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Chemometric-Based UV Spectrophotometric Approach for the Estimation of a Newly Developed Anti-Tubercular Liquisolid Formulation

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ABSTRACT

This study proposes a numerical approach based on spectroscopic data combined with partial least squares (PLS) multivariate analysis to simultaneously quantify quercetin and rifampicin in raw materials and liquisolid formulations. Spectral measurements were obtained for rifampicin and quercetin over a linear concentration range of 2–10 µg/ml for each compound. A total of 25 simulated mixtures were generated, including 16 calibration and 9 validation sets, using a wavelength interval of λ = 15 nm in 0.1 M hydrochloric acid and phosphate buffer at pH = 6.8, covering the spectral range of 200 to 630 nm. The suitability of the models was evaluated based on the root mean square errors (RMSE) obtained from the calibration and validation data sets. The analytical performance was further evaluated by comparing recovery percentages and relative prediction errors across different chemometric techniques. The results of the recovery analysis confirmed that this method effectively enabled pharmaceutical formulation without the interference of excipients. The proposed approach is rapid, user-friendly, and serves as a viable alternative to conventional analytical methods for formulation development and pharmaceutical quality control.

Keywords: Validation, Rifampicin (RIF), Partial least square (PLS), Quercetin (QUE), UV-spectroscopy

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Introduction

Tuberculosis (TB) is a potentially fatal infectious disease caused by *Mycobacterium tuberculosis*, primarily affecting the lungs. The disease spreads through respiratory droplets from individuals with lung or throat conditions, making it highly contagious. TB remains the second leading cause of death from infectious diseases worldwide, following AIDS. In 2019, there were 10 million new reported cases of tuberculosis, with Africa and Asia bearing the most significant burden. Almost 40% of TB cases globally are reported in China and India [1-4]. Rifampicin (RIF), a semisynthetic antibiotic derived from Streptomyces mediterranei, has a broad antimicrobial spectrum, although several strains of Mycobacteria exhibit resistance to it. RIF inhibits RNA synthesis initiation by forming a stable complex with DNA-dependent RNA polymerase, effectively blocking its activity in susceptible organisms [1-4].

However, some antituberculosis drugs, including rifampicin, can induce hepatotoxicity in certain individuals, potentially leading to acute liver failure and death. These adverse effects limit their clinical use and contribute to treatment failure, which can lead to drug resistance. In addition to hepatotoxicity, these drugs may cause neurotoxicity, ototoxicity, nephrotoxicity, gastrointestinal disturbances, and central nervous system damage. Moreover, rifampicin has a low bioavailability of only 50–60%, which makes enhancing its solubility and bioavailability through improved formulation strategies essential. Other challenges in TB treatment include poor patient adherence, prolonged treatment duration, lung tissue damage, and susceptibility to infections [5-8].

To address these issues, incorporating herbal bio-enhancers such as quercetin may enhance bioavailability while reducing the adverse effects of conventional antituberculosis treatments. Herbal bio-enhancers have been shown to improve the bioavailability and efficacy of various drugs, including antitubercular agents, anti-infectives, antivirals, antifungals, and anticancer drugs. The use of bioenhancers can reduce toxicity and shorten the treatment period, with potential additional benefits such as immunomodulation and hepatoprotection in the treatment of tuberculosis [9-12].

Quercetin, a flavonoid aglycone found in citrus fruits, works by inhibiting CYP3A4 and the P-glycoprotein efflux pump. It has antioxidant properties and can help prevent atherosclerosis. Quercetin has been shown to enhance the bioavailability and effectiveness of various drugs, including diltiazem, digoxin, verapamil, etoposide, and paclitaxel [13-15].

Numerous techniques have been described for quantifying rifampicin, either alone or in combination with other drugs, including UV spectroscopy [16, 17], RP-HPLC [18-20], and HPTLC [21, 22]. Similarly, methods such as HPLC [23-26], UV spectroscopy [27], and HPTLC [28-31] have been used to assess quercetin in different dosage forms, either alone or in combination with other drugs.

While chromatographic techniques are widely recommended and utilized, they often require sophisticated, expensive equipment, complicated sample preparation, solvent disposal, and specialized skills [32, 33]. In contrast, spectrophotometry is a simpler and more cost-effective approach for drug analysis, making it a preferred choice for routine quality control. However, traditional spectrophotometry cannot effectively analyze multiple active compounds simultaneously, especially in binary or tertiary mixtures, due to significant spectral overlap. To overcome this, methods like Q-absorbance ratio and simultaneous equation methods have been used for the simultaneous determination of drugs in binary mixtures. The chemometric multivariate partial least squares (PLS) method offers a more advanced solution by combining spectroscopy with mathematical models to provide both qualitative and quantitative data. This technique is especially useful for complex mixtures, offering rapid, simple, and non-destructive analysis with high accuracy and precision through a single-step decomposition and regression process [32, 33].

Given the lack of a simple UV spectrophotometric method with Partial Least Square (PLS) regression analysis for the simultaneous measurement of rifampicin and quercetin in combination dosage forms, the current study aimed to develop and validate a straightforward, rapid, reliable, and cost-effective UV spectrophotometric method for the quantification of rifampicin and quercetin in a newly formulated liquisolid dosage form.

Materials and Methods

Materials and reagents

The analytical-grade substances, RIF and QUE, were sourced from Swapnroop Drugs and Pharmaceuticals, located in Aurangabad, Maharashtra, India. Sodium hydroxide, potassium hydrogen orthophosphate, and hydrochloric acid (0.1 M) were purchased from Merck in Mumbai, India. The hydrochloric acid and phosphate buffer (pH = 6.8) were prepared according to the specifications outlined in the Indian Pharmacopeia. Throughout the experimental process, double-distilled water was utilized.

Instrumentation

Absorbance measurements were carried out using a UV-spectrophotometer (Shimadzu 1800, Japan), which features a spectral width of 2 nm and a wavelength precision of 0.5 nm, with quartz cells (10 mm) employed for the analysis. The spectra were captured in real time through the Ultra Violet System software (version 2.34). Additionally, an ultrasound bath (Frontline FS 4, Mumbai, India) and an electronic balance (Shimadzu AUX220, Japan) were used for the preparation of the solutions.

Preparation of standard drug solutions

Two distinct working standard solutions were prepared for RIF and QUE, each containing 100 µg/ml. To do this, 10 mg of each drug was dissolved separately in hydrochloric acid (0.1 M) for Method A and phosphate buffer (pH = 6.8) for Method B. Both solutions were then diluted to a final volume of 100 ml with the appropriate solvent.

Preparation of validation and calibration sets

The preparation of the calibration and validation sets involved mixing specific volumes of the working standard solutions of RIF and QUE, which were then diluted with phosphate buffer (pH = 6.8) or hydrochloric acid (0.1 M). This process generated 9 validation standards and 16 calibration standards, with the mixtures summarized in **Table 1**. Absorbance spectra for these solutions were recorded within a wavelength range of 200–800 nm in 15 nm increments. The calibration set's absorbance data were subsequently analyzed using the Unscrambler® software to develop a partial least squares (PLS) model. The validity of the PLS model was confirmed by testing it with the validation set, following the IUPAC and ICHQ2(R1) guidelines [32-34].

Table 1. Components of the validation and calibration 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

V= validation set solution, C = calibration set solution

The relative error of prediction (REP%), the square of the correlation coefficient (R^2), and the root mean square error of cross-validation (RMSECV) are statistical indicators used to evaluate the goodness of fit for a model. To assess the potential deviations in predicted concentrations, RMSECV serves as a diagnostic tool, offering insights into both the precision and accuracy of the model's predictions. The key to accurate quantification in PLS calibration is the correct selection of the number of components in the model. Typically, the model with the smallest RMSECV is chosen for optimal results. Various performance indicators, such as analytical sensitivity, detection limit, and sensitivity, have been detailed in the literature to quantify the effectiveness of specific multivariate models. These metrics also facilitate comparisons across different techniques, offering a deeper understanding of the quality of a given analytical approach [32, 33].

Analysis of liqui-solid dosage form

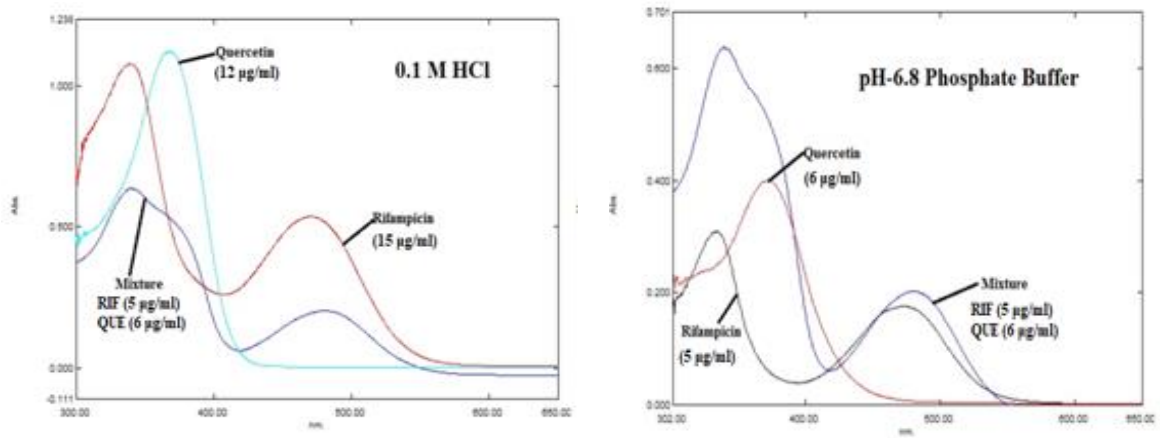
For the analysis, 800 mg of the Liqui-solid dosage form, containing 150 mg each of RIF and QUE, was accurately weighed and transferred into two separate 100 ml amber volumetric flasks. To each flask, 70 ml of either HCl (0.1

M) or phosphate buffer (pH = 6.8) was added. The solutions were then sonicated for approximately 15 minutes before being diluted to the final volume with their respective solvents. A 1 ml aliquot from each solution was then transferred to separate 10 ml amber volumetric flasks, followed by dilution to the mark with the same solvents to yield 150 µg/ml concentrations of RIF and QUE. Subsequently, 0.4 ml of the 150 µg/ml solution was diluted with the solvents to achieve a final concentration of 6 µg/ml for both RIF and QUE. The absorbance of the prepared solutions was measured across a wavelength range of 200 to 800 nm. Using the PLS model, the absorbance data of the test solutions were analyzed. This procedure was repeated multiple times with the Liqui-solid dosage form for reliability [32, 33].

Results and Discussion

Spectral range for PLS analysis and calibration matrix

The partial least squares (PLS) method is commonly applied as a chemometric approach to simultaneously analyze multi-component formulations, especially when the active ingredients exhibit significant overlap in their absorption spectra. By collecting absorbance measurements at specific wavelength ranges, this chemometric model can quantify the components in the mixture. A key advantage of the PLS approach lies in its ability to focus on the most relevant data while eliminating unnecessary information, which improves the model's efficiency. As a result, spectroscopic methods supported by chemometric techniques are increasingly preferred over traditional, time-consuming methods, as they offer cost-effectiveness, simplicity, greater sensitivity, and rapid results.



a) b)

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

Figure 1 presents the individual UV absorption spectra of RIF and QUE, along with their mixture, measured in HCl (0.1 M) and phosphate buffer (pH = 6.8). The spectra of RIF and QUE exhibit notable overlap in their absorption regions. Specifically, RIF shows peak absorbances at 338 and 469 nm in HCl (0.1 M) and 334 and 473 nm in phosphate buffer (pH = 6.8). QUE, on the other hand, absorbs maximally at 367 nm in HCl (0.1 M) and 368 nm in phosphate buffer (pH = 6.8). Due to this spectral overlap, direct spectroscopic analysis cannot distinctly differentiate the two components in mixtures. To address this, chemometric techniques were employed, leveraging the zero-order spectra for the simultaneous determination of both compounds.

Multivariate approach

The first phase of the multivariate analysis involved constructing the calibration matrix. The selected wavelength range spanned from 200 to 650 nm, with data recorded at 30 different wavelengths, each spaced 15 nm apart. The compositions of the calibration mixtures were randomly selected to maximize the diversity of the spectral data. The chosen spectral range and wavelength intervals were pivotal in optimizing the multi-component analysis. **Figures 2a and 2b** illustrate the UV absorption responses for RIF, QUE, and their mixture at standard concentrations, both in HCl (0.1 M) and phosphate buffer (pH = 6.8). The calibration and validation sets were created by randomly combining RIF and QUE in both solvents, as summarized in **Table 1**. Absorbances were recorded from 200 to 650 nm, with data collected at 30 intervals.

The PLS model was developed using the Unscrambler® software. The root mean square error (RMSE) for each technique was computed by comparing the predicted concentrations of the compounds to the actual values observed in the validation samples.

PLS model development

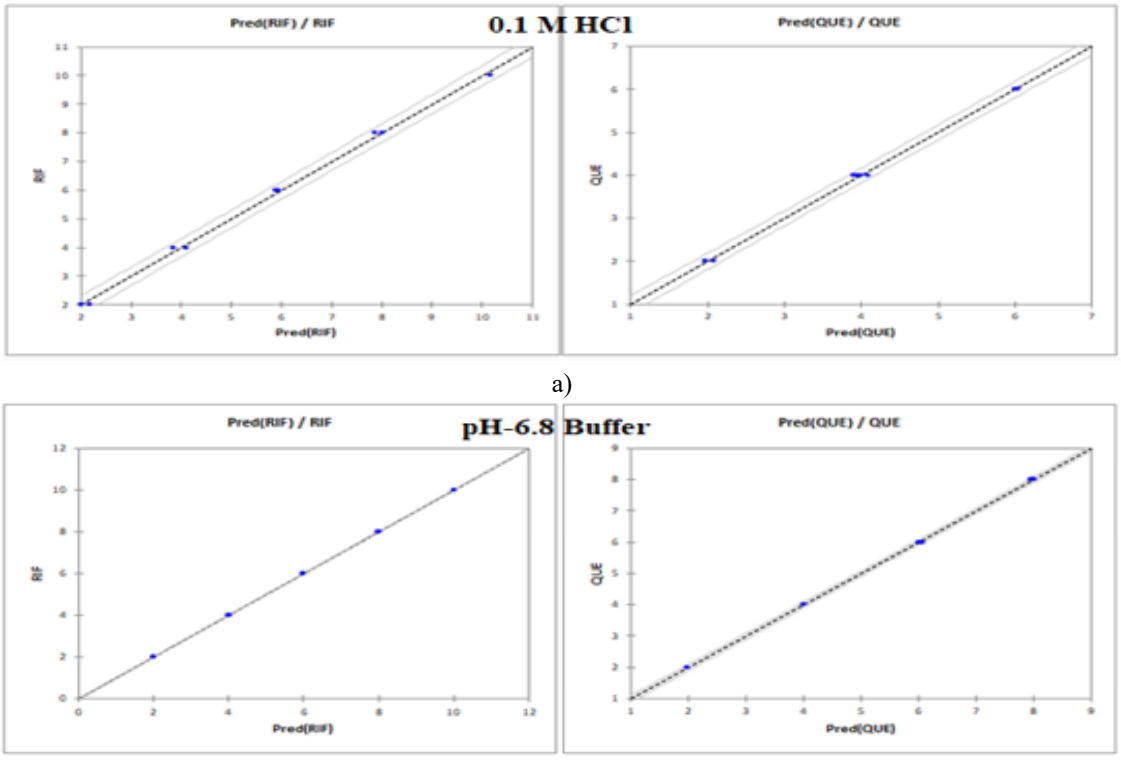
To ensure accurate quantification, the PLS calibration models were carefully constructed. Validation of the models involved predicting the concentrations of compounds in an independent set of samples, which were not part of the training data. The results of the prediction accuracy and recovery percentages are summarized in **Table 2**.

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

Conc. expected (µg/ml)		Conc. predicted (µg/ml)		Recovery %		Conc. residual (E-P) (µg/ml)	
RIF	**QUE**	**RIF**	**QUE**	**RIF**	**QUE**	**RIF**	**QUE**
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 = 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

Figure 2 illustrates the plot comparing the actual concentrations with the predicted concentrations. This graph was used to assess the performance of the models by correlating the predicted values with the true concentrations. As observed, there was a strong alignment between the predicted (calculated) and actual concentrations of the drugs.

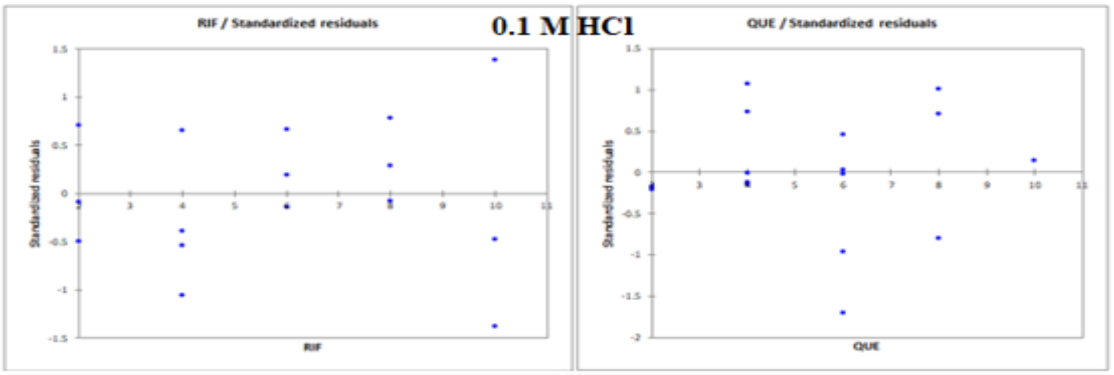
For both RIF and QUE, the relative standard deviation and average recovery values for the proposed methods were determined and are presented in **Table 3**. The PLS-optimized models yielded satisfactory correlation coefficients (r^2) for each component in the validation dataset, confirming that the models had a robust predictive capability. Another diagnostic evaluation involved plotting the predicted concentration against the residual concentrations, which showed a random scatter around zero, indicating that the model had been appropriately developed **(Figure 3)**. The statistical parameters RMSEC and RMSEV exhibited low values, further supporting the accuracy and precision of the proposed method. In addition, several analytical figures of merit, such as sensitivity and detection limits, were computed according to the IUPAC technical report, and these values are summarized in **Table 3**.



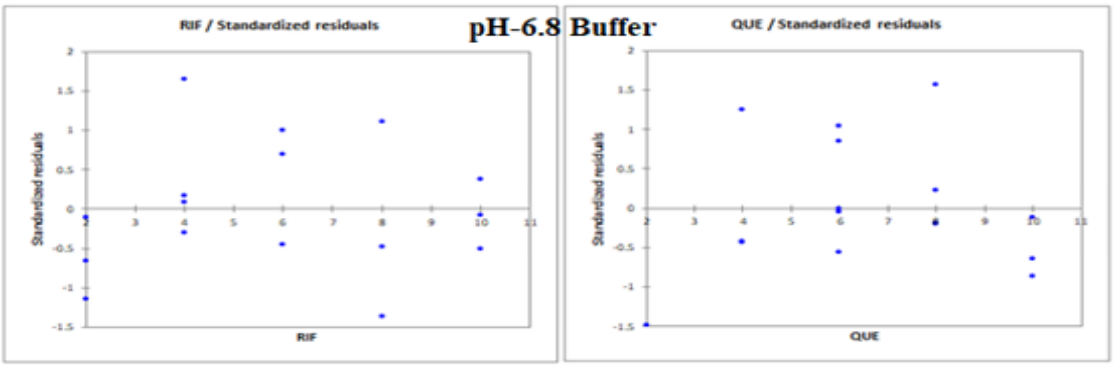
a)

b)

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



a)



b)

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 illustrates the statistical parameters of the calibration and validation set.

Table 3. Statistical parameters for the PLS method

Parameters	HCl (0.1 M)		Phosphate buffer (pH-6.8)	
	RIF	**QUE**	**RIF**	**QUE**
Range (µg/ml)	2-10			
Spectrum range	200 – 650 nm			
Δλ (nm)	15			
% 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
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
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

Liqui-solid dosage form analysis

The analysis of the liqui-solid dosage form, containing 150 mg each of RIF and QUE, was carried out using the chemometric-enhanced UV spectroscopic method developed in this study. The mean recovery rates for RIF and QUE in 0.1 M HCl were found to be 96.89% and 97.15%, respectively, in line with the label claims. The techniques developed here provide a significant improvement over traditional methods. They offer distinct advantages by simplifying the quality control process, routine analysis, and the testing of tablet formulations containing both drugs, while also being more cost-effective.

Conclusion

Traditional UV spectroscopic methods are not suitable for combination drugs with small differences in their maximum absorbance. In such cases, chemometric techniques, such as PLS, offer a viable alternative to more complex methods like HPTLC and HPLC. Once the calibration matrix is prepared and stored in the data processing system, samples can be prepared, diluted, and their absorbance recorded to determine the concentration. The chemometric PLS method has proven effective in the development of pharmaceutical formulations and in simultaneously quantifying RIF and QUE in laboratory mixtures. This approach can also be applied in dissolution studies. The primary advantages of these methods include their speed, cost-efficiency, and ability to analyze the pharmaceutical mixture simultaneously without requiring pre-treatment steps.

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Conflict of Interest: None

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Ethics Statement: None

References

1. WHO. Global tuberculosis report. 2021. Available from: https://www.who.int/publications/i/item/9789240037021
2. Wu IL, Chitnis AS, Jaganath D. A narrative review of tuberculosis in the United States among persons aged 65 years and older. J Clin Tuberc Other Mycobact Dis. 2022;28(9):100321.
3. Xin H, Zhang H, Liu J, Pan S, Li X, Cao X, et al. Mycobacterium tuberculosis infection among the elderly in 20 486 rural residents aged 50-70 years in Zhongmu county, China. Clin Microbiol Infect. 2019;25(9):1120-6.
4. Yogo N, Furukawa C, Hayano S. Paediatric progressive primary tuberculosis. J Clin Tuberc Other Mycobact Dis. 2022;28(367):100318.
5. Sanni S, Wachinou AP, Colette Merle CS, Bekou KW, Esse M, Gossa S, et al. Hepatic safety of high-dose rifampicin for tuberculosis treatment in TB/HIV Co-infected patients: a randomized clinical trial. Arch Pharm Pract. 2021;12(3):66-72.
6. Waggiallah HA, Eltayeb LB, BinShaya AS, Elmahi OM. A saga of Hepcidin anti-microbial effectiveness as iron acquisitor and anemia initiator in mycobacterium tuberculosis infection. J Biochem Technol. 2020;11(2):128-34.
7. Shahsavani M, Baghbani-Arani F, Sheikhpour M. The expression profile evaluation of Mir-125b in tuberculosis and non-small cell lung cancer patients. Clin Cancer Investig J. 2021;10(2):60-4.
8. Khawbung JL, Nath D, Chakraborty S. Drug resistant tuberculosis: a review. Comp Immunol Microbiol Infect Dis. 2021;74:101574.
9. Kulkarni D, Surwase S, Musale S, Giram P. Current trends on herbal bioenhancers. Drug Deliv Technol. 2022:275.
10. Shenoy AM, Mahurkar N. The mechanism of actions for herbal bioenhancers. RGUHS J Pharm Sci. 2021;11(2):1-4.
11. Prasad R, Singh A, Gupta N. Role of bioenhancers in tuberculosis. Int J Health Sci Res. 2016;6(6):307-13.
12. Shamama J, Ahsan W, Kohli K. The concept of bioenhancers in bioavailability enhancement of drugs: a patent review. J Sci Lett. 2016;1(3):143-65.
13. Dhanlaxmi P, Patel AB, Jagdish K. Role of Quercetin as an effective bioenhancer in Curcumin absorption, in vitro study. Res J Pharm Tech. 2022;15(11):4867-70.
14. Li H, Li M, Fu J, Ao H, Wang W. Enhancement of oral bioavailability of Quercetin by metabolic inhibitory Nanosuspensions compared to conventional Nanosuspensions. Drug Deliv. 2021;28(1):1226-36.
15. Butov D, Zaitseva S, Butov T. Efficacy and safety of Quercetin and polyvinylpyrrolidone in treatment of patients with newly diagnosed destructive pulmonary tuberculosis in comparison with standard antimycobacterial therapy. Int J Mycobacteriol. 2016;5(4):446-53.
16. Khan MF, Shamina AR, Kayser MS. Theoretically guided analytical method development and validation for the estimation of rifampicin in a mixture of isoniazid and pyrazinamide by UV spectrophotometer. Front Chem. 2017;5(6):1-12.
17. Swamy N, Basavaiah K, Vamshikrishna P. Stability-indicating UV-spectrophotometric assay of Rifampicin. Insight Pharm Sci. 2018;8(1):1-12.
18. Shah PA, Thakkar VT, Pandya TV. Development and validation of HPLC method for simultaneous estimation of Rifampicin and Ofloxacin using experimental design. J Taibah Univ Sci. 2019;13(1):146-54.
19. Khatak S, Khatak M, Rathi A. Development and validation of a RP-HPLC method for simultaneous estimation of antitubercular drugs in solid lipid nanoparticles. Indian J Pharm Sci. 2018;80(6):996-1002.

20. Shah U, Patel S, Raval M. Stability indicating reverse phase HPLC method for estimation of Rifampicin and Piperine in pharmaceutical dosage form. Curr Drug Discov Technol. 2018;15(1):54-64.
21. Strock J, Nguyen M, Sherma J. Transfer of minilab TLC screening methods to quantitative HPTLC-densitometry for pyrazinamide, ethambutol, isoniazid, and Rifampicin in a combination tablet. J Liq Chrom Rel Tech. 2015;38(11):1126-30.
22. Jadhav SG, Viswanathan V, Mukne AP. Validated HPTLC method for simultaneous quantification of isoniazid, Rifampicin and glabridin. J Pharm Biomed Sci. 2016;6(7):453-9.
23. Yue LM, Yu JC, Ding RW, Ping C. HPLC determination of Quercetin in three plant drugs from genus sedum and conjecture of the best harvest time. Pharmacogn J. 2017;9(6):725-8.
24. Jain V, Shaikh M. Simultaneous RP-HPLC analysis of Quercetin and kaempferol in different plant parts of cissus quadrangularis. Int J Pharm Sci. 2016;8(7):138-42.
25. Sahani S, Jain V. Novel RP-HPLC method for simultaneous estimation of berberine, Quercetin, and Piperine in an ayurvedic formulation. Int J Appl Pharm. 2019;11(1):94-9.
26. Baghel US, Nagar A, Pannu MS, Yadav R. HPLC and HPTLC methods for simultaneous estimation of Quercetin and Curcumin in polyherbal formulation. Indian J Pharm Sci. 2017;79(2):197-203.
27. Patil V, Angadi S, Devdhe S. Determination of Quercetin by UV spectroscopy as quality control parameter in herbal plant: cocculus hirsutus. J Chem Pharm Res. 2015;7(1):99-104.
28. Packia NCJ, Viveka S, Mini ST, Jeeva S. High performance thin layer chromatography profile of Quercetin in three cultivars of Allium cepa and its antimicrobial activity against bacterial cultures. Asian J Pharm Clin Res. 2015;8(3):213-8.
29. Dhar BL, Kumar RK, Manosi D, Jayram H. HPTLC method for quantitative determination of Quercetin in a polyherbal compound for urolithiasis. Int J Pharmacogn Phytochem Res. 2016;8(7):1187-90.
30. Doshi GM, Une HD. Quantification of Quercetin and rutin from Benincasa hispida seeds and Carissa congesta roots by high-performance thin layer chromatography and high-performance liquid chromatography. Pharmacogn Res. 2016;8(1):37-42.
31. Patel AA, Amin AA, Patwari AH, Shah MB. Validated high performance thin layer chromatography method for simultaneous determination of Quercetin and Gallic acid in Leea indica. Braz J Pharmacogn. 2017;27(1):50-3.
32. Shah UH, Patel S, Raval M. Chemometric assisted spectrophotometric methods for the simultaneous determination of Rifampicin and Piperine in bulk and capsule. Indian J Pharm Educ. 2015;49(3):200-7.
33. Shah PS, Patel KG, Shah PA. UV-spectrophotometry-assisted chemometric methods for simultaneous determination of Ambroxol hydrochloride and Doxofylline in pharmaceutical formulation. J Chem Metrol. 2020;14(2):106-13.
34. ICH. Validation of analytical procedure: methodology (Q2R1). Proceeding of the international conference on harmonization, November 2005, food and drug administration, USA; 2005.