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A Novel UV-Vis Spectrophotometric Method for Quantifying Rifaximin: Method Development and Validation

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Abstract— *This study evaluated the standardization and method validation of rifaximin using different acid and phosphate buffer conditions (pH 1.2, 6.8 and 7.4). Although all tested conditions showed excellent precision (less than 2% RSD), limitations in linearity and precision were observed. Although the correlation coefficients were high (0.9898 to 0.9972), they deviated from the ideal (1.0), indicating possible nonlinearities. Accuracy ranged from 89.45% to 94.16%, indicating slight under- or overestimation of rifaximin concentration. These limitations compromise the reliability of the rifaximin quantification method. Other optimization strategies are recommended, including exploring different pH conditions, refining the concentration range of the standard curve, and considering alternative analytical methods such as HPLC when possible. By addressing these limitations, a more robust and reliable method for rifaximin standardization can be achieved.*

Keywords— ***Rifaximin, Spectrophotometer, Standardization and method validation, Robustness, RSD***

I. INTRODUCTION

Rifaximin is an antibiotic that is semi-synthetic and produced from rifamycin. It is commonly used to treat gastrointestinal diseases, including hepatic encephalopathy, irritable bowel syndrome, and traveler's diarrhoea. Targeting pathogenic bacteria in the stomach is made easier by its broad-spectrum antibacterial action and low systemic absorption. The analytical techniques available for rifaximin's quantification and quality control are few and frequently intricate, despite its therapeutic importance. Thus, the development of an easy-to-use, trustworthy, and verified analytical technique is necessary for the regular analysis of rifaximin.

The majority of laboratories choose to use spectrophotometry, especially UV-Visible (UV-Vis) spectrophotometry, since it is an easy and affordable analytical technique. With this technique, the amount of medication present may be ascertained by measuring how much UV or visible light the analyte absorbs.

UV-Vis spectrophotometry has been shown in several studies to be useful in the study of different medicinal substances. For example, Bhavsar et al. (2015) demonstrated the sensitivity and specificity of their UV-Vis spectrophotometric approach for the accurate measurement of cefixime in pharmaceutical formulations[1]. Similar to this, Patel et al. (2017) proved the accuracy and strong linearity of a UV-Vis spectrophotometric approach for olmesartan medoxomil determination[2]. These investigations highlight UV-Vis spectrophotometry's promise as a trustworthy analytical instrument.

Prior analytical approaches for rifaximin have mostly relied on chromatographic techniques, such high-performance liquid chromatography (HPLC), which are accurate but need complex gear and thorough sample preparation. While HPLC techniques offer great sensitivity and specificity, Kumar et al. (2018) claim that they are frequently more difficult to use and require more time than

spectrophotometric techniques[3]. Thus, the creation of a UV-Vis spectrophotometric technique provides a more useful substitute for regular analysis in resource-constrained environments.

The present study aims to develop and validate a UV-Vis spectrophotometric method for the quantitative analysis of rifaximin in bulk and pharmaceutical dosage forms. The method will be optimized and validated according to ICH guidelines, ensuring its accuracy, precision, linearity, and robustness. The successful implementation of this method will provide a valuable tool for the efficient and cost-effective analysis of rifaximin, contributing to better quality control and therapeutic efficacy.

II. MATERIAL & METHODS

1-Materials:

- Spectrophotometer: Systronics 117 UV-Vis Spectrophotometer
- Cells: 1 cm matched quartz cells

2-Methods:

2.1Preparation of Standard Stock Solution:

- A standard stock solution of Rifaximin was prepared at a concentration of 1000 µg/ml (micrograms per milliliter) for each of the following:
  - 0.1N HCL (hydrochloric acid), pH 1.2
  - Phosphate buffer, pH 6.8
  - Phosphate buffer, pH 7.4
- 10mg of Rifaximin was dissolved in 10ml of the respective solvent to obtain these solutions.

2.2-Working Standard Solution:

- A working standard solution containing 100 µg/ml of Rifaximin was prepared from the standard stock solutions. The specific method of dilution is not mentioned here.

2. **Selection of Wavelength for Analysis:**

- The standard stock solution (1000 µg/ml) was further diluted using the same three solvents (0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4).
- Each dilution was scanned in the UV-Vis spectrophotometer over a wavelength range of 200-800 nm.
- For each scan, the corresponding solvent (without Rifaximin) was used as a blank to account for background absorption.

2.3. Preparation for Calibration Curve:

- This section details how the researchers prepared a set of solutions for creating a calibration curve.
- The standard stock solution (1000 µg/ml) was further diluted with each of the three solvents (0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4).
- The goal was to obtain a series of solutions with concentrations ranging from 2 to 10 µg/ml.
- The absorbance of each solution was measured using the corresponding solvent as a blank (similar to step 2.2).
- To ensure accuracy, each concentration was measured three times.

2.4. Assay of Rifaximin in Tablet:

- This section describes how the researchers analyzed the amount of Rifaximin present in a tablet.
- Twenty tablets were weighed, and the average weight was determined.
- The tablets were then finely powdered.
- An amount of the powder equivalent to 50mg of Rifaximin was accurately weighed.
- This weighed powder was dissolved in a small amount of methanol in a 50 mL volumetric flask.
- The flask was then filled to the 50 mL mark with methanol, resulting in a solution with a concentration of 1000 µg/ml (assuming all the Rifaximin dissolved).
- From this initial solution (1000 µg/ml), 10 mL was pipetted and diluted to 100 mL with methanol in another volumetric flask. This dilution step creates a solution with a concentration of 100 µg/ml.
- Finally, 2 mL of the 100 µg/ml solution was diluted to 10 mL with methanol.
- The concentration of Rifaximin in this final solution was then measured using the UV spectrophotometer.

2.5. Method Validation

- This section details the experiments performed to ensure the analytical method is reliable and accurate for Rifaximin analysis.

- The validation is based on the International Council for Harmonisation (ICH) guidelines, a recognized standard for drug analysis.[4,5&6]

Here are the specific parameters evaluated:

- **Linearity:** Similar to section 2.3, solutions with concentrations ranging from 2 to 10 µg/ml were prepared using each solvent (0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4).
    - Absorbance was measured at a specific wavelength chosen based on the scans from section 2.2 (likely around 440 nm).
    - A linear calibration curve is obtained by plotting the absorbance values against the corresponding concentrations.
    - Linearity ensures a proportional relationship between absorbance and concentration within a defined range.
- **Accuracy:** This step verifies if the method provides a true reflection of the actual Rifaximin amount in the sample.
    - A standard addition method is employed. A known amount of Rifaximin standard is added to pre-analyzed samples at three levels: 80%, 100%, and 120% of the expected concentration.
    - The spiked samples are then reanalyzed, and the recovery of the added standard is calculated for each level (usually expressed as a percentage).
    - Good accuracy translates to consistent recovery close to 100% across these levels.
- **Precision:** Precision reflects how close repeated measurements are under the same conditions.
    - There are three aspects of precision evaluated here:
        - **Repeatability:** This measures the agreement between multiple measurements of the same sample within a short time frame (e.g., same day).
        - **Intra-day precision:** This assesses the variability of measurements within a single day using different aliquots of the same sample solution.
        - **Inter-day precision:** This evaluates the variation in measurements across different days, potentially involving different analysts or instruments.
    - Precision is typically expressed by statistical measures like standard deviation or relative standard deviation (RSD%). Lower values indicate higher precision.
- **Limit of Detection (LOD) & Limit of Quantitation (LOQ):**
    - LOD refers to the minimum concentration of Rifaximin detectable in a sample.
    - LOQ represents the lowest concentration that can be reliably measured with acceptable accuracy and precision.
    - Both LOD and LOQ are calculated using equations based on the slope and standard deviation of the calibration curve.
- **Robustness:** This step assesses how the analytical method is affected by slight variations in the experimental conditions.
    - The analysis is performed with deliberate changes, such as using a slightly different wavelength or altering the time between sample preparation and measurement.
    - Robustness ensures the method is not overly sensitive to minor changes and delivers consistent results.

By evaluating these parameters, the researchers can ensure the analytical method is suitable for determining the amount of Rifaximin in Rifaximin-containing tablets.

III. RESULTS

3.1-Selection of Wavelength for Analysis

The UV spectrum of Rifaximin showed the maximum absorbance at the wavelength 440 nm,441 nm & 445 nm respectively for 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4 [Figure 1-3]. It was selected for the analysis of Rifaximin in bulk and tablet formulation.

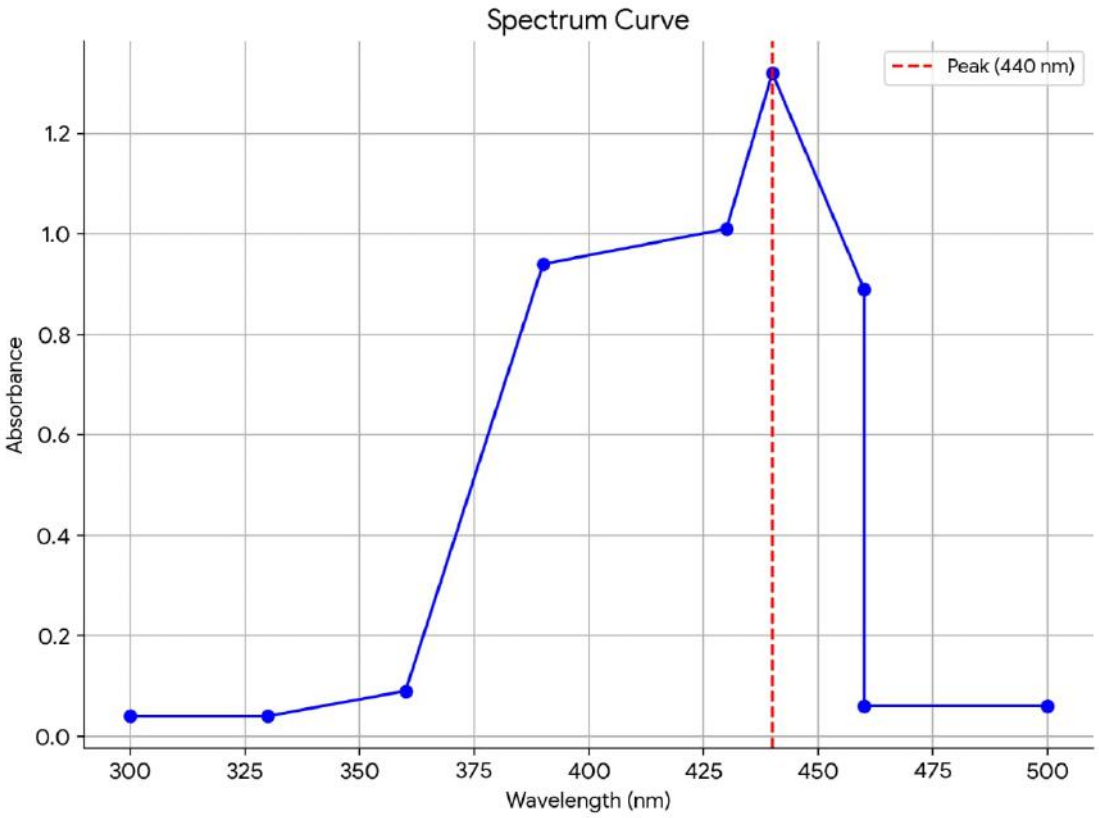

Fig.1: UV Spectrum of Rifaximin in 0.1N HCL (pH 1.2)

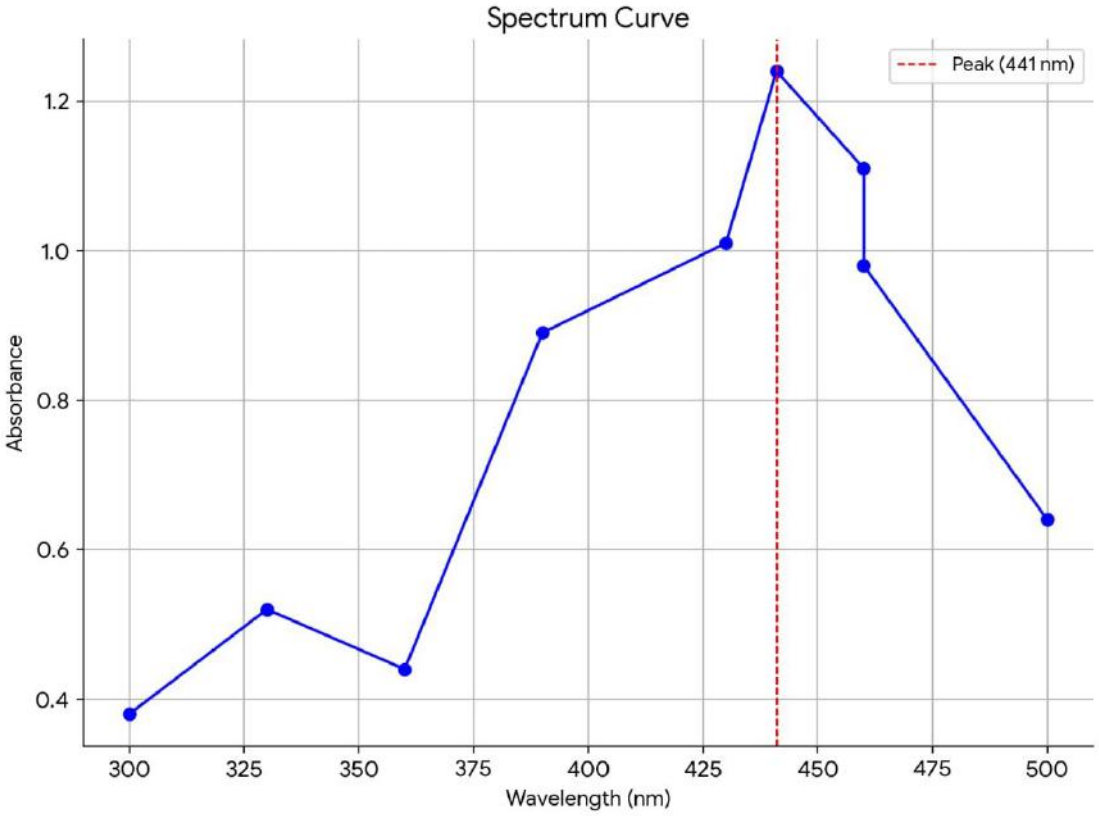

Fig.2: UV Spectrum of Rifaximin in Phosphate buffer (pH 6.8)

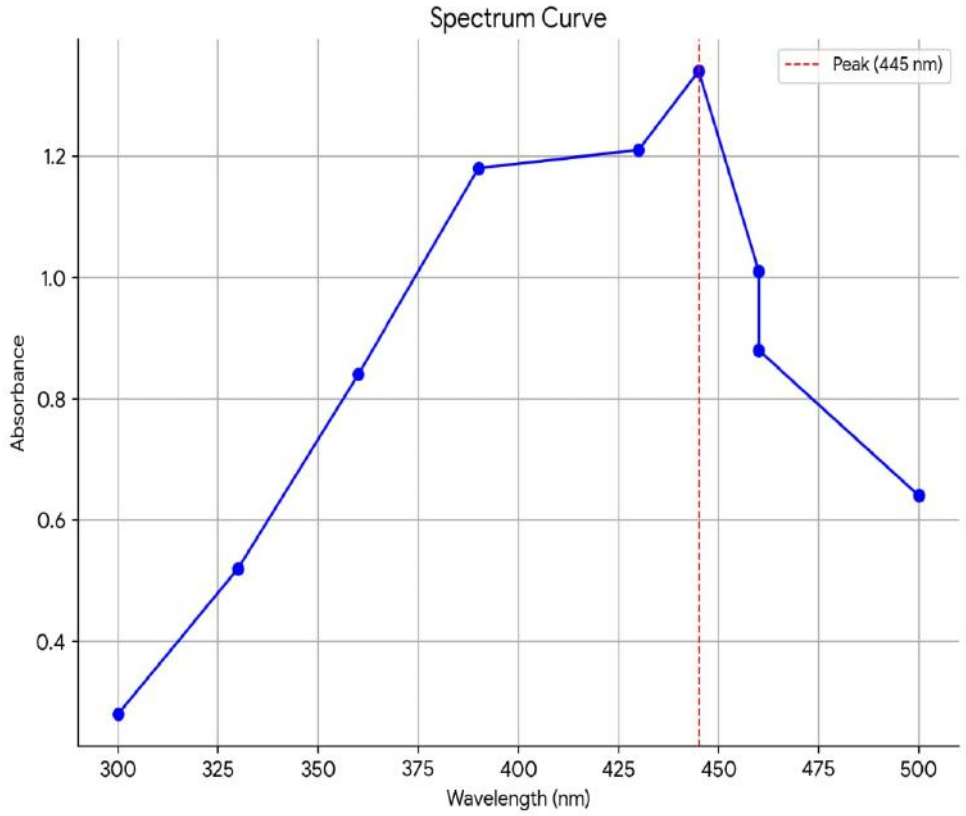

Fig.3: UV Spectrum of Rifaximin in Phosphate buffer (pH 7.4)

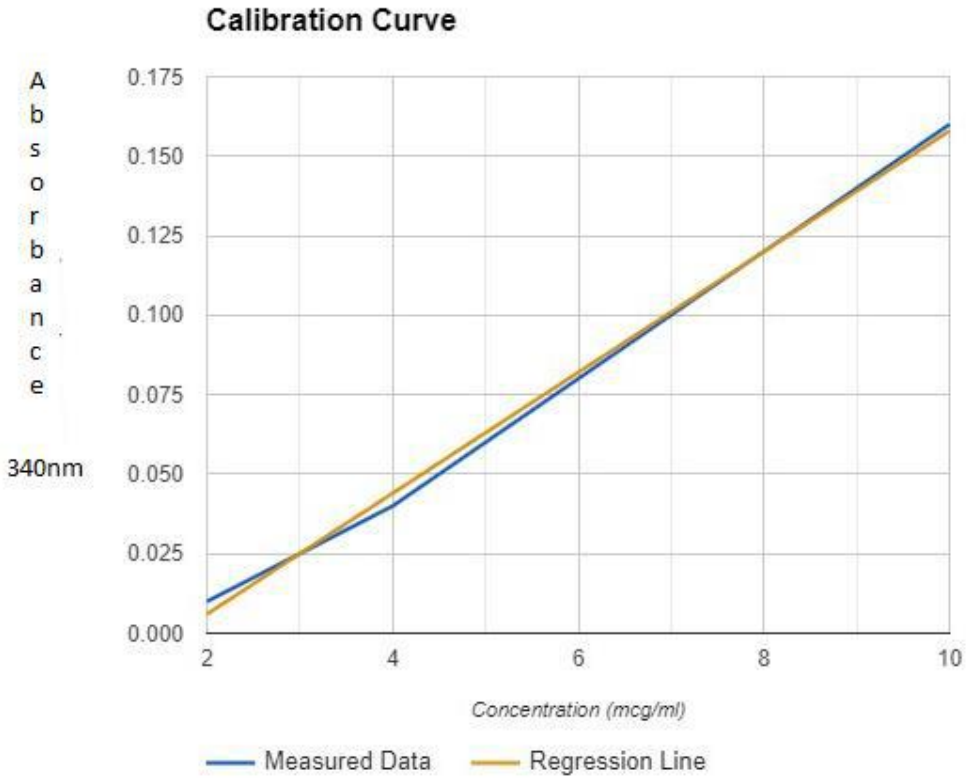

Fig.4: Calibration Curve of Rifaximin in 0.1N HCL (pH 1.2)

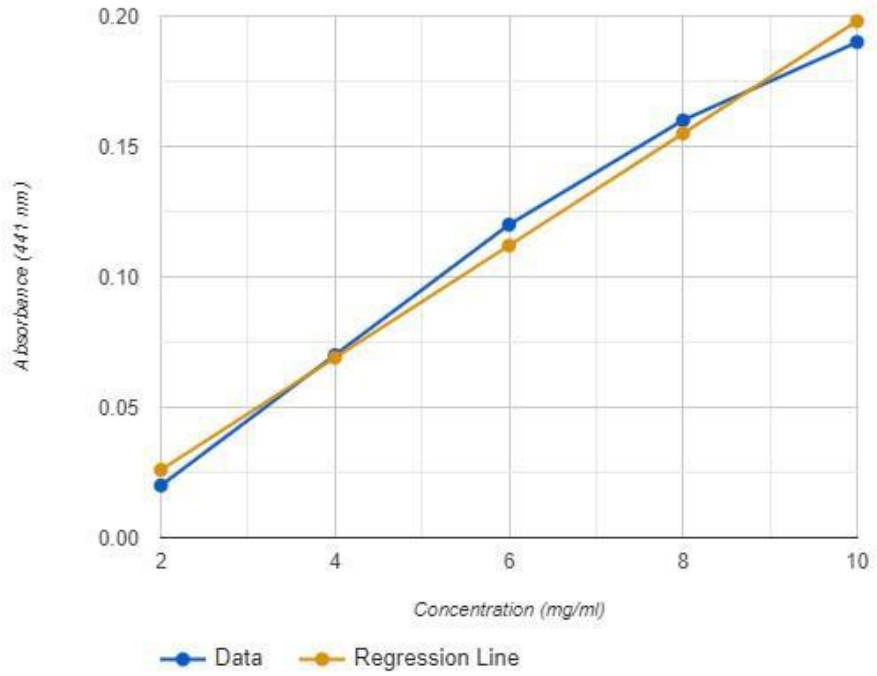

Fig.5: Calibration Curve of Rifaximin in Phosphate buffer (pH 6.8)

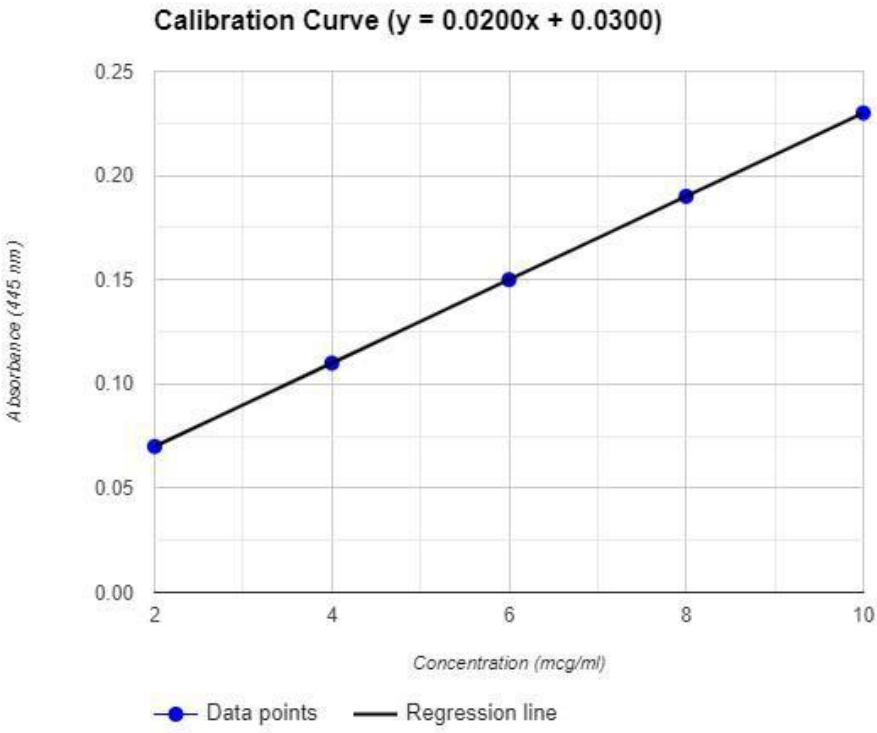

Fig.6: Calibration Curve of Rifaximin in Phosphate buffer (pH 7.4)

3.2-Preparation of the Calibration Curve

The calibration curve was constructed by plotting absorbance against corresponding concentration as shown in [Figure 4,5,&6] The calibration curve for Rifaximin. The drug obeyed Beer–Lambert's law in the concentration range of 2, 4, 6, 8, 10 µg/mL with coefficient of correlation (r2) of 0.998. [Table 1]

Table 1: Linearity data of Rifaximin in 0.1N HCL of 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4.

PARAMETERS		**RESULTS**	
	0.1N HCL pH 1.2	**Phosphate buffer pH 6.8**	**Phosphate buffer pH 7.4.**
Linearity range	2-10 µg/ml	2-10 µg/ml	2-10 µg/ml
Regression line equation	y = 0.0190x - 0.0320	y = 0.0215x - 0.0170	y = 0.0200x + 0.0300
Slope	0.0190	0.0215	0.0200
Y-intercept	-0.0320	-0.0170	0.0300
Correlation cofficient	0.9972	0.9898	1

3.3-Assay of Rifaximin in Tablet

The amount of Rifaximin present in formulation was calculated by comparing the absorbance of sample with standard absorbance. Content of Rifaximin in tablet formulation determined by developed method was in good agreement with the label claim. [Table 2]

Table 2: Assay of Tablet Formulation by UV method

Labelled claim (mg)	**200mg**
Drug content ± SD (mg)	200±0.0028
% Assay	101.42
% RSD	0.41

3.4-Method Validation

3.4.1. Accuracy

The responses were reanalyzed using the suggested method, and the accuracy results are shown in [Table 3-5], which demonstrate that the percentage amount recovered was between 98.60%-99.96%, 95.12% - 95.59% & 98.17%-98.87% with % RSD less than 2.

Table 3: Results of Accuracy for Rifaximin in 0.1N HCL (pH 1.2)

		Observation table for accuracy (0.1N HCL pH 1.2)				
Levels	**Conc. In ppm**	**Absorbance**	**Conc. Found**	**Mean**	**SD**	**% Recovery**
80	18	0.0655	16.4982			
		0.0663	16.5807	16.6391	0.1449	92.43
		0.0688	16.8384			
100	20	0.0866	18.6735			
		0.0871	18.7250	18.7353	0.0552	93.67
		0.0879	18.8075			
120	22	0.1077	20.8487			
		0.1079	20.8693	20.7164	0.0351	94.16
		0.1085	20.9312			

Table 4: Results of Accuracy for Rifaximin in Phosphate buffer (pH 6.8)

		Observation table for accuracy (Phosphate buffer pH 6.8)				
Levels	**Conc. In ppm**	**Absorbance**	**Conc. Found**	**Mean**	**SD**	**% Recovery**
80	18	0.1141	16.1030			
		0.1138	16.0669	16.102	0.0283	89.45
		0.1147	16.1361			
100	20	0.1203	18.5669			
		0.1205	18.5823	18.5617	0.0192	92.80
		0.1199	18.5361			
120	22	0.1626	19.8207			
		0.1622	19.7961	19.8202	0.0195	90.09
		0.1629	19.8438			

Table 5: Results of Accuracy for Rifaximin in Phosphate buffer (pH 7.4)

		Observation table for accuracy (Phosphate buffer pH 7.4)				
Levels	**Conc. In ppm**	**Absorbance**	**Conc. Found**	**Mean**	**SD**	**% Recovery**
80	18	0.1452	16.6883			
		0.1459	16.7448	16.8442	0.182	93.57
		0.1503	17.0996			
100	20	0.1641	18.2125			
		0.1649	18.2770	18.2655	0.0374	91.31
		0.1652	18.3012			
120	22	0.1866	20.0270			
		0.1888	20.2045	20.4394	0.533	93.13
		0.2016	21.2367			

3.4.2. Precision

The developed method's precision was reported as a % RSD. These findings demonstrate the assay's repeatability. % RSD values less than 2 shows that the method for determining rifaximin is precise. [Table 6-8]

Table 6: Results of Precision for Rifaximin in 0.1N HCL (pH 1.2)

Conc. (ppm)	Observation Table for Precision (0.1N HCL pH 1.2)			
	Intra-day precision		Inter-day precision	
	Conc. Found ± SD (µg/ml)	%RSD	Conc. Found ± SD (µg/ml)	%RSD
10	9.92±0.005	1.44	9.98±0.005	1.41
20	19.92±0.003	0.51	19.91±0.004	0.59
30	29.48±0.001	1.21	29.94±0.004	1.10

Table 7: Results of Precision for Rifaximin in Phosphate buffer (pH 6.8)

Conc. (ppm)	Observation Table for Precision (Phosphate buffer pH 6.8)			
	Intra-day precision		Inter-day precision	
	Conc. Found ± SD (µg/ml)	%RSD	Conc. Found ± SD (µg/ml)	%RSD
10	9.03±0.004	0.04	8.42±0.006	1.7
20	19.42±0.003	0.85	19.40± 0.003	0.76
30	28.41±0.002	0.38	28.23± 0.004	0.51

Table 8: Results of Precision for Rifaximin in Phosphate buffer (pH 7.4)

Conc. (ppm)	Observation Table for Precision (Phosphate buffer pH 7.4)			
	Intra-day precision		Inter-day precision	
	Conc. Found ± SD (µg/ml)	%RSD	Conc. Found ± SD (µg/ml)	%RSD
10	9.21±0.002	0.72	9.62±0.004	0.89
20	19.31±0.002	1.65	19.83±0.003	1.82
30	29.42±0.004	1.73	29.63±0.001	1.95

3.4.3. LOD & LOQ

By using the given formula, the LOD & LOQ were calculated for rifaximin in 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4 respectively in [Table 9]

Table 9: Results of LOD & LOQ

Conc (ppm)	Absorbance		
	(0.1N HCL pH 1.2)	(Phosphate buffer pH 6.8)	(Phosphate buffer pH 7.4)
0.1	0.0123	0.0800	0.1219
0.2	0.0141	0.0801	0.2578
0.3	0.0160	0.0808	0.3786
0.4	0.0215	0.0810	0.1188
0.5	0.0310	0.0814	0.2580
0.6	0.0324	0.0845	0.3773

SD	0.00675	0.0171	0.1286
Slope	0.0328	0.1087	0.0589
LOD	0.0203	0.5207	7.17
LOQ	0.0675	1.0414	21.77

3.4.4. Robustness

This method's robustness was tested using variations in wavelength change. The experimental results demonstrated that the suggested UV technique is robust, with the change since% RSD being less than 0.9%. [Table 10-12]

Table 10: Results of Robustness for Rifaximin in 0.1N HCL (pH 1.2)

Wavelength	**Chamber Saturation Time(Min)**			**Time form application to development (min)**		
440	14	15	16	0	30	60
0.246	1.071	1.019	0.941	0.748	0.968	1.311

Table 11: Results of Robustness for Rifaximin in Phosphate buffer (pH 6.8)

Wavelength	**Chamber Saturation Time(Min)**			**Time form application to development (min)**		
441	14	15	16	0	30	60
0.429	1.220	1.239	1.225	1.025	0.656	0.335

Table 12: Results of Robustness for Rifaximin in Phosphate buffer (pH 7.4)

Wavelength	**Chamber Saturation Time(Min)**			**Time form application to development (min)**		
445	14	15	16	0	30	60
0.211	0.331	0.268	1.015	0.979	0.325	0.435

3.5 The Summary of Validation Parameters by UV Method

NO	**PARAMETERS**	**(0.1N HCL pH 1.2)**	**(Phosphate buffer pH 6.8)**	**(Phosphate buffer pH 7.4)**
1	ABSORPTION MAXIMA	440	441	445
2	BEERS RANGE (µg/ml)	2-10 µg/ml	2-10 µg/ml	2-10 µg/ml
3	STANDARD REGRESSION EQUATION	y = 0.0190x - 0.0320	y = 0.0215x - 0.0170	y = 0.0200x + 0.0300
4	CORRELATION COFFICIENT	0.9972	0.9898	1
5	PRECISION	Below 2%	Below 2%	Below 2%
6	ACCURACY	92.43-94.16%	89.45-92.80 %	91.31-93.57%
7	ROBUSTNESS	0.246	0.429	0.211

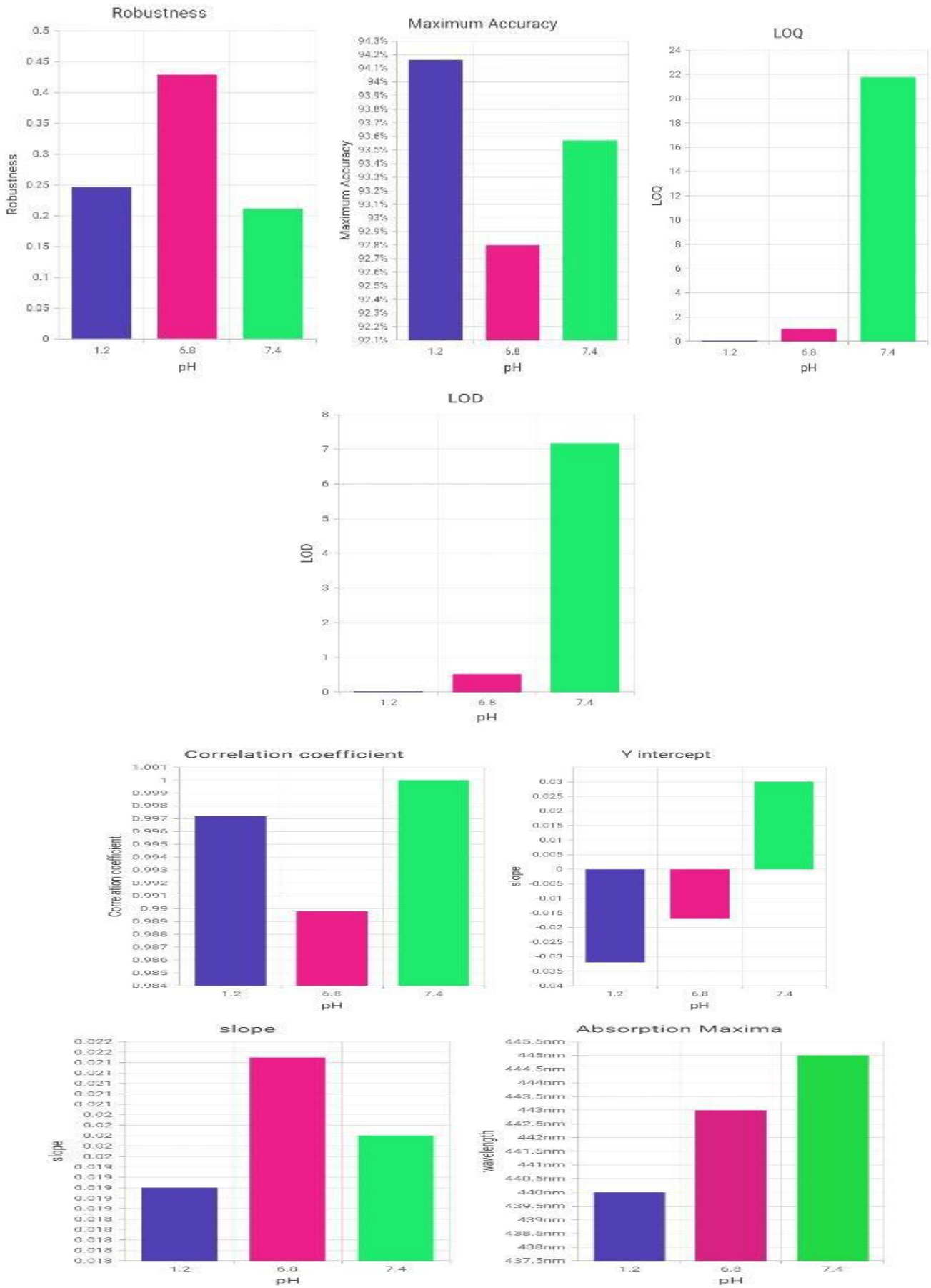

Fig-7 Graphical Representation Summary of Validation Method of Drug Rifaximin

IV. CONCLUSION

Validation of the rifaximin standardization method using different acid and phosphate buffer conditions (pH 1.2, 6.8 and 7.4) showed good accuracy (relative standard deviation less than 2%) but limitations in linearity and precision. Although the correlation coefficients were relatively high (0.9898 to 0.9972), they were below the ideal (1.0), indicating possible biases in concentration and absorption. In addition, accuracy ranged from 89.45% to 94.16%, suggesting a slight under- or overestimation of rifaximin concentration. Further optimization is needed to create a more robust method. This may include investigating different pH conditions, refining the concentration range of the standard curve, or even considering alternative analytical methods.

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