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A Review: Validated Analytical Methods Developed on Antitubercular Drug, Rifampicin

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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

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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 multidrugresistant (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)[2]

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. [3]

Table -1 Antimicrobials Used in the Treatment of Tuberculosis.[1]

Drug	Typical Adult Dosage[1]
First-line agents (in approximate order of preference)	
Isoniazid	300 mg/d
Rifampin	600 mg/d
Pyrazinamide	25 mg/kg/d
Ethambutol	15–25 mg/kg/d
Streptomycin	15 mg/kg/d
Second-line agents	
Amikacin	15 mg/kg/d
Aminosalicylic acid	8–12 g/d
Capreomycin	15 mg/kg/d
Ciprofloxacin	1500 mg/d, divided
Clofazimine	200 mg/d
Cycloserine	500–1000 mg/d, divided
Ethionamide	500–750 mg/d
Levofloxacin	500 mg/d
Rifabutin	300 mg/d[2]
Rifapentine	600 mg once or twice weekly

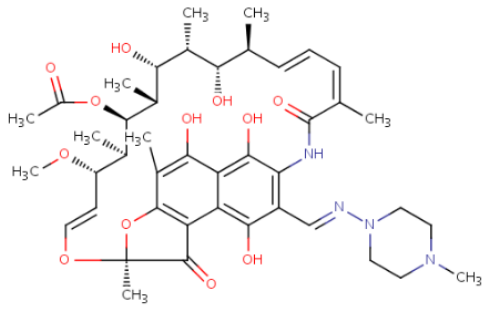
[1]Assuming normal renal function.

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

Table –2 Recommended Duration of Therapy for Tuberculosis.[1]

Regimen (in Approximate Order of Preference)	Duration in Months
Isoniazid, rifampin, pyrazinamide	6
Isoniazid, rifampin	9
Rifampin, ethambutol, pyrazinamide	6
Rifampin, ethambutol	12
Isoniazid, ethambutol	18
All others	24

Rifampicin (Rifampin):



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.[3]

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.[5]

Pharmacodynamics:

Rifampicin has high activity against many organisms, Mycobacterium tuberculosis and M.leprae, including Staphylococcus aureus, coagulase-negative staphylocci, 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. [6]

Indications:

Clinical Uses [2]

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 innmethicillin-resistant staphylococcal infections, such as osteomyelitis and prosthetic valve endocarditis.

Adverse Reactions 2

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:

SR NO.	DRUG	METHOD	DESCRIPTION	DETECTION AT	SPECIFICATION	REF NO.
1.	Rifapentine	UV	Chemito UV 2600 spectrophotometer with 1 cm matched quartz cells Y = 0.004x+ 0.024	478nm (0.1N HCl)	**R^2**- 0.999 **Linearity range**-5-50µg/ml **LOD**-3.28 µg/ml(2.15) **LOQ**-9.96 µg/ml(6.25) **%RSD**-0.003985	7.
2.	Rifampicin & Piperine	2nd dvt.	No intereference from the capsule excipients	341nm for rifampicin (0 cross point for piperine) 241nm for piperine (0 cross point for rifampicin)	**R^2**-0.999 **Linearity range**- 10-60 µg/ml for rifampicin & 2-20 µg/ml for piperine **LOD**= (µg/ml)- RIFA-1.65 PIPE- 0.57 **LOQ**=(µg/ml) RIFA-5.03 PIPE-1.75 **Repeatability(%RSD)**= RIFA-0.31 PIPE-0.37	8.
3.	Rifampicin	Visible	shimadzu double beam spectrophotometer UV-140 A=0.0009+0.0119C **Buffer solution (pH=7.0)**	400-650nm (max at 510nm)	**R^2**-0.9999 **Linearity range**- Conc range:5-50µg/ml Molar absorptivity(l/mole cm)- $9.83x10^3$ Sandell's sensitivity(µg / cm2 / 0.001 absorbance unit)-0.084 **Optimum photometric range**:7.9-39.1µg/ml **%RSD**=1.90	9.
4.	Rifampicin and Isoniazid	Simultaneous equation method)	Non interference of the excipients Solvent-ethanol	RIF- 337nm INH- 263nm	**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 **LOD**= RIF-1.653µg/ml INH-0.585 µg/ml **LOQ**= RIF-5.007 µg/ml INH-1.772 µg/ml **Precision(%RSD)=** RIF-0.578 INH-0.673	10.
5.	Rifampicin and Piperine	Q-Absorbance ratio	A shimadzu model 1700 double beam UV/Visible spectral width - 2 nm, wavelength accuracy-0.5 nm & pair of 10 mm matched	In methanol RIF- 337nm PIPE- 337nm RIFA+PIPE-387nm	**R^2**-0.999 **Linearity range-** RIF- 5-40 µg/ml PIPE-2-20 µg/ml **LOD=** RIF-1.51 µg/ml PIPE-0.28 µg/ml	11.

			quartz cell .		RIF+PIPE-0.80 and 0.32 **LOQ=** RIF- 4.6 µg/ml PIPE- 0.86 µg/ml RIFA+PIPE- 2.45 and 0.98 µg/ml **%RSD=** RIF-0.54 PIPE-0.091 RIF+PIPE-0.68 **Precision:** RIFA-0.14-0.71 PIPE-0.11-1.50 RIFA and PIPE-0.17-1.31	
6.	Rifampicin and Isoniazid	Visible & 1st derivative	Buffer solution pH 7.4 as solvent	257nm(1st derivative, HCl 0.012M)	Recovery- 99.03% for rifampicin & 100.01% for isoniazid.	12.
7.	Isoniazid, rifampicin and Piperine	UV & RPLC-PDA Methods	2 chemometric methods applied- ILS and CLS Reversed phase: phenomenex Luna C18 column- gradient elution with mobile phase 0.05M Na_2HPO_4 buffer pH 7 and acetonitrile Total run time: 12min	220-360nm with interval of 10nm	**Linearity range**- INH- 30-270 µg/ml RIF- 20-180 µg/ml PIPE- 1-9 µg/ml	13.
8.	Rifampicin, isoniazid, pyrazinamide	Simultaneous spectrophotometric by 1st derivative UV spectrophotometry	---	RIF at ZCP for INH and PYZ(262.2 and 268.8) PYZ at ZCP for RIF and PYZ (254 and 268.8nm) PYZ at ZCP for INH and RIF (262.2 and 254 nm)	---	14.
9.	Isoniazid, rifampicin and Piperine	UV, RP-HPLC	Methanol and distilled water was used as diluents 2nd RP-HPLC- acetonitrile as diluents Flow rate of 0.9mL/min	INH-262nm PIPE- 338nm RIFA-477nm	**Correlation coefficient**-0.995 **Linearity range-** INH- 12-34.5 µg/ml RIFA- 8-23 µg/ml PIPE-0.4-1.15 µg/ml	15.
10.	Rifampicin	HPLC	**SP**-C18 monolithic **MP**-Methanol: CAN :mono potassium phosphate (0.075 M):citric acid (1.0 M) (28:30:38:4, v/v) flow rate 2 mL/min	with UV detection at 254 nm	Linearity range- RQ- 1.5-60 µg/ml SV- 1-40 µg/ml RNO-1-40 µg/ml 3-FR-1-40 µg/ml RIF- 5-200 µg/ml LOD=0.2 µg/ml LOQ= 1 µg/ml %RSD= 2.5%	16.
11.	Rifampicin	HPTLC	**SP**- Al backed silica gel	254nm	**R^2**-INH- 0.994	17.

	and Isoniazid		60 F_{254} plates **MP**- n-hexane:2-propanol:acetone:ammonia:formic acid=3:3.8:2.8:0.3:0.1 (v/v) Resolved INH and RIF with R_F 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_2PO_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.	337nm	**Correlation coefficient**-0.9971 **Linearity range**- 0.31 – 25.48 µg/ml **LOD**=100ng/ml **LOQ**=1µg/ml	18.
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.	333nm	**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	19.
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	333.6	**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	20.
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	335nm	Linearity range- 2-20µmol/L	21.
16.	pyrazinamide ,isoniazid rifampicin ,and	RP-HPLC	Stationary phase- pre-column derivatization with phenethyl isocyanate	210nm	**R^2**- 0.9998 **Linearity range-** PYP : 16.0–160 µg/ml INH : 4.8–48.0 µg/ml	22.

	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	**R^2**- 0.2 **Linearity range-** 0.5-20µg/ml **LOQ=** 0.5 µg/ml in plasma 1.5 µg/ml in blood	23.
18.	Rifampicin	RP-HPLC	**SP**-C18 column **MP**-phosphate buffer pH 7.4: methanol(75:25 v/v) R_t - 2.54min	475nm	**Linearity range**- 0.05-20L1/4gm/mL for plasma Good reproducibility (both interday and intraday)	24.
19.	Rifampicin	RP-HPLC	SP-C18 column MP-Acetonitrile:monobasic potassium phosphate buffer solution 0.05 M (38:62 v/v) The R_t of RFP and RFP-QN were 7.81 and 12.26 minutes, resp.	at 335 nm in which standard rifampicin quinone (RFP-QN) was used.	**Linearity range**- 0.5-250µg/mL Good accuracy, precision, specific, sensitive, selective	25.
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	26.
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	27.
22.	Rifampicin	RP-HPLC	**SP**-a C18 column	254nm	**Correlation coefficient**-	28.

	(RMP) and desacetyl rifampicin(DRMP) Rifapentine(RPN)		**MP**-0.05 M phosphate buffer: acetonitrile (55:45 v/v) **R_t**= DRMP-2.9min RMP-4.8min RPN-10.5min		Plasma- 0.9996 urine- 0.9943 **linearity range**- 0.25-15µg/ml for plasma 2.5-80µg/ml for urine **LOD**- 0.1-0.25µg/ml **LOQ**- 1-2.5µg/ml Accuracy and precision good for both intra day and inter day	
23.	Rifampicin	HPLC-MS	**MP-**Acetonitrile :water		**linearity range**- 100-12800ng/mL	29.
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.	238nm using PDA detector	**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	30.
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 (min)- RIF= 3.5min PIPR= 7min Tailing factor- RIF= 1.1±0.3% PIPE=1±0.6%	341nm (200-400 nm)	**Correlation coeffient**- 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%	31.
26.	Isoniazid, Rifampincin	Isocratic 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 R_t= INH-2.953min RIF-3.382min	274nm	**Correlation coefficient**- 0.99 for both **linearity range**- Y= 16646x for Isoniazid and y = 19288x for Rifampicin **LOD**= INH-2.359 RIF-2.896 **LOQ**= INH-7.864 RIF-9.6541 Theoritical plates= INH-6642 RIF-10333	32.

27.	Rifampicin and a flavonoid glycoside(CC-I)	RP-HPLC	**SP**- RP-18 column **MP**- acetonitrile: phosphate buffer, 50 mM, pH 5.0 in a ratio of 60:40 v/v; oven temperature, 40 0C; flow rate 0.8 ml min total run time-15min **R_t**- RIF-4.779 CC-I-3.072	340nm	**Correlation coefficient**- >0.999 **linearity range**- 0.1-10 µg/mL for RIF 0.05-10 µg/ mL for CC-I in combination **Precision values**: RIF-1.08-2.77% CC-I-1.14-2.98% **LOQ**= RIF-0.10µg/mL CC-I-0.05µg/mL	33.
28.	Rifampicin, isonoazid	HPLC and wall-jet/ thin layer electrochemical detection	**SP**-Reversed phase C18 column (150mmx4.6mm, 5µm) **MP**- gradient elution Flow rate- 1mL/min	268nm	**linearity range**- 0.01-100µm for INH and RIF **LOD**= INH-0.3nM RIF-0.5nM (S/N=3)	34.
29.	Rifampicin, isonoazid, pyrazinamide	RP-HPLC	**SP-** A Hypersil C18, 5 mm, 250 mm x 4.6 mm internal diameter column was maintained at 40°C **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	254nm	Analysis time is 35 minutes.	35.
30.	Rifampicin, isoniazid	Isocratic HPLC	**SP-**A shimadzu liq-cromatographic unit equipped with a C18 GraceVydac analytical column(250mmx4.5 mm, 5µm particle size) **MP**-0.05M sodium dihydrogen phosphate sol(pH 3.1) and acetonitrile (20:80) flow-rate set at 0.6 ml/min.	254nm	**Correlation coefficient**- 0.999 **linearity range**- good Flow rate set at 0.6ml/min Good accuracy, precision	36.
31.	Rifampicin, isonoazid,	HPLC	**SP**- Micro-bondpack C18, 4.6x250mm column		Retention time were measured on different	37.

	pyrazinamide		Optimized using an artificial neural network(ANN) for data modelling. **MP**-Acetonitrile as solvent and tetrabutylammonium hydroxide(tBAH)-42.5:57.5v/v), used to adjust pH 3.10		experimental condition(solvent, buffer type and pH)	
32.	Rifampicin, 3-Formyl rifamycin SV (3-FRSV) and isoniazid	HPTLC	**MP**-chloroform:methanol:water (80:20:2.5 v/v)	RIF-475nm and 507nm 3-FRSV- 457nm and 492nm	**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.	38.
33.	Rifampicin	HPLC	**SP-**C18 (150x4.6mm Phenomenex Gemini) **MP**-1.49gm of monosodium phosphate monohydrate, 0.31gm of disodium phosphate hetahydrate,400ml of acetonitrile, using 85% phosphoric acid to pH 5.87 Flow rate- 1.2mL/min	254nm	**Correlation coefficient**-0.995 **Linearity range**- 10-200mcg/ml Peak tailing-1.183 Theoretical plates- 4769.02 %RSD=0.18 Specificity(USP)=4.32	39.

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 estimationn which is possible by chromatography method. In this way various analytical methods for the estimation of Rifampicin in bulk or in various matrixes 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.

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