
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<td>12</td>
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<td>220-360 nm with interval of 10nm</td>
<td>Linearity range: INH- 30-270 µg/ml RIF- 20-180 µg/ml, PIP- 1-9 µg/ml</td>
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<tr>
<td>13</td>
<td>Isoniazid and Piperine</td>
<td>UV &amp; RPLC-PDA Methods</td>
<td>99.03% for rifampicin &amp; 100.01% for isoniazid.</td>
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<tr>
<td>14</td>
<td></td>
<td>Simultaneous spectrophotometric by 1st derivative</td>
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<tr>
<td>15</td>
<td>UV, RP-HPLC</td>
<td>Methanol and distilled water was used as</td>
<td>INH-262nm RIFA-477nm</td>
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<td>Flow rate of 0.9mL/min</td>
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<tr>
<td>16</td>
<td>SP-C18 monolithic</td>
<td>with detection at 254 nm</td>
<td>Linearity range: RQ: 1.5-60 µg/ml SV- 1-40 µg/ml, RNO-1-40 µg/ml, RIF- 5-200 µg/ml</td>
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<tr>
<td></td>
<td>HPLC</td>
<td>UV detection at 254 nm</td>
<td>LOD, LOQ 0.2 µg/ml, 1 µg/ml, %RSD 2.5%</td>
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<tr>
<td>17</td>
<td>SP- Al backed silica gel</td>
<td>254nm</td>
<td>R&lt;sup&gt;2&lt;/sup&gt;-INH 0.994</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
and Isoniazid

60 F_{254} plates

MP-
n-hexane:2-
propanol:acetone:am-
monia:formic
acid=3:3.8:2.8:0.3:0.1
(v/v)

Resolved INH and RIF
with R_i values of 0.59 ±
0.02 and 0.73 ± 0.04,
respectively

RIF- 0.997

Linearity range-
100-700 ng per spot

LOD= INH- 20 ± 0.51ng
RIF- 25 ± 0.63ng

LOQ= INH- 60 ± 1.05 ng
RIF- 75 ± 1.12 ng

%RSD=
INH= Conc (ng per spot)
200- 0.17
300-0.26
600-0.19
RIF=
200- 0.37
300- 0.44
600- 0.38

12. Rifampicin & Hydrochlorothiazide

HPLC

SP- 150 X 4.6 mm i.d, 5
μm Phenomenex ODS 2
C18 column.

MP - 40:60 % v/v
acetonitrile and 10mM
KH_{2}PO_{4}
(pH 3.2), flow rate of 1.0
ml/min

R_t for RIF and HCTZ were
6.80 and 2.56 min,
respectively.

Correlation coefficient-0.9971

Linearity range-
0.31 – 25.48 μg/ml

LOD=100ng/ml

LOQ=1μg/ml

13. Rifampicin

HPLC

SP- An Ultrabase-C18
column

MP- water (pH 2.27):
acetonitrile= (40:60 v/v),
flow-rate - 1 ml/min

Rt- 4 min.

Linearity range-
0.1–1 and 1–50 g/ml for
plasma
0.6–40 g/g for liver

LOD=
0.025μg/ml for plasma
0.06 μg/g for liver

LOQ=
0.05 μg/ml for plasma
0.25μg/g for liver

14. Rifampicin in complex (isoniazide and serum)

HPLC

Stationary phase-
a 5 μm C18

Mobile phase-
water-methanol at
a flow rate of 1 ml/min.

Rt- 12.8 min

R^2 >0.98

Linearity range- 0.1- 0.3 mg/ml for
drug content in
Rifamazid and from 1 - 3
μg/ml for serum
%RSD=
1)RIF in rifamazid capsules-
0.0024
2) RIF in serum- 0.037

15. Serum rifampicin

HPLC

SP- Phenomenex
Prodigy ODS3 150mm x
4.6mm, 5μm, 100Å
MP- 70% 0.1mmol/L
phosphate buffer pH 4.8, 30% methanol

Flow: 1.0ml/min

Linearity range-
2-20μmol/L

16. pyrazinamide, isoniazid, rifampicin

RP-HPLC

Stationary phase-
pre-column derivatization with
phenethyl isocyanate

R^2- 0.998

Linearity range-
PYP : 16.0–160 μg/ml
INH : 4.8–48.0 μg/ml
Ethambutol 2HCl (PEIC)

Mobile phase-gradient consisting of acetonitrile: phosphate buffer (8 mM, pH 6.8) 10:90v/v at 0min (gradient) 60:40v/v for 18min (linear gradient) at a flow rate of 1.0 ml/min

RIF : 4.8–48.0 μg/ml
EMB : 10.1–101.0 μg/ml

LOD=
PYR- 0.13 μg/ml
INH- 0.08 μg/ml
RIF- 0.20 μg/ml
EMB- 0.10 μg/ml

LOQ=
PYR- 0.40 μg/ml
INH- 0.24 μg/ml
RIF- 0.60 μg/ml
EMB- 0.30 μg/ml

Reproducibility (%RSD)
PYR-0.83
INH-0.86
RIF-0.66
EMB-0.95

17. Rifampicin & papaverine HCl

HPLC

SP- Kromasil C18 column
MP- ammonium acetate (20mM, pH 4) & ACN

334nm

R² - 0.2

Linearity range- 0.5-20μg/ml

LOQ= 0.5 μg/ml in plasma
1.5 μg/ml in blood

18. Rifampicin

RP-HPLC

SP-C18 column
MP-phosphate buffer pH 7.4: methanol(75:25 v/v)
Rₜ - 2.54min

475nm

Linearity range- 0.05-20L1/4gm/mL for plasma
Good reproducibility (both interday and intraday)

19. Rifampicin

RP-HPLC

SP-C18 column
MP- Acetonitrile:monobasic potassium phosphate buffer solution 0.05 M (38:62 v/v)

The Rₜ of RFP and RFP-QN were 7.81 and 12.26 minutes, resp.

20. Isoniazid (INH), rifampicin (RIF), and pyrazinamide (PZA), deacetylrifampicin(DRIF)

HPLC

SP- a C8 reversed phase column
MP- RIF and DRIF=80% acetonitrile/0.1% trifluoroacetic acid (TFA)
INH and PZA =3% acetonitrile/0.6% TFA

Correlation coefficient- 0.9995

21. Rifampicin and Isoniazid

HPLC

SP-isocratic conditions with a octadecylsilane column
MP-Methanol (75%):0.02M Disodium Hydrogen Orthophosphate(25%) with pH4.5 adjusted with o-phosphoric acid.

254nm

Precise

22. Rifampicin

RP-HPLC

SP- a C18 column

254nm

Correlation coefficient-
(RMP) and desacetyl rifampicin (DRMP) and rifapentine (RPN)

<table>
<thead>
<tr>
<th>RMP-0.05 M phosphate buffer: acetonitrile (55:45 v/v)</th>
<th>Plasma- 0.9996 urine- 0.9943</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_t = ) DRMP-2.9min ( R_t = ) RMP-4.8min ( R_t = ) RPN-10.5min</td>
<td></td>
</tr>
<tr>
<td>linearity range- 0.25-15µg/ml for plasma 2.5-80µg/ml for urine</td>
<td></td>
</tr>
<tr>
<td>LOD= 0.1-0.25µg/ml LOQ= 1.2.5µg/ml</td>
<td></td>
</tr>
<tr>
<td>Accuracy and precision good for both intra day and inter day</td>
<td></td>
</tr>
</tbody>
</table>

23. Rifampicin HPLC-MS MP-Acetonitrile : water linearity range- 100-12800ng/mL

24. Rifampicin RP-HPLC SP- an ODS C18(4.6x150mm, 3.5µm) analytical column

| MP-potassium dihydrogen phosphate buffer( pH3 adjusted with o-phosphoric acid) and acetonitrile in the ratio of 50:50(v/v) The flow rate was 1ml/min. |
| Correlation coefficient- 0.9999 linearity range- 10-50 ppm |
| LOD= 0.026µg/ml LOQ=0.087 µg/ml |
| Theoretical plates- 4092.567 Tailing factor- 1.46 |

25. Rifampicin and piperine RP-HPLC SP-a Hypersil BDS C18 (25cm x 4.6mm, 5 µm) column,temp 25°C MP-Methanol: Acetonitrile(Buffer and Acetonitrile in the proportion of 55 : 45 (v/v) with apparent pH adjusted to 6.8) Flowrate-1.5ml/min

| \( R_t = \) INH-2.953min \( R_t = \) RIF-3.382min |
| Correlation coefficient- RIF=1 (Y= 23308x- 1898.2) PIPE=0.999 (y= 105066x-372.4) linearity range- RIF- 8-24µm/ml PIPE- 0.4-1.2 µg/ml Resolution= 8.93±0.8% RIF= LOD- 0.498 µg/ml LOQ= 1.51 µg/ml PIPE= LOD- 0.081 µg/ml LOQ= 0.246 µg/ml Theoretical plates- RIF= 5631±0.7% PIPE= 13218±0.8% |

26. Isoniazid, Rifampicin RP-HPLC SP- A Inertsil ODS (250*4.6*5µ) column Column temp. was 30°C MP-Water pH 4.5 adjusted with Sodium di hydrogen phosphate: Acetonitrile in the ratio of (40: 60, v/v) The flow rate was 1.0ml/min

<p>| ( R_t = ) INH-2.953min ( R_t = ) RIF-3.382min |
| Correlation coefficient- 0.99 for both linearity range- Y= 1664x for Isoniazid and y = 19288x for Rifampicin LOD= INH-2.359 RIF-2.896 LOQ= INH-7.864 RIF-9.6541 Theoretical plates= INH-6642 RIF-10333 |</p>
<table>
<thead>
<tr>
<th></th>
<th>Method</th>
<th>Column</th>
<th>Mobile Phase</th>
<th>Wave length</th>
<th>Correlation coefficient</th>
<th>Linearity Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.</td>
<td>Rifampicin and a flavonoid glycoside(CC-I)</td>
<td>RP-HPLC</td>
<td>SP-RP-18 column</td>
<td>340nm</td>
<td>&gt;0.999</td>
<td>0.1-10 µg/mL for RIF, 0.05-10 µg/mL for CC-I in combination</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MP-acetonitrile: phosphate buffer, 50 mM, pH 5.0 in a ratio of 60:40 v/v; oven temperature, 40°C; flow rate 0.8 ml min total run time 15 min.</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Retention time</td>
<td></td>
<td>R&lt;sub&gt;t&lt;/sub&gt;-RIF 4.779</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CC-I 3.072</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28.</td>
<td>Rifampicin, isoniazid</td>
<td>HPLC and wall-jet/thin layer electrochemical detection</td>
<td>SP- Reversed phase C18 column (150mmx4.6mm, 5µm)</td>
<td>268nm</td>
<td>0.01-100µg/mL for INH and RIF</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MP-gradient elution</td>
<td></td>
<td>LOD= INH-0.3nM RIF-0.5nM (S/N=3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Flow rate- 1mL/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29.</td>
<td>Rifampicin, isoniazid, pyrazinamide</td>
<td>RP-HPLC</td>
<td>SP- A Hypersil C18, 5 mm, 250 mm x 4.6 mm internal diameter column was maintained at 40°C</td>
<td>254nm</td>
<td>0.999</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MP-isocratic elution with potassium phosphate buffer (pH 6.0; 0.05 M) for 10 min, followed by linear gradient to potassium phosphate buffer (pH 6.0; 0.05 M)-methanol (40:60, v/v) in 5 min, isocratic elution at the same composition for a further 15 min and then linear gradient back to potassium phosphate buffer (pH 6.0; 0.05 M) in 5 min. The flow-rate was 1 ml/min.</td>
<td></td>
<td>linearity range- good</td>
<td>Analysis time is 35 minutes.</td>
</tr>
<tr>
<td>30.</td>
<td>Rifampicin, isoniazid</td>
<td>Isocratic HPLC</td>
<td>SP- A shimadzu liquid chromatographic unit equipped with a C18 GraceVydac analytical column(250mmx4.5 mm, 5µm particle size)</td>
<td>254nm</td>
<td>0.999</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MP-0.05M sodium dihydrogen phosphate sol(pH 3.1) and acetonitrile (20:80) flow-rate set at 0.6 ml/min.</td>
<td></td>
<td>linearity range- good</td>
<td>Flow rate set at 0.6ml/min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Good accuracy, precision</td>
<td></td>
</tr>
<tr>
<td>31.</td>
<td>Rifampicin, isoniazid</td>
<td>HPLC</td>
<td>SP- Micro-bondpack C18, 4.6x250mm column</td>
<td>254nm</td>
<td></td>
<td>Retention time were measured on different</td>
</tr>
</tbody>
</table>
Pyrazinamide was optimized using an artificial neural network (ANN) for data modelling. MP-Acetonitrile was used as solvent and tetrabutylammonium hydroxide (tBAH)-42.5:57.5 v/v, used to adjust pH 3.10.

32. Rifampicin, 3-Formyl rifamycin SV (3-FRSV) and isoniazid

**HPTLC**

**MP-** chloroform:methanol:water (80:20:2.5 v/v)

RIF-475 nm and 507 nm

3-FRSV-457 nm and 492 nm

**Linearity range**

3-FRSV was 2-10 µg/ml and 50-250 ng/spot for DW spectrophotometric method and HPTLC method, respectively, and 5-50 µg/ml for RIF using DW spectrophotometric method. The rate of degradation of RIF in presence of INH was almost two times more than that of RIF alone.

Specific, accurate and reproducible

RIF degrades by 12.4% to form 3-FRSV (RIF formulations) while in presence of INH the degradation is catalyzed to about 21.5% (RIF+INH formulations), in 45 min.

33. Rifampicin

**HPLC**

**SP-C18** (150x4.6 mm Phenomenex Gemini)

MP-1.49 gm of monosodium phosphate monohydrate, 0.31 gm of disodium phosphate hetahydrate, 400 ml of acetonitrile, using 85% phosphoric acid to pH 5.87

Flow rate - 1.2 mL/min

**Correlation coefficient** - 0.995

**Linearity range**

10-200 mcg/ml

Peak tailing - 1.183

Theoretical plates - 4769.02

%RSD = 0.18

Specificity (USP) = 4.32
DISCUSSION:

The presented review gives detail on various analytical methods published on Rifampicin and combination with other drug with different validation parameters. Various analytical methods like spectrophotometry, chromatography and in combinations are presented in under Table 2 . Developed spectroscopic methods mentioned in the above texts are rapid and far more economical than chromatographic methods but their destructive nature and lack of sensitivity is huge disadvantage for the estimation in biological fluids and impurities estimation which is possible by chromatography method. In this way various analytical methods for the estimation of Rifampicin in bulk or in various matrices like blood, serum, plasma, alone or in combination with other drugs is discussed. The presented information is useful for the researchers especially those involved in the development of different dosage forms and for quality control of rifampicin and combination with other drug.

REFERENCES:


[21] Woolard G, Madhavaram H, Chiu. W. Measurement of serum Rifampicin by high performance liquid chromatography with ultra violet detection, sample preparation and stability considerations, Department of Chemical Pathology, LabPlus, Auckland City Hospital, Auckland, New Zealand. geraldw@adhb.govt.nz
[34] Hongling Yan, Yaping Zhou, Qingji Xie, Yi Zhang, Pei Zhang, Hualing Xiao, Wen Wang and Shuozhuo Yao. Simultaneous analysis of isoniazid and rifampicin by high-performance liquid chromatography with gradient elution and wall-jet/thin-layer electrochemical detection, Anal. Methods, 2014; 6, 1530-1537.