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Development and Validation of UV Spectroscopic Method for Simultaneous Estimation of Berberine and Resveratrol

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ABSTRACT:

Simple spectrophotometric methods have been developed for simultaneous estimation of Berberine and resveratrol and dosage form. Method is a simultaneous estimation method in which absorbance is measured at two wavelengths, 422 nm at which Berberine and 310 nm at resveratrol which both the drugs have considerable absorbance. Berberine and resveratrol stock standard solutions were divided into aliquots and diluted with chloroform to provide a series of concentrations of 5–25µg/ml and 10–50µg/ml, respectively. The accuracy and precision were determined and found to comply with ICH guidelines. The methods showed good reproducibility and recovery with % RSD in the desired range. The methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of Berberine and resveratrol drug in their dosage form.

Keywords: Berberine, Resveratrol, UV Spectroscopic Method, Validation

INTRODUCTION

The simultaneous estimation of multiple active pharmaceutical ingredients (APIs) in combined dosage forms has gained significant importance in modern analytical chemistry. Accurate and precise quantification of each component is essential for ensuring the efficacy, safety, and quality of pharmaceutical formulations. Among various analytical techniques, UV–Visible spectrophotometry remains one of the most widely used methods due to its simplicity, cost-effectiveness, sensitivity, and reliability for routine quality control analysis. [1, 2]

Berberine is a natural isoquinoline alkaloid obtained from various medicinal plants such as *Berberis aristata*, *Coptis chinensis*, and *Hydrastis canadensis*. It possesses a broad spectrum of pharmacological activities, including antidiabetic, antimicrobial, anti-inflammatory, and cardioprotective effects. [3, 4] Resveratrol, a polyphenolic stilbene compound primarily found in grapes, berries, and peanuts, exhibits potent antioxidant, anti-inflammatory, cardioprotective, and anticancer properties. Owing to their complementary therapeutic benefits, formulations containing both berberine and resveratrol have attracted increasing interest for synergistic effects in metabolic and cardiovascular disorders. [5-8]

However, due to the overlapping absorption spectra of these compounds, their simultaneous estimation poses analytical challenges. Therefore, the development of a simple, rapid, accurate, and validated UV-spectroscopic method for the simultaneous quantification of berberine and resveratrol in bulk and pharmaceutical dosage form is essential. [9, 10] Such a method would provide an efficient tool for quality control and routine analysis in pharmaceutical industries. [11] The present study aims to develop and validate a UV-spectrophotometric method based on simultaneous equation or derivative spectroscopic techniques for the estimation of berberine and resveratrol in bulk and combined dosage formulations in accordance with ICH guidelines.

MATERIALS AND METHODS

Materials: The instruments used in the present study included an electronic balance for accurate weighing of samples, a Hamilton syringe (20 µL) for precise volumetric measurements, and a pH analyzer for determining the pH of various solutions. UV-Visible absorbance measurements were carried out using an Elico SL-164 UV-VIS spectrophotometer, and an ultra sonicator was employed to ensure complete dissolution and homogenization of the samples. The solvents and chemicals utilized in the study comprised berberine and resveratrol working solutions as standard reference compounds. Analytical reagent (AR) grade solvents such as chloroform, dichloromethane, and methanol were used throughout the analysis. Distilled water was employed for the preparation of

various solutions and dilutions. All chemicals and reagents used were of analytical grade to ensure reliability and accuracy of the results.

Analysis of the spectra and choice of the wave length: The aliquot portion of the standard stock solutions of berberine and resveratrol was appropriately diluted with chloroform to obtain 10 µg/ml of each, and the solutions were scanned in a 1.0 cm cell between 200 and 400 nm against chloroform as a blank. The λ max for berberine and resveratrol was discovered to be 422 nm and 310 nm, respectively. [12]

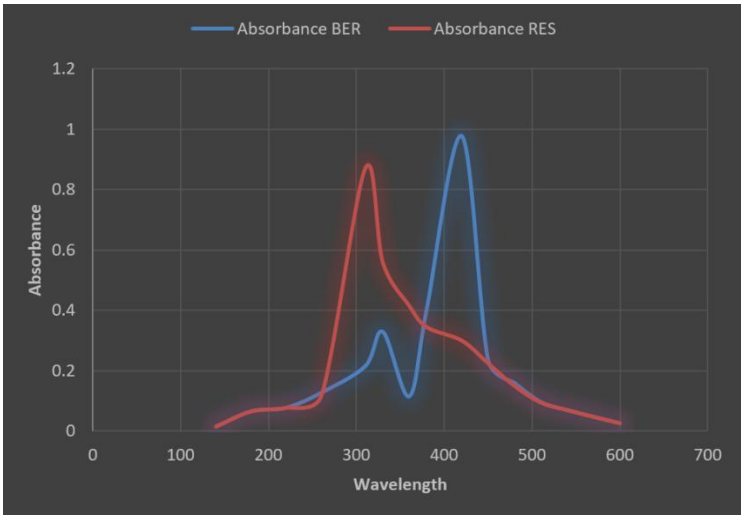

Figure 1: UV Spectra of Berberine and Resveratrol

Standard Solution: A standard stock solution of berberine was prepared by accurately weighing 100 mg of berberine and dissolving it in chloroform, followed by dilution to 100 ml with the same solvent to obtain a concentration of 1000 µg/ml. Similarly, a standard stock solution of resveratrol was prepared by accurately weighing 100 mg of resveratrol, dissolving it in chloroform, and making up the volume to 100 ml with the same solvent to achieve a concentration of 1000 µg/ml. To study Beer-Lambert's law, aliquots of the standard stock solutions of berberine and resveratrol were suitably diluted to obtain concentration ranges of 5–25 µg/ml and 10–50 µg/ml, respectively. The absorbance of these dilutions was measured at 422 nm for berberine and 310 nm for resveratrol using UV-Visible spectrophotometry. [13]

Amount and Concentration of Absorptive Value in Pure Drug:

By Simultaneous Equation Method (or) Vierodt's Method: Berberine and resveratrol were tested for their absorbance at 422 nm and 310 nm, respectively, and calibration curves were constructed. Then, ax_1, ax_2, ay_1 and ay_2 determine the absorptive values for the medications. The absorbance of the sample combination was measured at A_1 and A_2 and recorded as A_1 and A_2 by the following equation. Berberine and resveratrol pure drug samples' absorbance graphs were provided. [14]

$$C_x = A_1ax_2\text{-}A_2ax_1/ax_2ay_1\text{-}ax_1ay_2 \quad \text{.....1}$$

$$C_y = A_2ax_1\text{-}A_1ax_2/ax_2ay_1\text{-}ax_1ay_2 \quad \text{.....2}$$

Where,

ax_1 = the absorptivity valve of Berberine at 422 nm.

ax_2 = the absorptivity valve of berberine at 310 nm.

Analysis Of Formulation: Weighed and powdered twenty pills. In a 100 ml volumetric flask, 500 mg of berberine and 200 mg of resveratrol were combined, then they were dissolved in chloroform and subjected to a 10-minute sonication process. The volume was then adjusted to the proper level using the same solvent. The final product was filtered through Whatman filter paper grade I, and to create a solution with a about 20 µg/ml berberine concentration, 1 ml of the filtrate was taken and diluted to 100 ml. Two

medicines' concentrations in the sample were calculated using a simultaneous equation after absorbances of a 10 µg/ml sample solution were measured at 422 & 310 nm. [15]

Table 1: Analysis of Formulation

Form.	"Label Claim"	"Amount Found% RSD*"	"% Assay RSD*"
Inzufast	Berberine 500mg	496.6	99.2
	Resveratrol 200mg	197.5	98.75

* RSD for Three Determinations

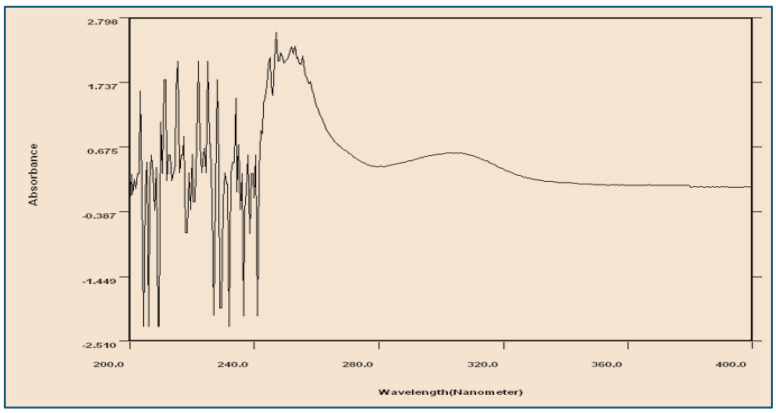

Figure 2: Berberine 10 µg/ml in Formulation (Inzufast)

Validation of Method: The created method's precision, accuracy, ruggedness and stability were all verified. In table 2, the validation parameters are displayed. [16]

Table. 2: Validation Parameters

Parameters	Berberine 422nm	Resveratrol 310 nm
"Linearity range (µg/ml)"	"5-25"	"10-50"
"Coefficient correlation"	.998	.997
"Slope"	.036	.018
"Intercept"	.012	.008
Repeatability (% RSD)	0.41-1.56	0.21-76
Intraday Precision	0.14-1.07	0.26-1.09
Interday precision	0.24-0.89	0.23-1.04
Standard error	0.000324322	0.000321336

Linearity and Range: Berberine and resveratrol stock standard solutions were divided into aliquots and diluted with chloroform to provide a series of concentrations of 5–25µg/ml and 10–50µg/ml, respectively. A standard graph was constructed by placing drug concentration on the x-axis and absorbance on the y-axis to create a Beer's law plot for berberine and resveratrol. The medicine complied with Beer's law. At 422 nm and 310 nm, respectively, the regression coefficients for berberine and resveratrol were reported to be 0.998 and 0.997. [17]

Table 3: Linearity and Range

S. No.	Conc. µg/ml		Abs. at	
	Berberine	Resveratrol	Berberine 422nm	Resveratrol 310 nm
1	5	10	0.234	0.167
2	10	20	0.444	0.350
3	15	30	0.612	0.486
4	20	40	0.784	0.654
5	25	50	0.997	0.843

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Accuracy: Recovery trials were conveyed out by including renowned quantities of pharmaceuticals to pre-analyzed samples at 3 levels, and the % recovery was computed in order to determine the precision and reproducibility of the suggested procedures. Table 4 provides a summary of the findings. The following formula was used to compute the percentage of recovery. [18]

$$\%\text{Recovery} = \frac{\text{Amount of drug found after addition of standard drug} - \text{Amount of drug found in sample before addition of standard drug}}{\text{Amount of standard drug added}} \times 100$$

Table 4: Recovery Studies

Rec. Lev.		"Amt. of drug added (mg)"		"Amt. of drug found (mg)"		"Mean"		"% Recovery * ±%RSD"	
		BER	RES	BER	RES	BER	RES	BER	RES
Lev. 1	80%	40	20	495.5	197.6	496.74	197.6	99.7±0.66	99.7±1.56
				498.3	195.7				
				496.4	193.6				
Lev. 2	100%	50	25	500.1	200.3	500.5	200.5	100.1±2.32	100±0.98
				500.5	200.7				
				500.6	200.5				
Lev. 3	120%	60	30	524.7	212.6	525.6	211.6	102.7±1.86	102.6±0.66
				526.8	211.6				
				528.6	215.7				

* RSD for three Determinations; BER=Berberine, RES=Resveratrol

Precision: The inaccuracy of a single determination is determined by precision and accuracy taken together. They rank among the most crucial standards for evaluating the effectiveness of analytical techniques. [17]

Repeatability of measurements inside a set or the spread of a set's dispersion around its core value are both examples of precision. A number (n) of independently replicated measurements of a particular property are referred to as a set in scientific terminology; the standard deviation of a population of observations is one of the most often used statistical phrases and the square root of the sum of the squares of deviations for each individual result from the mean, divided by one less than the total number of outcomes in the collection, is the standard deviation. The formula for the standard deviation S is

$$S = \sqrt{\frac{1}{n-1}\sum_{i-1}^{n}(x_i - \bar{x})^2}$$

The units of SD are the same as the unit of measurement.

Variance (S2) is the SD squared. The SD that is stated as a percentage of the mean is called the relative standard deviation, which is six. It is sometimes stated as a percent relative standard deviation and multiplied by 100. It becomes a more trustworthy way to represent accuracy.

$$\%\text{ Relative standard deviation} = S \times 100 / x$$

Table 5: Repeatability studies for Berberine

Conc. in µg/ml	Abs	%RSD
1	0.231	1.56
	0.223	
	0.229	
	0.232	
	0.221	
	0.223	
5	0.938	0.43
	0.945	
	0.958	
	0.976	
	0.987	
	0.996	

Table 6: Repeatability studies for Resveratrol

Conce. in µg/ml	Abs	%RSD
1	0.198	1.34
	0.199	
	0.201	
	0.199	
	0.198	
	0.201	
5	0.890	0.39
	0.902	
	0.900	
	0.889	
	0.902	
	0.903	

Table 7: Inter-day precision for Berberine

Concentration in µg/ml	Day	Abs	%RSD
1	1	0.212	1.44
	2	0.211	
	3	0.213	
	4	0.211	
	5	0.214	
	6	0.211	
5	1	0.899	0.36
	2	0.903	
	3	0.902	
	4	0.903	
	5	0.899	
	6	0.903	

Table 8: Inter-day precision for Resveratrol

Conc. in µg/ml	Day	Abs.	%RSD
1	1	0.189	0.67
	2	0.191	
	3	0.189	
	4	0.191	
	5	0.192	
	6	0.194	
5	1	0.885	0.35
	2	0.886	
	3	0.897	
	4	0.886	
	5	0.869	
	6	0.884	

Table 9: Intra-day precision for Berberine

Conce. in µg/ml	Day (6 times)	Abs.	%RSD
1	1	0.202	0.78
	2	0.201	
	3	0.201	
	4	0.199	
	5	0.202	
	6	0.198	
5	1	0.898	0.41
	2	0.899	
	3	0.887	
	4	0.904	
	5	0.897	
	6	0.889	

Table 10: Intra-day precision for Resveratrol

Conc. in µg/ml	Day (6 times)	Abs.	%RSD
1	1	0.184	0.56
	2	0.185	
	3	0.186	
	4	0.185	
	5	0.187	
	6	0.183	
5	1	0.903	0.47
	2	0.902	
	3	0.904	
	4	0.903	
	5	0.901	
	6	0.904	

Ruggedness: The analysis of the formulation performed using various analyzers tools and revealed the tenacity of the approach. RSD's total and percent were computed. [18]

Table 11: Ruggedness analysis of formulation

Drug	Condition	% obtained	S.D	%RSD	S.E
BER	"Analyst 1 Analyst 2"	99.8766 99.9976	1.1677 1.0857	1.1542 1.1456	0.0321 0.0325
	"Instrument 1 Instrument 2"	99.9887 99.9964	0.9865 1.0533	0.9463 1.1032	0.0149 0.0353
RES	"Analyst 1 Analyst 2"	98.9865 98.6322	0.9865 0.9586	0.9965 0.9075	0.0245 0.0165
	"Instrument 1 Instrument 2"	98.6433 98.9993	0.5604 0.4573	0.4986 0.4874	0.0145 0.0217

For BER and RES, the % RSD was determined to be 1.1542 and 0.9965, respectively. The low % RSD demonstrated that there was no interference from formulation excipients. Consequently, the method's correctness was verified.

Stability: Studies on the medication solution's stability were conducted. At room temperature, the drug solution was discovered to be stable, and the results are displayed in the table 14. [19]

Table 12: Stability Study

Drug Conc. (µg/ml)		Time (Min)	Abs.	
BER	RES		BER	RES
10	10	30	0.221	0.189
		60	0.225	0.194
		90	0.227	0.196
		129	0.224	0.195
		150	0.222	0.193

3. RESULTS AND DISCUSSION

Compared to separately estimating the two medications, two pharmaceuticals the simultaneous estimation of in a formulation offers better advantages in terms of accuracy, reagent usage, and processing time. New, simple, exact, and accurate analytical techniques were developed for the following combinations in order to validate the procedures in compliance with ICH regulations and apply them for its estimation in marketed formulations. The λmax of BER and RES was discovered to be 422 nm and 310 nm using the optical properties of the proposed approaches. From the results in table 3, it can be inferred that BER and RES obey linearity within the conc. ranges of 5–25µg/ml and 10–50µg/ml, respectively. The calibration curve demonstrated linearity, and the correlation coefficient (r^2) values for BER and RES, respectively, are 0.998 and 0.999. It was discovered from table 4 that the% RSD is less than 2%, proving the method's high repeatability. The fact that the percentage recovery values of pure medication from the pre-analyzed formulations ranged from 99 to 103% shows that the suggested approach is accurate and that the additives and excipients that are frequently employed in formulations were not taken into account in the proposed method. Table 2 summarizes the findings of the validation parameters for the created methods. The formulation study produced positive results with concentrations between 98 and 101%.

CONCLUSION

The majority of the work involved in developing a UV spectroscopic method should focus on method development and optimization, as these steps significantly influence the overall performance and reliability of the final analytical procedure. A well-developed method is simple to validate and can be effectively applied for the rapid preparation and analysis of preclinical samples, formulation prototypes, and commercial dosage forms. A review of the available literature on berberine (BER) and resveratrol (RES) revealed that no UV-spectrophotometric method has been reported for their simultaneous estimation and validation in bulk and pharmaceutical dosage forms. However, several analytical methods exist for the individual determination of BER and RES, or in combination with other drugs.

The analytical method developed in the present study is specific, accurate, linear, and system-appropriate for the simultaneous determination of BER and RES by UV-spectroscopy in bulk and dosage forms. The method was validated with respect to key parameters such as linearity (correlation coefficient), accuracy, and precision (expressed as %RSD), in accordance with ICH guidelines. The developed method was successfully applied for the analysis of pharmaceutical formulations, and the results obtained for the validation parameters were found to be within the acceptable limits as per Beer's law, ICH, FDA, and USP standards.

REFERENCES

1. Book of Pharmaceutical analysis, Beckett & Stenlake, Fourth Edition
2. Mehta Kalpesh K, Patel Navnik K, Ganatra Maulik H, Patel Tushar D, Patel N.J. Spectrophotometric method for formulation and process optimization of Gastro Retentive floating tablets of Ondansetron HCl. JPRHC Research article at www.jprhc.in.
3. Singh IP, Mahajan S. Berberine and its derivatives: a patent review (2009 – 2012). Expert Opinion on Therapeutic Patents. 2012; 23: 215–231: doi.org/10.1517/13543776.2013.746314.
4. Singh V, Chauhan I, Majhi S, Verma M, Yasir M. Development and Validation of UV-Spectrophotometric Method for Berberine Quantification. Journal of Applied Pharmaceutical Sciences and Research. 2024; 7(2):28-33 Doi: 10.31069/japsr.v7i2.05
5. Devi Violina, Bhupen Kalita. Simultaneous UV Spectrophotometric Estimation and Validation of Rutin and resveratrol in combined dosage form by Iso absorptive point method. Asian J. Pharm. Ana. 2018; 8(3): 147-152. doi: 10.5958/2231-5675.2018.00027.3
6. Iacopini P, Baldi M, Storchi P and Sebastiani. Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, in vitro antioxidant activity and interactions. Journal of Food Composition and Analysis. 2008; 21: 589-598.
7. Dar MA and Tabassum N. Rutin- potent natural thrombolytic agent. International Current Pharmaceutical Journal. 2012; 1(12): 431-435.
8. Shindikar A, Singh A, Nobre M and Kirolikar S. Curcumin and Resveratrol as Promising Natural Remedies with Nanomedicine Approach for the Effective Treatment of Triple Negative Breast Cancer. Journal of Oncology. 2016; 1-13.
9. Yeole P, Wadher S, Puranik M, Raval P. A validated HPTLC method for determination of ondansetron in combination with omeprazole or rabeprazole in solid dosage form. Asian J. Research Chem. 2020; 2(2): 20.
10. Ravikumar P, Muralikrishna M, Bhanuprakash P, Anilkumar B and Madhusudhan P. Derivative spectrophotometric Estimation of Ondansetron and Paracetamol. E.J. Chem., 2010; 7(1): 260- 264,
11. Saif MJ and Anwar J. A new spectrophotometric method for the determination of promethazine–HCl from pure and pharmaceutical preparations. Pharmazie. 2008; 63(8): 562-7
12. Chitlange Sohan S, Soni R, Wankhede SB, Amol A and Kulkarni P. Spectrophotometric Methods for Simultaneous Estimation of Dexibuprofen an Paracetamol. Journal of Pharmaceutical and Biomedical Analysis 2006; 41(1): 286–89
13. Pillai S and Singhvi. Spectrophotometric simultaneous estimation of ranitidine hydrochloride and ondansetron hydrochloride from tablet formulation. Scientific Publication of the Indian Pharmaceutical Association. 2007 69(4): 601-604
14. Sharma S, Goyal S and Chauhan K. A review on analytical method development and validation. Int J App Pharm, 2018; 10(6): 8-15.
15. Vichare V S, Choudhari V P and Venkat Reddy M. Development and Validation of UV-Visible Spectroscopic Methods for Simultaneous Estimation of Canagliflozin and Metformin in Pharmaceutical Formulation. Asian J. Research Chem. 2019; 12(1): 16-20. doi: 10.5958/0974-4150.2019.00004.X
16. Ramole R, Mohini B and Jain A. A Review: Analytical Method Development and Validation. Sys Rev Pharm 2021; 12(8): 450-454
17. Bijithra C, Ragan G and Shanmugasundaram P. Analytical Method Development and Validation of Modafinil in Pure and Tablet Dosage Form by UV Spectroscopy. Research J. Pharm. and Tech 2016; 9(8):1303-1308. doi: 10.5958/0974-360X.2016.00202.X
18. Nath L, Laldinchhana, Choudhury Abhijit D, Barakoti H and Devi Chanam M. Development and Validation of UV-Vis Spectrophotometric Method for Estimation of Amphotericin B. Research J. Pharm. and Tech. 2020; 13(1):55-59. doi: 10.5958/0974-360X.2020.00009.8
19. Ishaq BM, Ahad Hindustan A, Muneer S, Parveen S and Fahmida B. Analytical method development and validation for the estimation of Temozolomide in phosphate buffer pH 2.0 as a solvent by UV spectroscopy. Int. Res. J. Pharm. 2014; 5(1):17-20 http://dx.doi.org/10.7897/2230-8407.050104

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