Original Article

Development And Validation Of Simultaneous Uv-Spectrophotometric Method For The Determination Of Resveratrol And Piperine In Pharmaceutical Dosage Form

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Abstract

The purpose of current study is the development of a simple, rapid and precise UV-Visible spectrophotometric method for the simultaneous estimation of Resveratrol (RVT) and Piperine (PIP) present in the marketed formulation by using simultaneous equation. Methanol was used as a solvent for this study. The absorbances of the drugs were measured at their absorbance maxima (λmax), 310 nm for Resveratrol and 340 nm for Piperine. Calibration curve plotted in concentration range 10-50 µg/ml shows an excellent linear relationship for both Resveratrol and Piperine and follws Beers law. The method validation results found to obey all the validation parameters as per the ICH guideline, such as accuracy, precision, linearity and ruggedness. The method exhibits good reproducibility and recovery with % RSD in the favorable range indicating the sensitivity of the method towards analyte. This method can be effectively employed for the day to day analysis of both the drugs in the capsule dosage form.

KEYWORDS: Simultaneous equation method, Spectrophotometric, Validation, Resveratrol, Piperine

INTRODUCTION:

Resveratrol (RVT), a naturally occurring polyphenol. Structurally, Resveratrol is Trans-3,4′,5-trihydroxystilbene. The main sources of RVT includes, grapes (Vitis vinifera L.), a variety of berries, peanuts, medicinal plants such as Japanese knotweed and red wine.

It has anti-aging effects in animals and also potent antioxidant and anti-inflammatory effects, promotes vascular endothelial function, and has anticancer activity[1,2]. Since Resveratrol is present in wine, it has been postulated that it might be the reason for the "French Paradox," the epidemiological phenomenon in which the French population has a significantly lower incidence of cardiovascular disease, even though the French consume a diet higher in fat than other populations. The structure of Resveratrol was as shown below in figure-1.

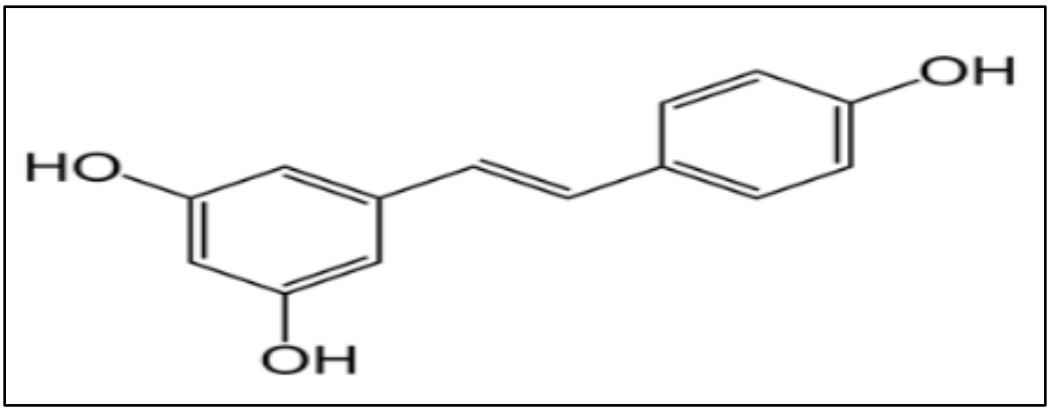

Fig. 1: Structure of Resveratrol

Piperine (PIP) is a major alkaloid isolated from Piper nigrum L. (Piperaceae). Structurally, Piperine is 1-[(2E,4E)-5-(1,3- Benzodioxol-5-yl)-1-oxo-2,4-pentadieneyl] piperidine[3]. The most interesting point is that Piperine increases the bioavailability of a number of therapeutic drugs, as well as phytochemicals, including Curcumin[4]

Piperine has several reported pharmacological activities like central nervous system depressant, antipyretic, analgesic, hepatoprotective, antioxidant, and anti-inflammatory[5]. It is also listed by the USFDA as a GRAS molecule[6]. The structure of Piperine is as shown in figure-2

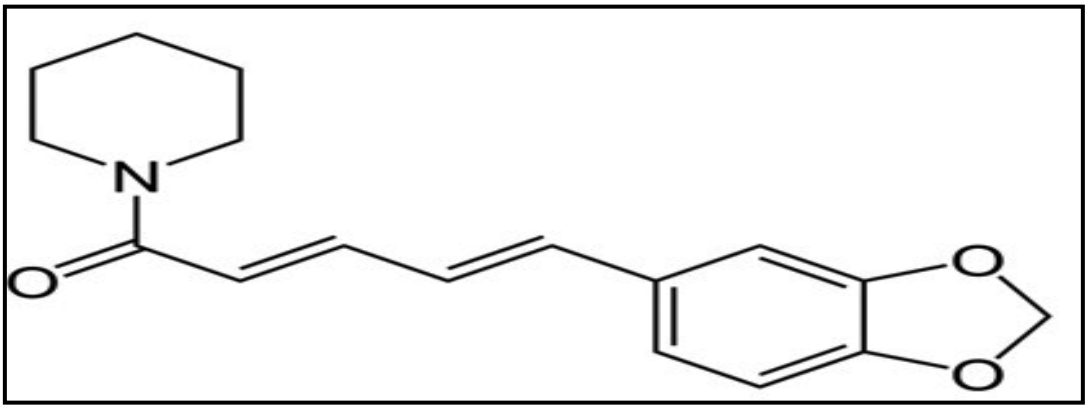

Fig. 2: Structure of Piperine

In this study, efforts were made to develop a simple, easy and economic UV spectrophotometric method for the determination of Resveratrol and Piperine. The developed method was then optimized and validated as per the International Conference on Harmonization (ICH) guidelines and established excellent specificity, linearity, precision and accuracy for Resveratrol and Piperine.[7]

The literature review showed there are some HPLC methods [8,9,10], UV and spectrophotometric methods [2,11], HPTLC[12] method for the estimation of Resveratrol and Piperine.

Resveratrol when used in combination with Piperine successfully reduces some measures of morbidity with little or no effect on mortality associated with Systemic lupus erythematosus (SLE) is a chronic multi-system inflammatory disease associated with autoantibody formation[13]. Piperine significantly improves the in vivo bioavailability of Resveratrol[14].

MATERIALS AND METHOD:

Instrumentation:

The instrument used to measure the absorbance of the working solutions was a UV-VIS Double beam spectrophotometer [Model: Shimadzu 1800]. Digital analytical balance [Model: PGB 600] and Equitron ultra sonicator were used in the study.

Chemical and Reagents:

Resveratrol was procured from Fine chem. labs, Mumbai and Piperine from Sigma Aldrich, Mumbai. AR grade methanol of purity 99.80% was bought from Merck, Mumbai. Formulation of Resveratrol and Piperine were purchased from HealthyHey Foods LLP, Andheri-Mumbai.

Method Development:

Selection of Solvent:

Different solvents were used to check the solubility of RVT and PIP. Both the drugs found to be soluble in methanol. So, methanol was selected as the solvent and diluent for this work.

Preparation of Standard stock solutions:

100 mg of Resveratrol and Piperine was precisely weighed and transferred into two clean and dry 100 ml volumetric flask, methanol was added to produce 100 ml of stock solution to get a concentration of 1000 µg/ml [Stock solution-1]. Then 1 ml of the stock solution-1 was transferred into 10 ml volumetric flask, and the volume was made up to 10 ml to get a concentration of 100 µg/ml [Stock solution-2].

Preparation of Sample Solution:

The contents of twenty capsules were emptied into a mortar and finely crushed using a pestle and the weight of the powder is noted. The quantity of the powder equivalent to 100 mg of RVT was transferred to a 100 ml volumetric flask and filtered through Whatmann filter paper. Required concentration of 10 µg/ml of both the drugs was made by making necessary dilutions with methanol.

Determination of wavelength of maximum absorbance (λmax) and iso-absorptive Point:

For determination of λmax of both RVT and PIP , working solutions (40 µg/ml) were prepared and scanned in spectrum mode in the UV range of 200 nm to 400 nm. Both RVT and PIP showed the maximum absorbance at 310 nm and 340 nm respectively as shown in Fig. 3.

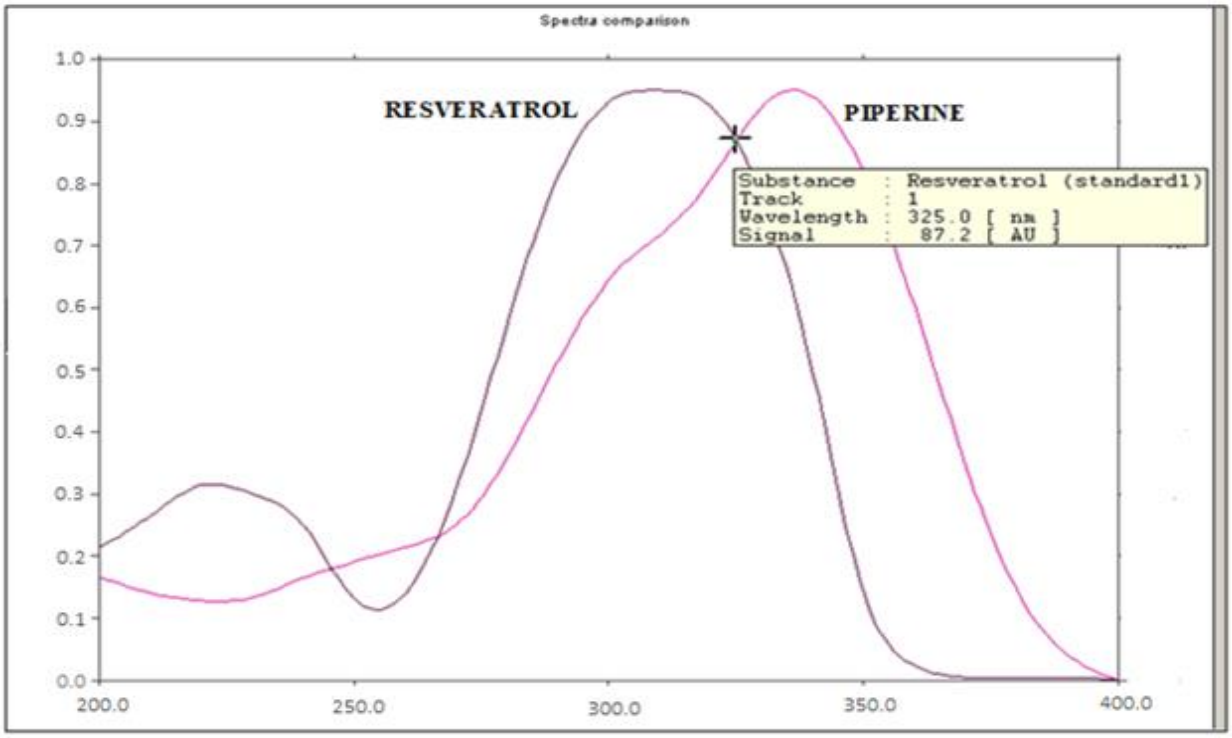

Fig. 3: Overlay spectrum of Resveratrol and Piperine

Construction of calibration curve for RVT and PIP:

Volumes of 1, 2, 3, 4 & 5 ml were pipetted out from stock solution-2 of both RVT and PIP to separate 10 ml dry and clean volumetric flasks and diluted to 10 ml to get the concentrations of 10,20,30,40 and 50 µg/ml respectively. The wavelength selected to measure the absorbance of both working standard was 310 nm and 340 nm. Methanol was used as the blank. The calibration curve for RVT and PIP were plotted by taking respective absorbance v/s concentration (µg/ml) and the regression equation was found out.

Simultaneous Equation Method:

If a sample contains two absorbing drug constituents, each of which absorbs at the λmax of the other, then it is possible to determine both drugs simultaneously using multicomponent analysis UV Spectrophotometric 'Simultaneous Equation Method'.

The research is focused on the absorption of actives Resveratrol and Piperine at their wavelength maxima in this simultaneous equation method[15]. Two chosen wavelengths for the simultaneous calculations are 310 and 340 nm. Concentrations in the sample (Cx and Cy) were obtained by using the following equations (1) and (2) by putting the values absorptivities and absorbances (A1 and A2)of sample at given wavelengths.

$$Cx = \frac{A2*ay1 - A1*ay2}{ax2*ay1 - ax1*ay2} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$Cy = \frac{A1*ax1 - A2*ax1}{ax2*ay1 - ax1*ay2} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Where,

Cx and Cy are the concentrations of Resveratrol and Piperine in µg/ml in sample solution, respectively.

A1 and A2 are the absorbances of sample solutions at 310 nm and 340 nm, respectively.

ax1 is the absorptivity of Resveratrol at 310 nm.

ax2 is the absorptivity of Resveratrol at 340 nm.

ay1 is the absorptivity of Piperine at 310 nm.

ay2 is the absorptivity of Piperine at 340 nm.

Method Validation:

The methods mentioned in this study have been validated using International Conference on Harmonization (ICH) parameters for the assay of the two active constituents of the mixture[7].

Accuracy:

Accuracy is the closeness of the measured value to the actual value. The accuracy of the method was determined by carrying out the recovery studies. The recovery tests were carried out in triplicate by spiking samples previously studied with three separate concentrations of standards.[16].

While precision is the closeness of the measurements to each other. Inter-day and intra-day precision: It was calculated by assay of sample solution on the same day and on different days at different time intervals[7].

Precision:

Precision is the closeness of the measurements to each other. Intraday and interday precision are considered for the precision studies. It was calculated by assay of sample solution on the same day and on different days at different time intervals[7].

Intraday Precision: The absorbance of the sample solutions of RVT and PIP at the concentrations of 10, 20 and 30 µg/ml was measured three times on the same day and % RSD was determined.

Interday Precision: The absorbance of the sample solutions of RVT and PIP at the concentrations of 10, 20 and 30 µg/ml was measured on three alternate days and % RSD was determined.

Linearity and Range:

Linearity is the ability of the method to elicit test results that are proportional to the concentration of the analyte in the sample. The standard solutions of five different concentrations of RVT and PIP ranging between 10-50 µg/ml was prepared to study the linearity of both the drugs. Calibration curves were constructed using the standard solutions and linear regression analysis was conducted.

Ruggedness: It is the degree of reproducibility of test results obtained by the analysis of the same samples under different conditions, such as different laboratories, different instruments or different analysts. In this work, ruggedness study is done by two different analysts.

Limit of detection (LOD) and Limit of Quantitation (LOQ):

According to ICH guidelines, the LOD of the actives were obtained by calculating the signal-to-noise ratio (S/N) i.e., 3.3 for LOD and 10 for LOQ using the following equation.

$$\text{LOD} = \frac{3.3\,\sigma}{\text{S}}$$

$$\text{LOQ} = \frac{10\,\sigma}{\text{S}}$$

Where, σ = standard deviation response and S = slope of the calibration curve.

RESULTS AND DISCUSSION:

Method development:

Determination of wavelength of maximum absorbance (λmax) and Iso-absorptive point:

RVT shows maximum absorption at a wavelength 310 nm, while PIP shows maximum absorption at a wavelength 340 nm. As per the overlay spectra (Fig.: 3), the iso-absorptive point is as 325 nm

Analysis of marketed formulation:

Analysis of marketed capsule formulation by using UV Spectrophotometric 'Simultaneous Equation Method' is carried out. The results of analysis obtained shown in Table-1.

Table 1: Drug content (assay) of Resveratrol and Piperine in marketed formulation

Marketed formulation	Name of drug	Label claim (mg)	Amount of drug found (mg)	% of drug content
	Resveratrol	250	247.31	99.85
	Piperine	10	8.90	98.84

Method validation:

Linearity and Range:

The linearity of both RVT and PIP was found to be in the range of 10-50 µg/ml. The data for linearity of RVT and PIP is shown in Table-2 and 3 respectively. The overlay spectrum is as shown in Fig. 3 and calibration curves of RVT and PIP are shown in Fig. 4 and 5 respectively.

Table 2: Linear regression analysis of Resveratrol

Drug	Concentration (µg/ml)	Absorbance	
		At 310 nm	At 340 nm
RVT	10	0.5253	0.1485
	20	0.6532	0.2796
	30	0.7901	0.4175
	40	0.9375	0.5813
	50	1.1073	0.6869
Regression Equation		y = 0.0145x + 0.3682	y = 0.0138x + 0.0092
Correlation Coefficient (R^2)		0.9969	0.9967

Table 3: Linear regression analysis of Piperine

Drug	Concentration (µg/ml)	Absorbance	
		At 310 nm	At 340 nm
PIP	10	0.1653	0.3197
	20	0.3458	0.5246
	30	0.5063	0.7336
	40	0.6631	0.8516
	50	0.8022	1.0529
Regression Equation		y = 0.0159x + 0.0192	y = 0.0179x + 0.1585

Correlation Coefficient (R^2)	0.9978	0.9927

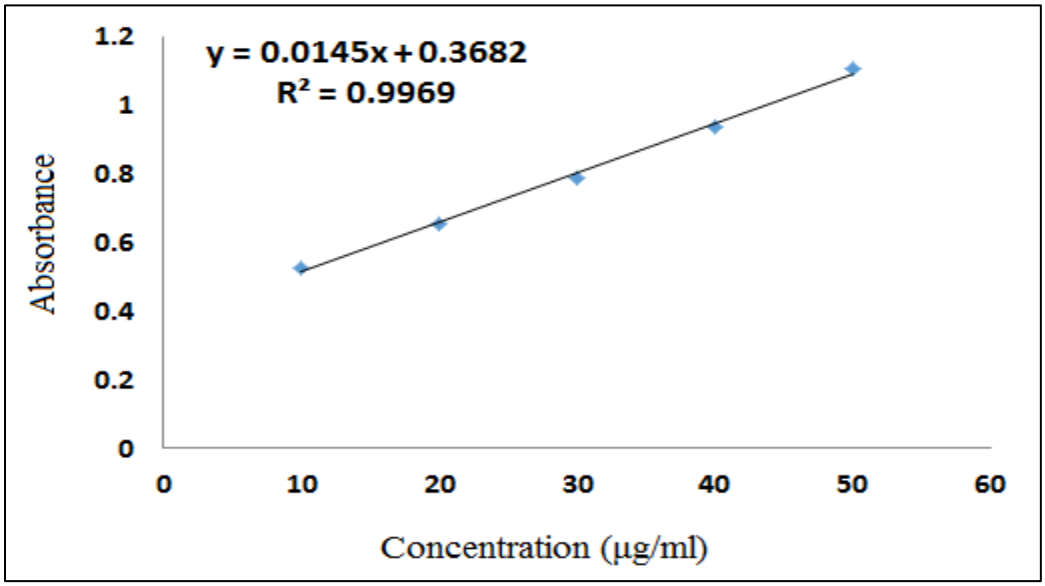

Fig. 4: Calibration curve of Resveratrol at wavelength (λmax) 310 nm

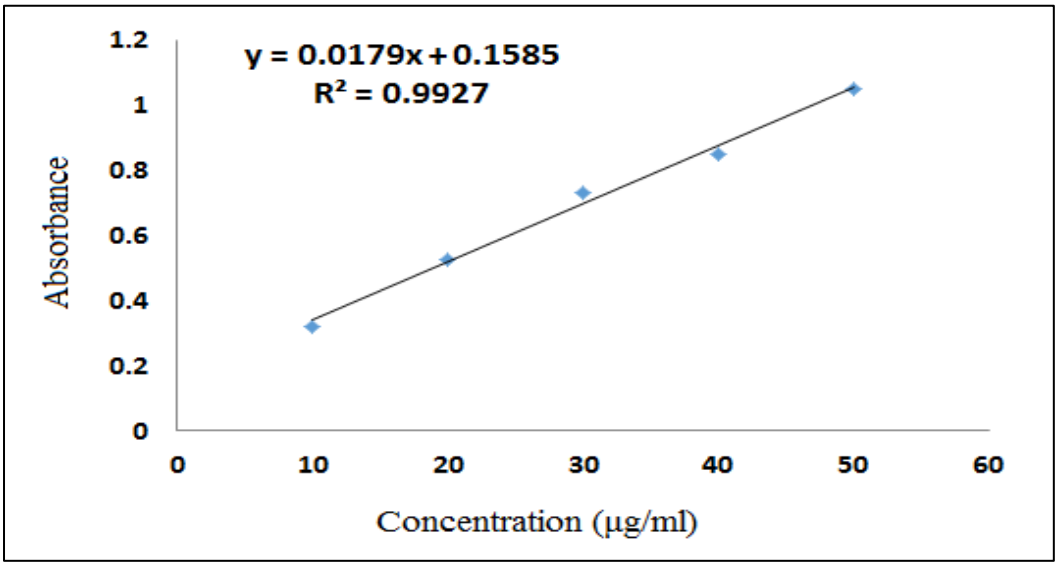

Fig. 5: Calibration curve of Piperine at wavelength (λmax) 340 nm

Accuracy:

Recovery studies evaluated the ability of the method to give accurate results after spiking the marketed formulation at 80%, 100%, and 120% with the standard drug solution. The % mean recovery of RVT and PIP was found to be in the

range of 95.36-99.11% and 97.61-99.13%. For both RVT and PIP,% RSD < 1.1. The recovery studies data of RVT and PIP is shown in Table-4.

Table 4: Accuracy studies for Resveratrol and Piperine

Drug	Recovery level (%) (n=3)	Amount of formulation	Amount of pure drug added	Total amount	Mean of Drug conc. recovered	% Mean Recovery	% RSD
RVT	80	10	8	18	17.84	99.11	0.792
	100	10	10	20	19.48	97.40	0.929
	120	10	12	22	20.98	95.36	1.069
PIP	80	10	8	18	17.57	97.61	0.571
	100	10	10	20	19.89	99.45	0.697
	120	10	12	22	21.81	99.13	0.802

Precision:

The results of precision study were reported in Table-5 in terms of % relative standard deviation. Intraday Precision: The intraday precision of RVT and PIP in terms of % RSD was found to be 1.16-1.55 % and 1.21-1.83% respectively.

Interday Precision: The interday precision of RVT and PIP in terms of % RSD was found to be 0.52-1.64 % and 0.98-1.37 % respectively.

Table 5: Intraday and Interday precision study of Resveratrol.

Drug	Precision	Wavelength	Conc. (µg/ml)	Abs.-1	Abs.-2	Abs.-3	% RSD
RVT	Intraday	310 nm	10	0.5253	0.5180	0.5301	1.16
			20	0.6532	0.6405	0.6589	1.44
			30	0.7901	0.8067	0.7826	1.55
	Interday	310 nm	10	0.5253	0.5095	0.5306	0.52
			20	0.6532	0.6659	0.6481	1.39
			30	0.7901	0.7859	0.8103	1.64
PIP	Intraday	340 nm	10	0.3197	0.3312	0.3228	1.83
			20	0.5246	0.5184	0.5411	1.21
			30	0.7336	0.7462	0.7199	1.79
	Interday	340 nm	10	0.3197	0.3054	0.3301	0.98
			20	0.5246	50.78	53.32	1.37
			30	0.7336	0.7183	0.7501	1.15

LOD and LOQ:

The values of LOD and LOQ for RVT and PIP at 310 nm and 340 nm were shown in Table-6.

Table 6: LOD and LOQ of Resveratrol and Piperine

Drug	LOD (µg/ml)		LOQ (µg/ml)	
	At 310 nm	At 340 nm	At 310 nm	At 340 nm
Resveratrol	0.78	1.61	2.39	4.88
Piperine	1.20	1.31	3.66	3.98

Ruggedness:

Ruggedness studies were carried out by changing the analyst for both the drugs by scanning each concentration three times at wavelength of maxima of each drug and then the % RSD was found out. Ruggedness study data obtained from two different analysts is shown in Table-7.

Table 7: Ruggedness study data for both Resveratrol and Piperine

Drug	Wavelength	Conc. (µg/ml)	Analyst-1	Analyst-2	Mean	% RSD	Average % RSD
RVT	310 nm	0	0	0	0	0	0.7483
		10	0.5253	0.5088	0.5170	0.89	
		20	0.6532	0.6405	0.6468	1.38	
		30	0.7901	0.8067	0.7984	1.47	
		40	0.9375	0.9451	0.9413	0.57	
		50	1.1073	1.0012	1.0542	0.18	
PIP	340 nm	0	0	0	0	0	0.6850
		10	0.3197	0.3306	0.3251	0.36	
		20	0.5246	0.5194	0.5220	0.50	
		30	0.7336	0.7403	0.7369	0.76	
		40	0.8516	0.8396	0.8456	0.80	
		50	1.0529	1.2141	1.1335	1.69	

CONCLUSION:

A simple, rapid and accurate UV-Spectrophotometric method for estimation of Resveratrol and Piperine in marketed formulation was developed. The developed method was found to be accurate, precise, simple and rapid. This method is suitable for the simultaneous analysis of Resveratrol and Piperine in the multicomponent formulation without the interference of one another. This method is recommended for the routine quality control analysis of the marketed formulation.

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