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**Original Article** 

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# Chemometric-Assisted UV-Spectrophotometric Method for Quantification of Novel Anti-Tubercular Liquisolid Formulation

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#### ABSTRACT

To simultaneously determine quercetin and rifampicin in raw material and liquid-solid formulation, a numerical technique built on spectroscopic data associated with partial least squares (PLS) multi - variate measurement is suggested in this work. Rifampicin and quercetin spectra were captured at concentrations that fell within the 2–10 µg/ml linear range for each compound. They were used to compute 25 simulated mixtures containing 16 calibration and 9 validation sets, with wavelength spacing of  $\lambda$ =15 nm in hydrochloric acid (0.1 M) and phosphate buffer pH-6.8. The wavelength ranges were 200 to 630 nm. Based on the validation and calibration data's (RMSE) root mean square errors, the models' appropriateness was determined. Recovery studies (%) and relative prediction errors were used to compare and contrast the analytical capabilities of various chemometric techniques. The recovery study's findings showed that this technique was successfully used to formulate pharmaceuticals without excipient interference. The suggested technique is quick, easy to use, and can be utilised as a replacement for traditional analytical tools in the formulation and quality control of pharmaceuticals.

Key words: Rifampicin (RIF), Quercetin (QUE), Partial Least Square (PLS), UV-spectroscopy, Validation

# INTRODUCTION

Tuberculosis (TB) is a conceivably deadly infectious disease brought about by *Mycobacterium tuberculosis*, which most regularly influences the lungs. Through droplets out from lungs and throat of people with fluctuating respiratory conditions, it travels from person to person. It is the world's second most regular cause of death from an irresistible malady after AIDS. In 2019, an unexpected 10 million new instances of tuberculosis were reported. Geographically speaking, Africa and Asia bear the brunt of TB. Nearly 40% of TB cases worldwide are reported in China and India [1-4].

Rifampicin is a semisynthetic anti-microbial delivered from *Streptomyces mediterranei*. A few different forms of *Mycobacteria* are resistant to its broad antimicrobial range. It stifles the beginning of RNA synthesis in defenceless living things by impeding DNA-subordinate RNA polymerase action by creating a stable complex with the molecule [1-4].

Antituberculosis drugs cause hepatotoxicity in certain people prompting intense liver disappointment, which brings about death. Such marvels limit the clinical utilization of medications, adding to treatment disappointment that conceivably causes drug opposition. Additionally, these drugs might cause adverse effects such as neurotoxicity, ototoxicity, touchiness, nephrotoxicity, GI poisoning and CNS damage. RIF's bioavailability is also only about 50–60%, making formulation strategy for boosting solubility and subsequently bioavailability

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necessary. Additionally, powerless patient consistency, lengthy action times, lung tissue destruction and susceptibility in a safe environment are major issues with tuberculosis treatment [5-8].

In order to combat the aforementioned problems, using a herbal bioenhancer like quercetin will increase bioavailability and lessen the negative effects of the existing medical therapy. Homegrown bio-enhancers have been used to upgrade bio-accessibility and bio-proficiency of various medications, for example, hostile to tubercular drugs, anti-infective agents, antiviral, antifungal anticancer medications. Bioenhancers will diminish the poisonousness and abbreviate the treatment time frame. Immunomodulation and hepatoprotective properties might be further advantages in treating tuberculosis [9-12].

Quercetin, an aglycone type of flavonoid glycosides, is found in natural citrus products. Repression of CYP3A4 and the P-glycoprotein efflux syphon is how it functions. It displays aversion to extremist searching, oxidants and restraint toward atherosclerotic activities. Quercetin has appeared to build bioavailability, blood levels and adequacy of various medications, including diltiazem, digoxin, verapamil, etoposide, and paclitaxel [13-15].

According to the literature, numerous methods, including UV spectroscopy [16, 17], RP-HPLC [18-20], HPTLC [21, 22] and others, are available for determining RIF both alone and in conjunction with various drugs in a variety of dose forms. A few methods, including HPLC [23-26], UV spectroscopy [27] and HPTLC [28-31], have been taken into consideration for the assessment of QUE both alone and in conjunction with different drugs in different forms of dosage.

The previously mentioned chromatographic strategies are broadly utilized and suggested; however, these techniques require mind-boggling and costly instrumentation, arrangement for use and removal of solvents, work escalated test planning methodology and individual aptitudes [32, 33].

The spectrophotometric strategy is one of the most favoured methodologies for drug investigation because it is effortless and reasonable compared to other expository techniques and hence can be used in most quality control investigations of medications. The traditional spectrophotometry strategy cannot perform the synchronous examination of a few active substances in drugs due to considerable overlapping of a spectral region in binary or tertiary mixtures. In this way, a few diverse methodologies like the Q-absorbance ratio and simultaneous equation method were used for the synchronous assurance of drugs in binary mixture. The chemometric multivariate PLS method has extended applications by hyphenation with spectroscopy to obtain qualitative and quantitative information about the analyte. Hence, a mathematical approach is applied to unravel the contribution to the spectra of drugs in the mixture. It can be used for very complex mixtures with accuracy and precision involving single step decomposition and regression due to its desirable characteristics, as are rapid, simple and non-destructive methods [32, 33].

Since basic UV spectrophotometric strategy with Partial Least Square regression analysis for the measure of RIF and QUE in consolidated dose structure is not accessible, the objective of the current examination was to create and approve an essential, quick, dependable and conservative UV spectrophotometric technique for the investigation of RIF and QUE in newly developed Liquisolid dosage form.

# MATERIALS AND METHODS

# Materials and reagents

Analytical grade RIF and QUE were bought from Swapnroop Drugs and Pharmaceuticals, Aurangabad, Maharashtra, India. Merck, Mumbai, India, bought sodium hydroxide, potassium hydrogen orthophosphate, and hydrochloric acid. (0.1 M) Hydrochloric acid and Phosphate buffer pH-6.8 was set up as per Indian Pharmacopeia. Throughout the test, double-refined water was used.

# Instrumentation

The absorbance of each solution was measured using a UV-spectroscopy (1800, Shimadzu, Japan) with a 2 nm spectral width, 0.5 nm wavelength precision and a set of quartz cells (10 mm). With the help of Ultra violet system software version 2.34, spectrum was immediately recorded. In the investigation, an ultrasound bath (Frontline FS 4 from Mumbai, India) and electronic balance (Model AUX220, Shimadzu Ltd., Japan) were both employed.

#### Standard drug solution preparation

Two working standard solutions of 100  $\mu$ g/ml of RIF and QUE were set up by dissolving 10 mg each independently in both Hydrochloric acid (0.1 M) (Method-A) and Phosphate buffer (pH-6.8) (Method-B) diluted independently to 100 ml with particular dissolvable in the adjusted flagon.

# Preparation of validation and calibration set

A set (Two) of validation and calibration of standard solutions were created. By combining appropriate amounts of the working standard solutions of RIF and QUE and individually dilute to volumes with phosphate buffer (pH-6.8) and HCl (0.1 M), 9 validation standards and 16 calibration standards were created. **Table 1** provides an overview of the RIF and QUE mixture. The solution's absorbance spectra were estimated to range from 800 to 200 nm with 15 nm stretches. The Unscrambler® programme for the PLS model was then given access to the calibration set's absorbance data. The proposed PLS model was used to predict the groupings of RIF and QUE in the validation set in order to approve the PLS model. IUPAC and ICHQ2(R1) rules were used to validate the method [32-34].

Table 1. Components of the varidation and canoration set data						
Sr. No.	RIF (µg/ml)	QUE (µg/ml)				
1C	2	2				
2C	2	4				
3C	2	6				
4C	4	4				
5C	4	6				
6C	4	8				
7C	4	10				
8C	6	2				
9C	6	4				
10C	6	6				
11C	8	4				
12C	8	6				
13C	8	8				
14C	10	4				
15C	10	6				
16C	10	8				
17V	2	2				
18V	2	4				
19V	4	4				
20V	4	6				
21V	6	2				
22V	6	4				
23V	8	4				
24V	8	6				
25V	10	4				

Table 1. Components of the validation and calibration set data

V= validation set solution, C = calibration set solution

The (REP%) relative error of prediction, square of correlation coefficient (R2) and root mean square difference (RMSECV) are statistical measures of how well the data fit the model. To check for inaccuracies in the expected concentrations, the RMSECV was utilised as a diagnostic test. It shows both the accuracy and precision of prediction. Accurate quantification in calibration of PLS depends on selecting the right number of components to build the model. Selecting the number of elements that produce the smallest RMSECV is the standard procedure. A variety of figures of merit, such as analytical sensitivity, sensitivity and detection limit have been described in

the literature to accurately measure the effectiveness of a specific multivariate model. These figures of merit are also used to compare techniques to understand the quality of a particular analytical technique [32, 33].

# Analysis of liqui-solid dosage form

Precisely weighed 800 mg of Liqui-solid dosage form comparable to 150 mg RIF and 150 mg QUE was moved into two 100 ml amber colour quantitative flasks independently. 70 ml of HCl (0.1 M) and phosphate buffer (pH-6.8) into isolated flask was added, sonicated around 15 min and diluted volume up to the mark with HCl (0.1 M) and phosphate buffer (pH-6.8) independently. From this 1 ml aliquot in independent amber colour 10 ml quantitative flask was transferred and dilution to the mark with HCl (0.1 M) and phosphate buffer (pH-6.8) to get 150  $\mu$ g/ml of RIF and QUE. Further, pull back 0.4 ml of 150  $\mu$ g/ml was withdrawn and diluted with the above solvents to get 6  $\mu$ g/ml of RIF and QUE. Between 200 and 800 nm, the solution's absorbance was measured. The absorbance of the test solution was determined utilizing the PLS equation. The examination technique was rehashed multiple times with Liqui-solid dosage form [32, 33].

# **RESULTS AND DISCUSSION**

# Spectral zones to be analyzed by pls and calibration matrix

The PLS approach is frequently employed as a chemometric model to estimate simultaneously multi-component dosage form containing medicines that display significant overlap in their absorption spectra. By taking measurements of absorbances at particular wavelength region, this chemometric model calculates the amount of medicines contained in the mixtures. Selecting the most insightful data and eliminating the unneeded ones, which gives the PLS chemometric model meaning, is one of the key advantages. Therefore, chemometric supported spectroscopic approaches are preferred over traditional time-consuming analytical procedures because they are more cost-effective, straightforward, sensitive, and quick.



Figure 1. Combine spectra of RIF, QUE and mixture in HCl (0.1 M) and phosphate buffer pH-6.8

**Figure 1** shows UV spectra for RIF and QUE individually and their mixture in HCl (0.1 M) and phosphate buffer (pH-6.8) independently. There is considerable overlapping in the absorption region of RIF and QUE spectra. RIF exhibits absorption maxima at 338, 469 nm in HCl (0.1 M) and 334, 473 nm in phosphate buffer (pH-6.8). QUE exhibits absorption maxima at 367 nm in HCl (0.1 M) and 368 nm in phosphate buffer (pH-6.8). Direct spectroscopic studies cannot resolve the mixes due to the spectrum overlap of these medications. Due to this, chemometric calibrations were applied separately to the simultaneous identification of both medicines in mixtures utilising the zero-order spectra.

# Multivariate analysis

Building the calibration matrix was the first phase in multivariate techniques. The employed wavelengths were between 200 and 650 nm. Within such a range, 30 spectral spots with 15 nm spacing were chosen. The calibration mixtures' compositions were chosen at random in order to get the most data from their spectra. The spectrum mode and wavelength range were employed affect the efficacy of multi - component assessment. **Figures 2a and** 

**2b** depict the UV spectral response of RIF, QUE and the mixture at their standard concentrations in HCl (0.1 M) and phosphate buffer (pH-6.8). The mixture of RIF and QUE in (0.1 M) HCl and (pH-6.8) phosphate buffer was used to generate the validation and calibration set at random (**Table 1**). In the range between 200 and 650 nm, the UV spectra were examined, and the absorbances were calculated at 30 wavelength spots spaced 15 nm apart. The Unscrambler® program developed the PLS model. The (RMSE) root mean square error for each technique was obtained by comparing the expected concentrations of the component in every sample with the true concentrations of the component in all of the validation samples.

#### Development of PLS analysis

To achieve precise quantification in PLS calibrations, the model is essential. By forecasting the amount of analysis in a different validation set that was not included in model building, the resulting models were additionally validated. **Table 2** show the accuracy studies' prediction findings and recovery percentages.

Conc. Expected (µg/ml)		Conc. Predicted (µg/ml)		Recov	Recovery %		Conc. Residual (E-P) (µg/ml)	
RIF	QUE	RIF	QUE	RIF	QUE	RIF	QUE	
			C	0.1 M HCl				
2	2	2.008	1.975	100.38	98.77	-0.008	0.025	
2	4	2.165	3.909	108.27	97.72	-0.165	0.091	
4	4	3.833	4.099	95.83	102.47	0.167	-0.099	
4	6	4.099	5.999	102.47	99.98	-0.099	0.001	
6	2	5.949	2.090	99.14	104.51	0.051	-0.090	
6	4	5.892	3.961	98.20	99.04	0.108	0.039	
8	4	8.008	3.994	100.10	99.86	-0.008	0.006	
8	6	7.883	6.049	98.53	100.81	0.117	-0.049	
10	4	10.164	3.924	101.64	98.10	-0.164	0.076	
			pH	I-6.8 Buffer				
2	2	1.99	1.97	99.69	98.66	0.006	0.027	
2	4	2.00	4.00	100.03	100.06	-0.001	-0.002	
4	6	4.02	6.08	100.44	101.29	-0.018	-0.077	
4	8	4.00	7.99	99.93	99.85	0.003	0.012	
6	6	6.00	5.99	99.97	99.87	0.002	0.008	
6	8	5.99	7.96	99.83	99.44	0.010	0.044	
8	6	8.00	6.01	100.03	100.19	-0.003	-0.011	
8	8	8.00	8.00	99.99	99.95	0.001	0.004	
10	8	10.00	8.00	100.01	100.05	-0.001	-0.004	

 Table 2. Recovery study of RIF and OUE in 0.1 M HCl and pH-6.8 buffer by PLS technique

The plot of the actual known concentrations against the expected concentrations is given in **Figure 2**. This evaluation of the models' prediction abilities was done by charting the predicted concentration vs. actual known concentrations. As was seen, the expected (calculated) and actual drug concentrations were in good agreement. For RIF and QUE, the relative standard deviation and mean recoveries of our suggested approaches were calculated and are shown in **Table 3**. PLS-optimized models produced acceptable correlation coefficient (r<sup>2</sup>) value for every component in the validation set, demonstrating good model predictive power. Plotting the predicted concentration vs. residuals concentrations served as another diagnostic test. The residuals have a random distribution near zero, indicating that the model has been built appropriately (**Figure 3**). The statistical measures RMSEC and RMSEV lesser value demonstrate the accuracy and precision of the suggested method. Various analytical figures of merits, including sensitivity and detection limit, have been computed (**Table 3**) as per IUPAC technical report.



b)

**Figure 2.** The graph of the concentration value's actual vs. predicted of RIF and QUE in Phosphate buffer (pH-6.8) and HCl (0.1 M)







Figure 3. A graph of residual vs. expected concentration of RIF and QUE in HCl (0.1 M) and pH-6.8 Phosphate buffer

Table 3 provides an illustration of the statistical param	neters of the calibration and validation set.
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	Tuble 5. Stati		Dhamk-4-1-	
Parameters —	HCI (	0.1 MI)	Phosphate bi	uner (pH-6.8)
	RIF	QUE	RIF	QUE
Range (µg/ml)		2-	-10	
Spectrum range		200 -	650 nm	
$\Delta\lambda$ (nm)		1	.5	
% Recovery	100.51	100.14	99.99	99.93
SD	3.5096	2.1866	0.20	0.69
%RSD	3.49	2.18	0.201	0.691
		Calibration set		
RMSEC	0.115	0.064	0.171	0.125
$\mathbb{R}^2$	0.998	0.998	1.000	1.000
Intercept	2.318	0.332	0.302	0.311
Slope	0.9946	0.9988	0.9962	0.9971
Press	0.6661	0.0927	0.4681	0.2499
REP%	0.3139	0.1286	0.2631	0.1780
Bias	0.1683	0.0556	0.1375	0.0975
		Validation set		
RMSEP	0.115	0.064	0.007	0.032
$\mathbb{R}^2$	0.998	0.998	1.000	1.000
Intercept	2.318	0.332	0.302	0.311
Slope	0.9981	0.9977	1.000	0.9997
Press	0.1199	0.0365	0.0004	0.0090
REP%	0.1878	0.1511	0.0118	0.0485
Bias	0.0985	0.0528	0.005	0.021
		Figures of merit		
LOD (µg/ml)	0.9588	0.3568	0.7155	0.5229
Sensitivity (µg/ml)	0.9946	0.9988	0.9962	0.9971

 Table 3. Statistical parameters for the PLS method

# Analysis of liquid-solid dosage form

With a label claims of 150 mg RIF and 150 mg QUE per dosage form, the liqui-solid dosage form was analysed using the established chemometrics-assisted UV spectroscopic method. The mean percentage recovery results were 96.89 % and 97.15 % for RIF and QUE in 0.1 M HCl respectively, accepting the label's claim. The created techniques can make a great difference from the ones currently being used. The approaches are advantageous because they make quality control of mixtures, routine analysis and tablet formulations incorporating these two medications simple to do and less expensive.

# CONCLUSION

Combination medicines with a minor difference in max are not suited for traditional UV spectroscopic techniques. Chemometric can be used in place of more complex techniques like HPTLC and HPLC in certain situations. The sample can be produced, diluted, and their absorbance recorded after the calibration matrix has been created and saved in the data computing device. The recorded matrix is read to determine the sample concentration. Chemometric procedure PLS has been effectively used to construct pharmaceutical formulations and to simultaneously determine RIF and QUE in laboratory combinations. The technique can be used to identify medicines in dissolving research. On the other hand, the core benefits of the researched approaches include their speed, cost-effectiveness, and simultaneous analysis of a mixture of the subject pharmaceuticals without any pre-treatments.

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