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A Comprehensive Review on Analytical Methods of Rifampicin

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Abstract: *Rifampicin, a cornerstone in the treatment of tuberculosis and other bacterial infections, necessitates robust analytical methods for its accurate determination in pharmaceutical formulations and biological matrices. This review aims to provide a comprehensive summary of the analytical methods developed and validated for rifampicin quantification, encompassing various chromatographic, spectroscopic, and other analytical techniques reported in the literature. The review begins with an overview of the physicochemical properties, pharmacological significance, and regulatory requirements pertinent to rifampicin analysis. Additionally, the review highlights the importance of method robustness, specificity, sensitivity, and stability-indicating capability in ensuring the quality and safety of rifampicin-containing formulations. Overall, this review serves as a comprehensive reference for researchers, analysts, and regulatory authorities involved in the development, validation, and quality control of analytical methods for rifampicin, facilitating the advancement of pharmaceutical sciences and therapeutic interventions aimed at combating infectious diseases effectively.*

Keywords: Rifampicin, Mechanism of action, UV-visible spectroscopy, HPLC, LC-MS/MS, HPTLC.

I. INTRODUCTION

Tuberculosis (TB) is caused by Mycobacterium tuberculosis (Mtb) and primarily affects the lungs, although it can also impact extra-pulmonary sites. Transmission occurs through the inhalation of aerosols containing Mtb from an infected individual. According to the World Health Organization (WHO) 2021 TB report, TB stood as the leading cause of death globally from a single infectious agent in 2019. In 2020, there were approximately 5.8 million new TB cases diagnosed and reported, with around 1.3 million of these cases resulting in fatalities. [1]

An increasing body of evidence indicates that rifampicin, when administered at the dose currently advocated by international guidelines, often results in sub-therapeutic drug concentrations. These low concentrations are significantly linked to unfavorable treatment outcomes, particularly in individuals coinfected with HIV and those with low body weight. Conversely, higher levels of rifampicin exposure have shown good tolerability and have recently been associated with shorter durations to sputum culture conversion and improved clinical outcomes. [2]

IUPAC name:
(2S,12Z,14E,16S,17S,18R,19R,20R,21S,22E,24Z)-6,22,24-trihydroxy-2'-methyl-3,4,10,16,20-pentaoxo-2',7',14',19'-tetrahydro-3'H-spiro[cyclopentane-1,9'-pyrazino[1',2':1,6]pyrido[3,4-b]indole-21,4'-piperidine]-5-carboxamide.
Molecular weight:823.92 g/mol
Formula:C43H58N4O12
Structure:

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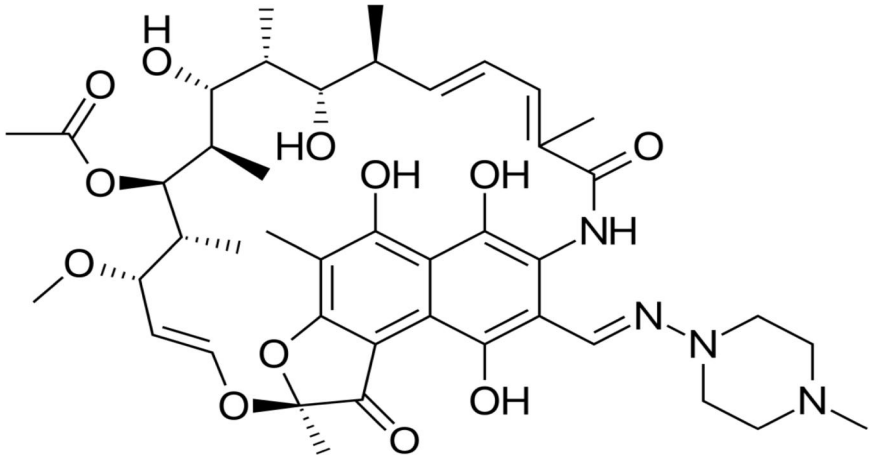

Figure 1: Structure of Rifampicin [4]

Pharmacodynamics:

While the pharmacokinetic rationale is convincing, it relies on the premise that rifampicin remains active when serum concentrations surpass the minimum inhibitory concentration (MIC) throughout the dosing interval. This implies that the ratio of the trough concentration (Cmin) to the MIC serves as the pertinent pharmacodynamic index, indicating time-dependent inhibition. Constans' assertion that the 900-mg dose utilized in a pilot trial was "unnecessarily high" aligns with a similar line of reasoning. [4] Rifampicin's bacteriostatic or bactericidal effects depend on the concentration of the drug achieved at the site of infection. Its bactericidal actions arise from interference with nucleic acid synthesis by inhibiting bacterial DNA-dependent RNA polymerase at the B-subunit. This inhibition prevents the initiation of RNA transcription while allowing chain elongation to proceed. (Fahr et al., 1985; Drug Information for Health Care Provider, 1984). [5]

Pharmacokinetics:

The pharmacokinetic parameters of rifampicin and desacetylrifampicin were determined using non-compartmental methods. The area under the plasma concentration-time curve from time 0 hours until 6 hours after the dose (AUC0–6 h) was calculated using the linear trapezoidal rule. The highest observed plasma concentration was denoted as Cmax, and the time at which this maximum concentration occurred was recorded as Tmax. Cmax and Tmax were directly obtained from the plasma concentration-time data. [3] Orally administered rifampicin typically leads to peak plasma concentrations within approximately two to four hours. However, when co-administered with 4-aminosalicylic acid, another anti-tuberculosis drug, the absorption of rifampicin is significantly diminished, potentially preventing peak concentrations from being reached. Consequently, when these two drugs must be used concurrently, as is often the case in TB treatment, they should be administered separately with an interval of eight to 12 hours between administrations. [5]

Mechanism of action:

Resistance to rifampicin can occur through three main mechanisms: Mutation of the target protein RNA polymerase, particularly the rpoB gene, either at various sites or in the promoter region, leading to overproduction, ADP ribosylation of the rifampicin molecule itself, Efflux mechanisms. Laboratory selection of spontaneous rifampicin-resistant mutants typically ranges from 10^-10 to 10^-7, depending on the organism and the methodology used. Resistance can develop independently or alongside resistance to other antimicrobials. However, resistance may carry a fitness disadvantage since rpoB is an essential gene. Mutations in rpoB predominantly occur in hotspots conserved across species, known as the rifampicin resistance-determining region (RRDR). These mutations are primarily point mutations, although small insertions and deletions have also been identified. Clinical isolates of various bacteria, including Escherichia coli, Staphylococcus aureus, Mycobacterium tuberculosis, Haemophilus influenzae, Streptococcus pneumoniae, and

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Neisseria meningitidis, have been reported to harbor mutations in rpoB. Some species are inherently resistant to rifampicin due to a refractory RpoB, such as spirochetes and mollicutes. Cross-resistance between rifampicin, rifabutin, and rifapentine has been observed in tuberculosis patients due to mutations in rpoB, while complete cross-resistance has been reported among these drugs for Staphylococcus aureus. [6] The constitutive androstane receptor (CAR) is another receptor involved in the transcriptional regulation of CYP3A4. CAR belongs to the steroid/retinoid/thyroid hormone receptor superfamily. Research has shown that CAR has the ability to activate CYP3A4 gene expression both in laboratory settings (in vitro) and within living organisms (in vivo). Interestingly, the response elements for CAR are also targeted by another receptor, the pregnane X receptor (PXR), suggesting a dynamic interplay between these receptors in regulating CYP3A4 expression. [7]

Analytical techniques in pharmaceutical analysis:

It seems like you're discussing analytical techniques used to estimate the concentration of rifampicin in various contexts, including bulk form, pharmaceutical formulations, and biological samples. UV/Visible Spectrophotometry, High-Performance Liquid Chromatography (HPLC), Ultra High-Performance Liquid Chromatography (UPLC), and High-Performance Thin Layer Chromatography (HPTLC) are some of the techniques commonly employed for this purpose. These methods are crucial for ensuring the accurate measurement of RTV, whether it's in standalone form or in combination with other anti-retroviral medications, across different dosage forms. [8]

UV-Visible spectrophotometry:

Spectroscopy, as a scientific discipline, traces its origins back to Isaac Newton's groundbreaking experiments with prisms, which led to the understanding of visible light and the study of color, initially known as optics. Over time, thanks to the work of scientists like James Clerk Maxwell, the scope of spectroscopy expanded to encompass the entire electromagnetic spectrum. At its core, spectroscopy deals with the interaction between electromagnetic radiation and matter. One of the fundamental outcomes of this interaction is the absorption or emission of energy by matter in discrete units known as quanta. These processes occur across the electromagnetic spectrum, from the gamma region, involving phenomena like nuclear resonance absorption or the Mossbauer effect, to the radio region, where nuclear magnetic resonance occurs. Experimental measurement of radiation frequency provides insight into the energy changes involved, allowing for conclusions to be drawn about the discrete energy levels of matter. The practice of spectroscopy involves both the experimental measurement of radiation frequency, whether emitted or absorbed, and the deduction of energy levels from these measurements. This comprehensive approach forms the foundation of spectroscopic analysis. [9]

High-performance Liquid Chromatography:

High Performance Liquid Chromatography (HPLC) has emerged as one of the most potent tools in analytical chemistry, offering the capability to separate, identify, and quantify compounds present in liquid-dissolvable samples. Widely utilized for both quantitative and qualitative analyses of drug products, HPLC operates on the principle of injecting a sample solution into a column packed with porous material (stationary phase), while a liquid (mobile phase) is propelled through the column under high pressure. The separation of the sample relies on varying rates of migration through the column, which stem from differing partitioning of the sample between the stationary and mobile phases. HPLC boasts several advantages: Simultaneous Analysis, High Resolution, High Sensitivity, Good Repeatability, Small Sample Size, Moderate Analysis Conditions, Ease of Fractionating and Purifying Samples. Classification of HPLC can be based on various criteria: Scale of operation: Preparative HPLC and Analytical HPLC, Principle of separation: Affinity Chromatography, Adsorption Chromatography, Size Exclusion Chromatography, Ion Exchange Chromatography, Chiral Phase Chromatography, Elution technique: Gradient Separation and Isocratic Separation, Modes of operation: Normal Phase Chromatography and Reverse Phase Chromatography. [10]

High-performance Thin Layer Chromatography:

High Performance Thin Layer Chromatography (HPTLC) represents the most advanced iteration of Thin Layer Chromatography (TLC), characterized by chromatographic layers boasting exceptional separation efficiency. HPTLC

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employs sophisticated instrumentation throughout the procedure, encompassing accurate sample application, standardized reproducible chromatogram development, and software-controlled evaluation. HPTLC embodies a standardized methodology founded on scientific principles, along with the adoption of validated methods for both qualitative and quantitative analyses. By meeting stringent quality standards, HPTLC equips modern analytical laboratories with enhanced resolution capabilities and facilitates more precise quantitative measurements. [11] Qualitative information in analytical chemistry refers to descriptors such as types of atoms, molecules, functional groups, or other qualitative measures. On the other hand, quantitative information provides numerical data, such as the quantities of various chemicals present in a sample. In contemporary analytical chemistry, the analytical process typically involves the use of analytical devices for conducting the analysis, with computer software handling data processing and instrument control. This integration of computer technology into analytical chemistry has led to its computerization. Moreover, the presentation format of analytical chemical study results has evolved. Nowadays, a single sample analysis can yield vast amounts of data within a short period of examination. [12]

Bio-analytical studies:

Included in the collection are various steps such as processing, storing, and analyzing biological data, as well as conducting bioassays to examine samples of compounds. Bioanalysis Method Validation (BMV) entails establishing a suitable quantitative method for biochemical applications. Assurance of method quality and reliability is derived from conducting a minimum series of validation experiments and attaining satisfactory outcomes. Advances in analytical methods and validation are pivotal in drug discovery, enhancement, and manufacturing. The foremost objective of analytical measurement is to procure consistent, accurate, and reasoned insights. Proven analytics strategies play a significant role in achieving this objective. Results derived from validation techniques can aid in selecting standards and ensuring the authenticity and uniformity of analytical outcomes. [13]

Mass spectrometry:

Mass spectrometry (MS) serves as a robust analytical technique, both qualitative and quantitative, employed to identify and measure a diverse range of clinically relevant analytes. When coupled with gas or liquid chromatography, mass spectrometers broaden analytical capabilities across various clinical applications. Furthermore, due to its ability to identify and quantify proteins, MS emerges as a pivotal analytical tool in proteomics research. In mass spectrometry data representation, the mass-to-charge ratio (m/z) is commonly utilized, where 'm' signifies the molecular weight of the ion (in daltons), and 'z' represents the number of charges present on the measured molecule. For small molecules (<1000 Da) typically carrying a single charge, the m/z value aligns with the mass of the molecular ion. However, larger molecules such as proteins or peptides, often bearing multiple ionic charges, exhibit a z value greater than 1. Consequently, in such cases, the m/z value denotes a fraction of the ion's mass. [14]

Nuclear magnetic resonance:

Nuclear Magnetic Resonance (NMR) spectroscopy stands out as one of the most versatile methods of analysis. Initially, until the early 1970s, NMR spectroscopy found exclusive use in structure elucidation and purity testing of synthesized compounds. However, in contemporary times, its successful applications have expanded significantly, encompassing the identification and structure elucidation of organic and biochemical molecules, precise quantitative determination of individual analytes, multicomponent analysis, and what's known as "non-targeted screening," often combined with various chemometric techniques.

The selectivity of NMR as an analytical tool, attributed to each molecule possessing its own spectral fingerprint, not only facilitates ingredient quantification but also enables comparison, discrimination, or classification of foods, beverages, and other consumer products. This capability extends to authenticity evaluation, determination of origin, and botanical variety of certain products. Through "non-targeted" NMR analysis, rapid and highly selective sample screening is achievable, yielding a wealth of information surpassing that obtained by any other previously employed analysis technique for similar purposes. [15]

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Fourier transform infrared (FTIR) spectroscopy:

Infrared (IR) or Fourier transform infrared (FTIR) spectroscopy offers a broad operational range, spanning from the analysis of small molecules or molecular complexes to the examination of cells or tissues. [16] FTIR difference spectroscopy finds extensive application in photosynthesis research and related fields. This method complements the three-dimensional structural data acquired through X-ray diffraction or Nuclear Magnetic Resonance (NMR). Analyzing active sites in proteins via reaction-induced FTIR difference spectroscopy provides insights into subtle structural alterations, hydrogen-bonding interactions, and proton transfer reactions, often surpassing the sensitivity of X-ray diffraction analyses. Moreover, advancements in time-resolved techniques, with current time resolutions reaching the femtosecond range, enable the observation of structural changes in protein active sites while they are actively engaged in their functions. [17]

Summary of Analytical methods used for Rifampicin:

Table 1: Analytical methods development and validation for Rifampicinin combined and single dosage formed by UV-visible spectroscopy and RP-HPLC

Sr. No.	Drug/Drugs	Method	Description	References
1	Rifampicin, Isoniazid, pyrazinamide	Theoretically guided analytical method development and validation for the estimation of rifampicin in a mixture of isoniazid and pyrazinamide by UV spectrophotometer	**Wavelength:** Rifampicin: 344 nm **Solvent:** Ethyl acetate **Linearity:** Rifampicin: 2.5–35.0 µg/mL	18
2	Isoniazid, Rifampicin	Simultaneous determination of isoniazid and rifampicin by UV spectrophotometer	**Wavelength:** Isoniazid: 263 nm Rifampicin: 338 nm **Solvent:** Methanol **Linearity:** 5-50 µg/mL	19
3	Pyrazinamide, Rifampicin	Development of derivative spectrophotometric method for simultaneous determination of pyrazinamide and rifampicin in cubosome formulation	**Wavelength:** Pyrazinamide: 247 nm Rifampicin: 365 nm **Solvent:** Methanol: Water (99:1v/v) **Linearity:** 4-12µg/mL	20
4	Isoniazid, Rifampicin	A simple Simultaneous UV Spectrophotometric Method to Determine Isoniazid and Rifampicin Contents in One Combined Tablet	**Wavelength:** Isoniazid: 261 nm Rifampicin: 337 nm **Solvent:** Methanol **Linearity:** 5-25µg/mL **Correlation coefficient:** 0.998	21
5	Rifampicin, Piperine	Q-Absorbance Ratio Spectrophotometric Method for the Simultaneous Estimation of Rifampicin and Piperine in their Combined Capsule Dosage	**Wavelength:** Rifampicin: 387 nm Piperine: 337 nm **Solvent:** Methanol **Linearity:** 2-20µg/ml	22
6	Rifampicin, Isoniazid	Simultaneous estimation of rifampicin and isoniazid in combined dosage form by a simple UV spectrophotometric method	**Wavelength:** Rifampicin: 337 nm Isoniazid: 263 nm **Solvent:** Ethanol	23

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			Linearity: Rifampicin: 5-35 µg/mL Isoniazid: 5-25 µg/mL	
7	Rifampicin	Development and Validation of a Simple and Sensitive RP-HPLC Method for Determination of Rifampicin in Bulk and Tablets	**RP-HPLC Wavelength:** 480 nm **Column:** Eclipse Plus C18 (4.6×250 mm, 5µm) **Flow rate:** 0.4ml/min **Mobile phase:** Acetonitrile: 0.1% Orthophosphoric acid (80:20v/v) **Linearity:** 1.95-250 µg/mL **Retention time:** 4.7 min **Correlation coefficient:** 0.9996	24
8	Isoniazid, Rifampicin	Analytical method development and validation of Isoniazid and Rifampicin by RP HPLC method	**RP-HPLC Wavelength:** 283 nm **Flow rate:** 1ml/min **Mobile phase:** Methanol: Water (72:28v/v) **Linearity:** Isoniazid: 10- 50µg/mL Rifampicin: 15-5µg/mL **Retention time:** Rifampicin: 5.667 min Isoniazid: 8.002 min	25
9	Rifampicin	Method validation of rifampicin analysis in human plasma and its application in bioequivalence study	**RP-HPLC Wavelength:** 337 nm **Flow rate:** 1.5ml/min **Mobile phase:** Acetonitrile: Phosphate buffer (45:55v/v) **Linearity:** 0.05-10.26µg/mL **Correlation coefficient:** 0.9984	26
10	Rifampicin, Isoniazid, Pyrazinamide, Ethambutol Hydrochloride	Development and Validation of an HPLC Method for Simultaneous Determination of Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol Hydrochloride in Pharmaceutical Formulations	**RP-HPLC** **Column:** Purospher STARRP18e (250mm × 4.6mm id, 5 µm, Merck) **Wavelength:** 238 nm **Flow rate:** 1.5 ml/min **Mobile phase:** Monobasic sodium phosphate buffer: Acetonitrile **Retention time:** Isoniazid: 3.5 min Pyrazinamide: 4.6 min Ethambutol Hydrochloride: 6.1 min	27

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			Rifampicin: 11.6 min **Correlation coefficient:** 0.99	
11	Rifampicin, Ofloxacin	Development and Validation of HPLC method for simultaneous estimation of Rifampicin and Ofloxacin using experimental design	**RP-HPLC Wavelength:** 230 nm **Column:** Kinetex C18, 100 A Phenomenex (250mm × 4.6 mm, 5 µm) **Flow rate:** 0.8ml/min **Mobile phase:** Potassium dihydrogen phosphate buffer: Acetonitrile (55:45v/v) **Linearity:** Rifampicin: 1-5µg/mL Ofloxacin: 2-10µg/mL **Retention time:** Ofloxacin: 2.91 min Rifampicin: 4.87 min	28
12	Rifampicin, Isoniazid, Pyrazinamide, Ethambutol Hydrochloride	Optimisation and validation of HPLC method for simultaneous quantification of rifampicin, isoniazid, pyrazinamide, and ethambutol hydrochloride in anti-tuberculosis 4-FDC tablet	**RP-HPLC Wavelength:** 210 nm **Column:** Waters Symmetry C8 (250mm × 4.6 mm i.d.; 5 µm) **Mobile phase:** Acetonitrile: Phosphate buffer **Flow rate:**1.5ml/min	29
13	Rifampicin, Isoniazid	Development and validation of HPLC method for the simultaneous estimation of rifampicin and isoniazid in bulk and tablet dosage form	**RP-HPLC Wavelength:** 239 nm **Column:** CosmosilC18 (250mm x 4.6ID, 5 micron) **Mobile phase:** Phosphate buffer: Methanol: Water (45:30:25v/v/v) **Flow rate:** 0.8ml/min **Retention time:** Rifampicin: 2.8 min Isoniazid: 3.7 min **Linearity:** Rifampicin: 450µg/mL Isoniazid: 300µg/mL	30
14	Rifampicin	A stability indicating RP-HPLC method development and validation for simultaneous quantification for assay of rifampicin in pharmaceutical solid dosage form	**RP-HPLC Wavelength:** 254 nm **Column:** Phenomenex Luna C18 (250mm x 4.6, 5 µm) **Mobile phase:** Acetate buffer: Acetonitrile (60:40v/v) **Flow rate:**1.0ml/min **Linearity:** 0.15mg/mL- 0.30	31

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			mg/mL	
15	Azithromycin, Rifampicin	Development and Validation of a New Reversed Phase HPLC Method for the Quantitation of Azithromycin and Rifampicin in a Capsule Formulation	**RP-HPLC Wavelength:** 254 nm **Mobile phase:** Acetonitrile: Potassium dihydrogen phosphate (60:40v/v) **Flow rate:** 1ml/min **Correlation coefficient:** 0.998	32
16	Rifampicin, Flavonoid Glycoside	Development and Validation of a RP-HPLC Method for the Simultaneous Determination of Rifampicin and a Flavonoid Glycoside - A Novel Bioavailability Enhancer of Rifampicin	**RP-HPLC Wavelength:** 340 nm **Column:** RP-18 (250 mm × 4.6 mm, 5 µm) **Mobile phase:** Acetonitrile: Phosphate buffer (60:40v/v) **Flow rate:**0.8ml/min **Retention time:** Rifampicin: 4.779 min Flavonoid Glycoside: 3.072 min **Linearity:** Rifampicin: 0.1-10µg mL Flavonoid Glycoside: 0.05-10 µg mL **Correlation coefficient:** 0.999	33
17	Rifampicin, Isoniazid	Validation of a simple isocratic HPLC-UV method for rifampicin and isoniazid quantification in human plasma	**RP-HPLC Wavelength:** 339 nm **Column:** Reversed-phase Luna C8 (250 × 4.6 mm, 5 µm) **Mobile phase:** Methanol **Flow rate:**0.5ml/min **Retention time:** 5.8 and 4.7 min **Linearity:** Rifampicin: 0.31-37.80 µg/mL Isoniazid: 0.89-71.36 µg/mL **Correlation coefficient:** 0.9989	34
18	Rifampicin	Estimation for method development and validation of rifampicin in oraldosage form by RP-HPLC	**RP-HPLC Wavelength:** 211 nm **Column:** Prontosil C18 (250 x 4.6mm, 3µm) **Mobile phase:** Acetonitrile: Sodium dihydrogen phosphate buffer (60:40v/v) **Flow rate:**1ml/min	35

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			Retention time: 2.38min, 2.747min and 3.660min **Linearity:** 8-38 µg/mL, 18-53 µg/mL and 32- 116 µg/mL **Correlation coefficient:** 0.9999	
19	Isoniazid, Rifampicin, Piperine	UV spectrophotometric and RP-HPLC methods for simultaneous estimation of isoniazid, rifampicin and piperine in pharmaceutical dosage form	**RP-HPLC Wavelength:** 282 nm **Column:** LC18 100 A^0 column (250 x 4.6 mm, 5 µ) **Mobile phase:** Dihydrogen Orthophosphate: Acetonitrile (40:60v/v) **Flow rate:**0.9 ml/min **Retention time:** Isoniazid: 2.702 min Rifampicin: 3.883 min Piperine:8.701 min **Linearity:** Isoniazid: 12-34.5 µg/mL Rifampicin: 8-23 µg/mL Piperine: 0.4-1.15 µg/mL **Correlation coefficient:** 0.995	36

Table 2: Analytical methods development and validation for Rifampicin in single dosage formed by LC-MS-MS

Sr. No.	Drug/Drugs	Method	Description	References
1	Rifampicin	Fast and Simple LC-MS/MS Method for Rifampicin Quantification in Human Plasma	**Chromatographic condition:**Core-shell Kinetex C18 column (50 × 2.1 mm, 2.6 µm) **Detected by:** Tandem mass spectrometry **Internal standard:**0.1% Formic acid in water: Acetonitrile **Run Time:** 2.4 min **Injection Volume:** 1µL **Linearity:**5-40000 µg/L **Correlation coefficient:** 0.9993	37
2	Rifampicin	Development and Validation of Liquid Chromatography-Mass Spectrometry Method for the Estimation of Rifampicin in Plasma	**Chromatographic condition:**BDS Hypersil Gold C18 (3×50 mm) **Run Time:** 2.5 min **Injection Volume:** 5µL **Mobile phase:** Methanol: Ammonium	38

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			acetate (80:20 v/v) **Flow rate:**0.2 mL/min	

Table 3: Analytical methods development and validation for Rifampicin in combined and single dosage formed by HPTLC.

Sr. No.	Drug/Drugs	Method	Description	References
1	Isoniazid, Ethambutol, Rifampicin, Pyrazinamide	Optimization of a reversed-phase-high-performance thin-layer chromatography method for the separation of isoniazid, ethambutol, rifampicin and pyrazinamide in fixed-dose combination anti-tuberculosis tablets	**Wavelength:** 280 nm **TLC Plate:** Silica gel 60 RP–18 W F_{254} **Mobile phase:** Ethanol: Water: Glacial acetic acid: Ammonia solution (70/30/5/1 v/v/v/v) **R_f-values:** 0.20–0.80	39
2	Rifampicin, Isoniazid, Pyrazinamide	Development and Validation of High-Performance Thin-Layer Chromatographic Method for the Simultaneous Determination of Rifampicin, Isoniazid, and Pyrazinamide in a Fixed Dosage Combination Tablet	**UV detection:** 277 nm **TLC Plate:** 20 cm × 10 cm HPTLC glass plates coated with 200-µm layer thickness of silica gel 60 F_{254} **Mobile phase:** Ethyl acetate: Acetone: Methanol: Glacial acetic acid (18:7:7:5 v/v/v/v) **R_f-values:**0.25, 0.44, and 0.74.	40
3	Isoniazid, Rifampicin	Development and Validation of a stability indicating HPTLC method for analysis of antitubercular drugs	**Wavelength:** 254 nm **TLC Plate:** Aluminum backed silica gel 60 F_{254} plates **Mobile phase:** n-hexane: 2-propanol: Acetone: Ammonia: Formic acid (3:3.8:2.8:0.3:0.1 v/v) **Rf value:** Isoniazid: 0.59 ± 0.02 Rifampicin: 0.73±0.04	41
4	Rifampicin	High-performance thin-layer chromatographic method for monitoring degradation products of rifampicin in drug excipient interaction studies	aluminium plates pre-coated with silica gel 60 F 254 aluminium plates pre-coated with silica gel 60 F 254 aluminium plates pre-coated with silica gel 60 F 254 aluminium plates pre-coated with silica gel 60 F 254 aluminium plates pre-coated with silica gel 60 F	42

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			254 10 cm × 10 cm aluminum plates precoated with 250-µm layers of silica gel 60 F254 **Wavelength:**490 nm **TLC plate:** Thin-layer plates (silica gel) **Mobile phase:** Chloroform: Methanol: Water (80:20:2.5 v/v/v)	

II. CONCLUSION

The present review explores various analytical methodologies utilized in the evaluation of Rifampicin. This comprehensive analysis seeks to provide guidance to researchers, pharmaceutical experts, and regulatory bodies in choosing the optimal analytical techniques for rifampicin analysis.

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