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Visible spectrophotometric method development and validation of rifampicin - A stability study

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Abstract
Background: Rifampin, also known as Rifampicin, belongs to the antimicrobial class of drugs. This medication is used to manage and treat diverse mycobacterial infections and gram-positive bacterial infections.
Objective: A simple and sensitive Visible Spectrophotometric method has been developed for the quantitative estimation of Rifampicin in its bulk and pharmaceutical dosage form.
Method: The proposed work was carried out on a TG Ultraviolet visible spectrophotometer, T-60 Model, which is a double beam double detector configuration with a 1 cm quartz matched cell.
Results: The drug showed maximum absorbance at 475nm. Regression analysis of Beer's plot showed correlation of 0.999 in the concentration range of 10-30µg/ml and the percentage recovery were between 98-102% indicting high degree of accuracy. The % RSD of intra-day and inter-day precision was found to be less than 2 as per ICH guidelines. Sensitivity of the method was confirmed by accurate LOD and LOQ values which were obtained 0.569 and 1.770 respectively.
Conclusion: The method was validated as per ICH guidelines provided and the results were found to be satisfactory. Studies on the drug's stability reveal that it is unstable in a variety of stress situations, including oxidation, base, acid and water.

Keywords: Rifampicin, Visible Spectroscopic method development, validation, ICH guidelines

Introduction

UV-Visible spectroscopy is the absorption or reflectance spectroscopy of the ultraviolet and adjacent visible regions of the electromagnetic spectrum. It is also known as UV-visible spectrophotometry (UV-Vis or UV/Vis). Because of its low cost and ease of implementation, this methodology is widely used in applied and fundamental applications. The only requirement is that the sample absorb in the UV-Vis range, indicating that it is a chromophore. Absorption spectroscopy supplements fluorescence spectroscopy. Aside from the wavelength, the parameters of interest are absorbance (A), transmittance (%T), and reflectance (%R), as well as their variations over time.[1]

The UV-visible range is only a small part of the total electromagnetic spectrum, and is generally defined from wavelength of 190nm at the high energy VISIBLE end to about 750nm at the low energy red end of the spectrum. In ultraviolet/visible spectroscopy, a molecule absorbs ultraviolet light, leading an electron to be promoted from a ground electronic state to an excited electronic state. This absorption spectroscopy divides the electromagnetic spectrum into the ultraviolet (UV, 190-400nm) and visible (VIS, 400-800nm) sections.

Ultraviolet light: wavelengths between 190 and 400 nm
Visible light: wavelengths between 400 and 800 nm[2-10]

1. Drug Profile

Rifampin, also known as rifampicin, belongs to the antimicrobial class of drugs. This medication is used to manage and treat diverse mycobacterial infections and gram-positive bacterial infections. Rifampin exhibits antibacterial activity against a wide range of gram-positive cocci including Mycobacteria and Clostridium difficile, and specific gram-negative organisms, including Neisseria meningitidis, N gonorrhoeae, and Hemophilus influenza. Rifampin exerts bactericidal antimicrobial effects by inhibiting DNA-dependent RNA polymerase (RNAP). This inhibition occurs either by sterically obstructing the path of the elongating RNA at its 5′ end or by reducing the RNAP's affinity for short RNA transcripts. Rifampin uniquely targets microbial RNAP, effectively arresting ongoing RNA synthesis. This activity describes the indications, mechanism of action, and contraindications of rifampin as a valuable drug for treating tuberculosis, leprosy, and methicillin-resistant Staphylococcus aureus. This activity also highlights the adverse event profile, off-label applications, dosage, pharmacodynamics, pharmacokinetics, monitoring, and pertinent interactions of rifampin, which are essential for healthcare team members caring for patients with infectious diseases [11].

The very high burden of rifampicin resistance tuberculosis (RR-TB) and the very low detection of RR-TB cases are a major challenge that China has been facing. This study analyzed the characteristics of RR-TB detection in China after the change of RR-TB detection strategy since 2015, aiming to provide reference and evidence for the development of more precise national drug resistance tuberculosis prevention and control policy[12].

Table 1: Drug Profile of Rifampicin

Category	Antimicrobial class of drugs.
IUPAC Name	5,6,9,17,19,21-Hexahydroxy-23-methoxy- 2,4,12,16,18,20,22-heptamethyl- 8-[N-(4-methyl-1-piperazinyl) formimidoyl]- 2,7-(epoxypentadeca [1,11,13] trienimino)- naphtho[2,1-b]furan-1,11(2H)-dione 21-acetate
Description	Red-Orange powder.

Chemical Formula	$C_{43}H_{58}N_4O_{12}$
Molecular Mass	822.94g/mol.
Storage	Stored in room temperature (25±3°C) or in a refrigerator (2-8°C) for four weeks.
Maximum Wavelength (λ_{max})	475nm (Visible)
Brand Name	RIFADIN and RIMACTANE
Solubility	Water (pH 4.3) (1.3 mg/ml), methanol (25 mg/ml), DMSO (25 mg/ml), dimethyl sulfoxide, tetrahydrofuran, and water (pH 7.3) (2.5 mg/ml at 25 °C).

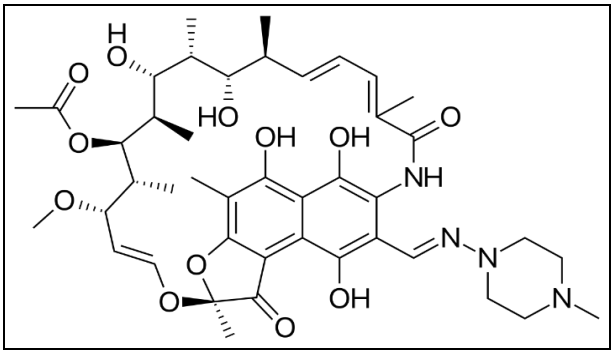

Fig 1: Structure of Rifampicin

A search through the literature revealed that the drug has been examined with a variety of analytical approaches, notably HPLC, RP-UPLC, UPLC-MS-MS, and LC-tandem mass spectrometry. The development of a basic, precise, reliable, and repetitive Visible Spectrophotometric method for the quantification of Rifampicin in bulk and in combined dosage form is described in the current work. According to ICH Guidelines, the developed approach was validated. [13-31]

Material and Methods

1. Chemicals and reagents

1.1 Instrumentation: The proposed work was carried out on a UV-Visible Spectrophotometer, which is a double beam double detector configuration with a 1 cm glass cell. All weighing was done on PGB-200 model weighing balance.

1.2 Selection of Solvents: On the basis of solubility study Methanol was selected as the solvent for dissolving Rifampicin.

Method Development

1. Preparation of Standard Stock Solution

1.1 Stock Solution A (1mg/ml)

10mg of the API is accurately weighed and transferred into clean 10ml volumetric flask and solubilized in 5ml of methanol and make it up to 10ml by usingdistilled water which gives us the concentration of 1000µg/ml.

1.2 Stock Solution B (100µg/ml)

From the above stock solution 1ml is transferred into a 10ml volumetric flask, added 4ml of methanol and made up to 10ml withdistilled water which gives us the concentration of 100µg/ml.

1.3 Preparation of serial dilutions

From the above solution 1,1.5,2,2.5,3ml transferred into a 10 ml volumetric flask and made up to 10ml with the diluent which gives us the concentration of 10,15,20,25,30µg/ml.

1.4 Selection of Analytical Wavelength

An appropriate aliquot portion of 2ml from stock solution B was transferred to 10 mL volumetric flasks; the volume was made up to the mark using Methanol and water (20µg/ml working standard). Drug solution was scanned against a Methanol blank between 400 nm to 800 nm range. The drug showed λ_{max} at 475nm.

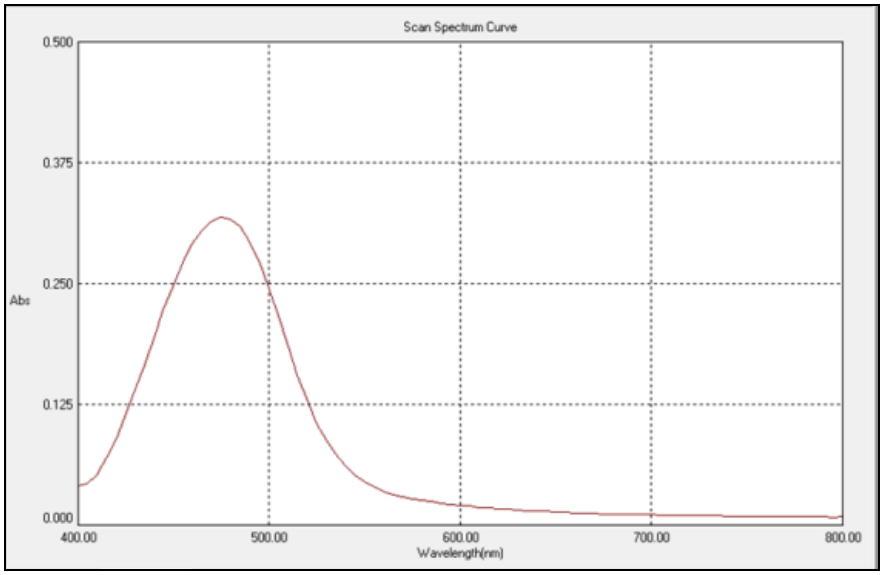

Fig 2: Spectrum of Rifampicin (475nm)

Degradation Studies1.

Stock preparation: Prepared 7µg/ml stock solution by taking 0.7ml ml of stock B solution and dissolving in methanol and water (5:5) in 10ml volumetric flask.

2. Acid degradation: Added 1ml of 1N HCl to 1 ml of working standard and made up the volume to 10 ml with solvent and kept the prepared solution aside for 24 hrs.

3. Alkaline degradation: To 1 ml of working standard solution, 1 ml of 1N NaOH was added and made up the volume to 10 ml with solvent and kept aside for 24hrs.

4. Oxidative degradation: To 1 ml of the working standard solution added 1 ml of H_2O_2 and made up the volume to 10 ml with solvent.

5. Degradation by hydrolysis: To 1 ml of the working standard solution added 1 ml of water and made up to the mark with solvent.

Method Validation

The suggested approach has undergone rigorous validation in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness, ruggedness and assay.

Linearity

A working standard solution of the drug was divided into five separate 10 mL volumetric flasks using an appropriately measured proportionate fraction. Methanol and water were used to make up the volume to the required level in order to produce concentrations (10-30 µg/mL). The absorbance of these solutions was measured at 475nm. The calibration curve was displayed in a concentration versus absorbance graph.

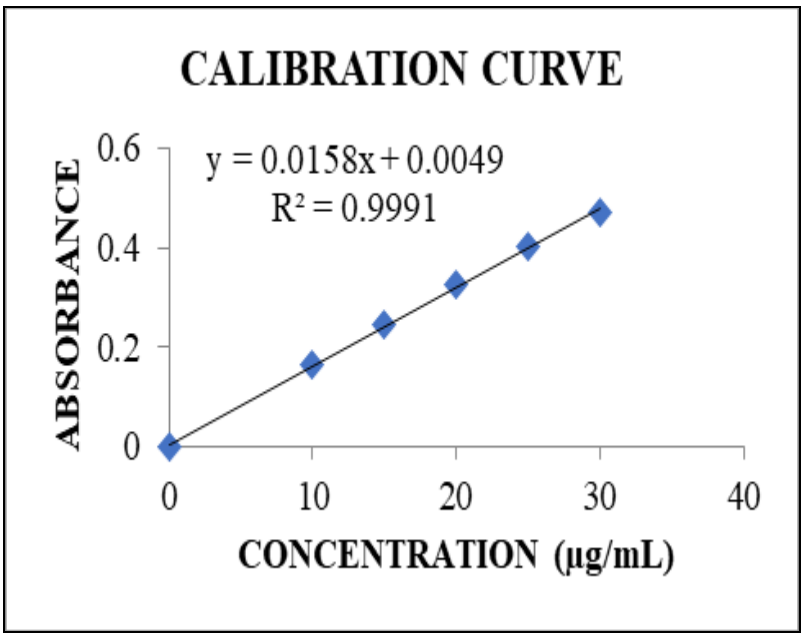

Fig 3: Calibration Curve of Rifampicin

2. Accuracy

On the basis of a recovery, research carried out using the conventional addition approach, the proposed method's accuracy was determined. The tablet powder was re-analyzed using the recommended method after being mixed with a known quantity of standard medication solutions to make final concentrations of 50%, 100%, and 150%. The absorbance recorded and the % recoveries were calculated.

3. Precision

Precision was determined as intra-day and inter-day variations. The results of the intra-day and inter-day precision research were determined by calculating absorbance at the same drug concentrations (7 ppm) three times on the same day and three different days over the course of a week at a wavelength of 240nm. The results were reported.

4. Ruggedness

The suggested method's durability was assessed through the analysis of portions from uniform slots by two different analysts under similar operational and environmental circumstances. The results were reported accordingly.

5. Robustness

Robustness was obtained by performing the analysis at two different wavelengths (±5 nm). The results were reported.

6. Lod & Loq

LOD & LOQ gives information about the sensitivity of the method. The values reported indicate that the method is highly sensitive.

7. Assay

Five capsules of Rifampicin were weighed and grounded to a fine powder. Weighed andtransferred the powder equivalent to 10 mg of capsule powder into a 10 mL volumetric flask, added4mL ofmethanoland made upto 10ml with distilled water. This solution was checked for absorbance for threetimes. Themean absorbance and its content in the formulation was calculated.

Table 2: Assay Results

Formulation	Labelclaim mg/tab	Amountfound Mean ±S.D	Assay	%RSD
Capsule	300	99.89 ± 0.0054	99.57	0.0054

Results and Discussion

1. Method Validation

1.1 Specificity: The analyte was assessed in the presence of components and does not show any interaction.

1.2 Linearity: Various standards in the range 10-30µg/ml of Rifampicin were observed in the system. A graph of absorbance (on Y-axis) versus concentration (on X-axis) is plotted and the correlation coefficient was calculated. The regression coefficient was found to be 0.999.

Table 3: Linearity values

Concentration (µg/ml)	Absorbance
10	0.165
15	0.245
20	0.325
25	0.403
30	0.471

1.3 Accuracy: The % RSD of the present work was found to be within and less than 2. The % recovery was between 99-100%.

Table 4: Accuracy Values

%Sample Spiked	Sample (Tablet)	Standard (20 ppm)	Mean	SD	%RSD	%Recovery
50%	1 1 1	1 1 1	0.095	0.001	1.219	99.16
100%	1 1 1	2 2 2	0.135	0.0005	0.426	100.4
150%	1 1 1	3 3 3	0.174	0.0005	0.33	99.7

1.4 Precision: The % RSD of Rifampicin was found to be 0.323 for intraday precision and. 0.158 for interday precision, respectively.

Table 5: Intraday Precision

	10AM Mea± SD	%RSD	1PM Mean± SD	%RSD	4PM Mean± SD	%RSD
LQC 10PPM	0.1653± 0.0008	0.493	0.1651± 0.0007	0.4557	0.165± 0.0008	0.542077
MQC 20PPM	0.3248± 0.0007	0.231	0.3241± 0.0007	0.2322	0.3245± 0.001	0.323208
HQC 30PPM	0.4708± 0.0007	0.159	0.4713± 0.0008	0.1732	0.471167± 0.0009	0.208672

Table 6: Interday Precision

	10AM MEAN ± SD	%RSD	1PM MEAN ± SD	%RSD	4PM MEAN ± SD	%RSD
LQC 3PM	0.1645± 0.000548	0.332962	0.165± 0.000894	0.542077	0.165333± 0.000816	0.493849
MQC 7PM	0.324833± 0.001169	0.359891	0.325± 0.000894	0.275208	0.325667± 0.000516	0.158566
HQC 11PM	0.4715± 0.001049	0.222441	0.471167± 0.000753	0.159768	0.4715± 0.000837	0.177446

1.5 Robustness: A small variation of the wavelength (±5nm) was applied to the presented method and it was found that the %RSD was within the limits and the values were 0.177 and 0.178.

Table 7: Robustness

S.no.	Concentration (µg/ml)	Absorbance	
		470nm	480nm
1	20	0.325	0.325
2	20	0.324	0.324
3	20	0.325	0.325
MEAN		0.32466	0.32433
STD		0.000577	0.000577
%RSD		0.17782	0.17801

1.6 Ruggedness: Two different analysts performed the ruggedness studies under same conditions and it was found that the % RSD was within the limits

Table 7: Ruggedness Result

S.no	Concentration (µg/ml)	Analysist -1	Analysist -2
1	20	0.323	0.324
2	20	0.323	0.326
3	20	0.324	0.325
	MEAN	0.32333	0.325
	STD	0.000577	0.001
	%RSD	0.17856	0.30769

1.7 LOD & LOQ: The study for LOD & LOQ was carried out and the obtained results are described in the table below.

Table 8: LOD & LOQ Values

Drug	LOD	LOQ
Rifampicin	0.569	1.77

1.8 Stability Studies: A 20µg/ml solution of Rifampicin was obtained and its stability was tested (24hours) in 0.1N HCl, 0.1N NaOH, Hydrogen Peroxide, and water. The results are shown in below table.

Table 9: Stabilities Studies of Rifampicin

Solution	% Degradation	
	Day 1	Day 2
HCl	8%	11%
NaOH	14%	10%
H_2O_2	8%	3%
Water (H_2O)	2%	5%

Conclusion

The %RSD values were within the limits and the method was found to be precise. The results expressed in the visible method were promising. The method is more sensitive, accurate and precise. This method can be used for the routine determination of Rifampicin in bulk drug and in pharmaceutical dosage forms. Degradation studies has also been studied. Method development and validation is followed according to the ICH guidelines and the all the parameters were validates and found to be within the limits.

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