REVIEW ARTICLE ISSN: 2349-2678

Contents lists available at www.ijpba.in
International Journal of Pharmaceutical and Biological Science Archive
NLM (National Library of Medicine ID: 101732687)
Index Copernicus Value 2019: 71.05
Volume 9 Issue 4; July-August; 2021; Page No. 34-39

DEVELOPMENT AND VALIDATION OF A SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF RESVERATROL IN BULK AND MARKETED DOSAGE FORMULATIONS

Sachin Bhusari*, Vikas Bhise, Pravin Wakte

[1]University Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India

Conflicts of Interest: Nil
Corresponding author: Sachin Bhusari
DOI: https://doi.org/10.32553/ijpba.v9i4.243

ABSTRACT

A simple, precise and cost effective UV- visible spectrophotometric method has been developed and validated according to the guidelines of the International Conference on Harmonization (ICH Q2 (R1) for the estimation of resveratrol in bulk and formulation. Spiked resveratrol solution was scanned over UV-visible range for its wavelength of maximum absorbance. Various calibration standards of resveratrol were prepared. Calibration curve of concentration vs. absorbance was plotted. Various analytical method validation parameters viz. linearity, accuracy, precision, robustness, ruggedness, limit of detection and limit of quantitation were calculated. The maximum wavelength of resveratrol was found to be 215 nm. The correlation coefficient over the concentration range of 0.5-6 µg/ml was found to be 0.999. Developed UV method was found to be precise for the intra-day and inter-day study and shows percent relative standard deviation in the range of 0.58 & 1.20 to 0.28 to 1.08 respectively. The total percent recovery of resveratrol was found to be 98.18 %. A simple, precise and cost effective UV- visible spectrophotometric method was developed and validated as per ICH guidelines. The results suggest that the developed method was found to be robust and it can be applicable in routine analysis and efficiently used for the estimation of resveratrol in bulk as well as combined dosage form.

Keywords: Resveratrol, HPLC method development, Validation

Introduction

Resveratrol (trans-3, 4', 5,-trihydroxystilbene) is a polyphenol molecule. 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]. Trans-resveratrol holds a broad range of pharmacological properties without harmful effects and is well known for its antioxidant, anti-inflammatory, analgesic, cardio protective, neuroprotective, anti-aging, and anticancer activities [3-5]. It inhibits the oxidation of low-density lipoprotein and platelet aggregation, and protects isolated rat hearts from ischemia-reperfusion injury [6-10]. The structure of Resveratrol was as shown below in fig. 1.

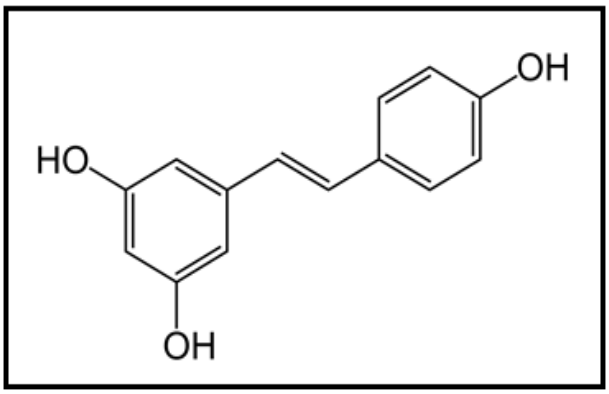

Figure 1: Structure of Resveratrol

For the formulation development of any drug molecule, analysis is an essential component. One of the most frequently employed technique in pharmaceutical analysis is UV-Visible spectrophotometry. The amount of ultraviolet or visible radiation which is absorbed by a substance in solution is measured by UV-Visible spectrophotometer [10-12]. In this study, efforts were made to develop a simple, easy and economic UV spectrophotometric method for the determination of Resveratrol using different solvents. 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.

Materials and Methods

Trans-Resveratrol was procured from TCI Chemicals (India) Pvt. Ltd, Chennai. HPLC grade methanol, water were used for the purposed study.

Instruments

A double beam UV-visible spectrometer (UV-530, Jasco) connected to a computer loaded with spectra manager software was used for the analysis. Quartz cells having 3 cm length with 1 cm path length were used for spectral measurement. Weighing balance (Essae, Vibra HT) with internal calibration mode has been utilized for accurate weighing purposes.

Preparation of Mobile Phase

The mobile phase Methanol and water were mixed in the ratio of 75:25 v/v and filtered through membrane filter (Millipore Nylon disc filter of 0.45 µ). This filtered mobile phase was sonicated for 15 min in ultrasonic bath.

Preparation of standard stock solution

Stock solutions (1 mg/mL) of resveratrol prepared in HPLC grade methanol and filtered through 0.45-m nylon membrane syringe filter.

Preparation of standard calibration curve

Calibration curve was prepared by diluting the stock-I solution to achieve the seven different calibration standards representing 0.5, 1, 2, 3, 4, 5 & 6 µg/ml strength of resveratrol. Absorbance of each calibration standard was measured at pre-identified λ_{max}; 215 nm using fixed wavelength measurement mode. The calibration curve representing concentration vs. absorbance was plotted. Above mentioned procedure was repeated three times so that reproducible results can be obtained.

Method Validation

The validation of pre-optimized chromatographic method was performed according to the Q2 (R1) guidelines of International Conference on Harmonization (ICH).Various analytical method validation parameters like, linearity range, accuracy, precision, robustness, ruggedness, LOD and LOQ were assessed [13-14].

Linearity& Range

Linearity of the proposed UV method was calculated by using seven different calibration standards of resveratrol The calibration curves were constructed using the Calibration Standards representing 0.5, 1, 2, 3, 4, 5 & 6 µg/ml strength of resveratrol absorbance vs. concentration were plotted, subjected to linear regression analysis and linearity in terms of R-squared values and respective range were reported.

Accuracy (% Recovery):

Accuracy of pre-optimized HPLC method was assessed using recovery studies by standard addition method. To the solutions with predefined amount of resveratrol (1, 3 and 6 µg/ml), its 80, 100 and 120 % amount was added externally and the % recovery of the drugs was calculated.

Precision

The precision of the developed method was evaluated by performing Intra-day and Inter-day studies. Intra-day precision study was carried out by analyzing five replicates of three different concentrations (1, 3 and 6 µg/ml of resveratrol) at morning, afternoon and evening time of the same day. Similarly, inter-day precision study was carried out by analyzing the samples on three consecutive days. Intra- and inter-day precision results were expressed in terms of % RSD.

Robustness

The robustness of the developed UV method was established using different percentages of methanol in the co-solvent system. Methanol percentage in the co-solvent system was kept at 80 and 70% and resveratrol was dissolved in said co-solvent system separately. Triplicate samples were analyzed at 215 nm for absorbance. Levels of resveratrol in each sample were estimated using a respective calibration curve. The results were calculated in terms of % RSD.

Ruggedness

Ruggedness study of the method was carried out by analyzing triplicate samples of resveratrol solution (3 µg/ml) on two different Instruments (V-530, Jasco and BA-UV-2600, Bioage) and absorbance were noted in terms of % RSD.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. LOQ is the lowest concentration of analyte that can be determined with

acceptable accuracy and precision. LOD and LOQ were calculated using following formula

LOD = 3.3 × SD/S

LOQ = 10 × SD/S

Where SD = standard deviation of response (peak area) and S = slope of the calibration curve.

Estimation of resveratrol content in pharmaceutical formulation

The resveratrol content in its marketed formulation (Resvita) was estimated using proposed method. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 10 mg of Resveratrol was transferred into a 10 mL volumetric flask and dissolved in 5 mL of a 75:25 v/v mixture of methanol and water. The contents of the flask were sonicated for 15 min. The volume was made up with the diluent and the solution was filtered through a 0.45 µm membrane filter. Said solution was suitably diluted with co-solvent system and analyzed for the resveratrol content using proposed UV method. The amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug.

Results and Discussion

Determination of wavelength of maximum absorbance:

Identification of wavelength of maximum absorbance is a prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of the wavelength of maximum absorbance. Considering the prerequisite and the suitability, determination of maximum wavelength for resveratrol solution was carried out using full scan mode of UV-Visible spectrophotometer. The full scan was processed using UV software and the λmax was identified with the help of software. It was found to be 215 nm for resveratrol (Fig.2).

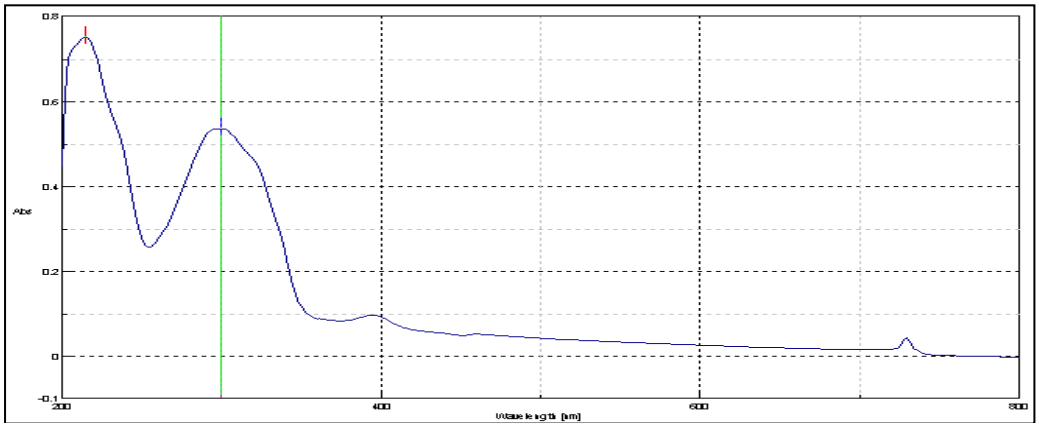

Figure 2: UV-visible spectra of Resveratrol

Preparation of calibration curve

Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and an equation stating the correlation between concentration and the response. As compared to the graphical method, above stated method is widely accepted and reproducible. Considering the utility of quantitative analysis of resveratrol, a calibration curve for resveratrol was developed using seven different calibration standards. The absorbance of different calibration standards at 215 nm was recorded using the fixed wavelength mode of UV-Visible spectrophotometer. The calibration curve was repeated three times and the mean values ± deviation was reported as shown in Table 1.

Table 1: Calibration standard data for Resveratrol

Concentration (µg/ml)	Absorbance
1	0.0758 ±0.027
2	0.1509 ±0.059
4	0.3141 ±0.029
6	0.4519±0.037
8	0.6037±0.048
10	0.7653±0.014
12	0.9028 ±0.041

Method validation

Linearity and Range

Linearity and range are the important parameters of analytical method that demonstrates the limit within

which the intended method is to be used for its optimum performance. Considering the prime importance of linearity and the range, seven point calibration curve of resveratrol (0.5-6 µg/ml) were constructed. Details of concentrations and the respective mean absorbance values are depicted in Table 1. Calibration curve when subjected to least square regression analysis yielded an equation; y = 0.1521x + 0.0007 with correlation coefficient 0.9995 respectively (Fig. 3). From the linearity study, it was revealed that, there is a linear relationship between response and amount of drug within the range 0.5-6 µg/ ml.

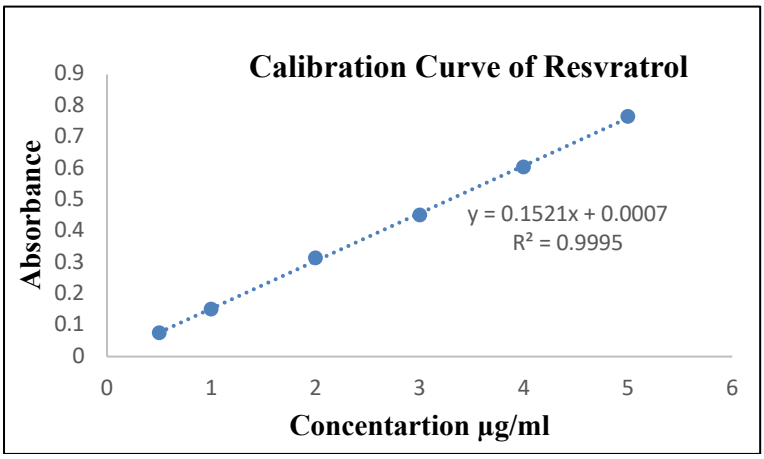

Figure 3: Calibration curve for Resveratrol

Accuracy (percentage Recovery)

Accuracy is the closeness of test results to the true value obtained by proposed method. The accuracy of an analytical method should be established over its calibration range so that at any point of determination, results obtained would be accurate. For resveratrol, accuracy was determined using recovery studies. At 80, 100 and 120 % standard addition, mean recovery of resveratrol was found to be 99.18%. The relative standard deviation (% RSD) was found to be less than 2 (Table 4). From the results of accuracy studies, it was concluded that the proposed method is accurate.

Table No. 4: Recovery studies of Resveratrol

Sr. No.	Sample	Spiked level	Theoretical Conc. (µg/mL)	Practical Conc. (µg/mL)	% Recovery	Mean % Recovery	% RSD
1	Resveratrol	80%	2.4	2.38	99.16	99.18	0.48
		100%	3	2.96	98.66		
		120%	3.6	3.59	99.72		

Precision

Precision was studied by analysis LQC, MQC and HQC STDs containing both the drugs at concentrations covering the entire calibration range**.** The results expressed in terms of % RSD for the intra- and inter-day precision study (Table 4 and 5).Percent RSD values of intra-day precision study were found to be in between 0.58 to 1.20, whereas inter-day precision was found to be in between 0.28 to 1.08. It was concluded that the analytical technique showed good repeatability.

Table 3: Intra-day precision data of UV method for Resveratrol

	Morning			Afternoon			Evening		
Concentration Range (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	0.978	97.80	0.68	0.982	98.20	0.79	0.994	99.40	1.05
3	2.948	98.26	0.97	2.995	98.83	1.20	2.986	99.53	0.58
6	6.014	100.23	0.58	5.984	99.73	0.89	5.981	99.68	0.68

Table 4: Inter-day precision data of UV method for Resveratrol

	Day 1			Day 2			Day 3		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1	0.992	99.20	0.94	0.986	98.60	1.08	1.010	101.00	0.55
3	3.011	100.36	0.38	2.998	99.93	0.84	2.997	99.90	0.49
6	5.994	99.90	1.02	6.012	100.20	0.66	5.974	99.56	0.28

Robustness

The robustness of an analytical method is the ability of a method to resist the change in its performance despite the small, deliberate change in method parameters. It is an important parameter of the analytical method as a small, unintentional change in method parameters like solvent composition; pH, etc. may occur during routine use and may hamper the performance of the said method. It is expected that such change should not alter the performance of the analytical method. Therefore, a robust analytical method is preferred. The robustness of the proposed UV method was established by modifying the composition of the co-solvent system. Change in Methanol percentage (80 to 70 %) in the co-solvent system did not affect the method performance. % RSD values were found to be in between 0.18 and 1.21 as shown in Table 5. % RSD values below 2 showed that the proposed UV method is robust.

Table 5: Robustness data of UV method for Resveratrol

Robustness Study of Resveratrol					
Concentration=3µg/ml					
Solvent Ratio (Ethanol: Water)	**I**	**II**	**III**	**Mean**	**%RSD**
(80:20)	3.01	2.97	3.02	3.00	0.18
(75:25)	2.99	2.98	3.01	2.99	0.58
(70:80)	2.96	2.95	2.97	2.96	1.21

Ruggedness

The ruggedness of the analytical method is the ability of a method to resist the change in its performance despite influential environmental factors like temperature. Rugged analytical methods are preferred as these methods are free from the impact of environmental/external factors. To establish the ruggedness of the proposed UV method, the Resveratrol solution was analyzed using two different UV-Visible spectrophotometers of two different labs. Sample analysis and data processing resulted in % RSD values between 0.46 and 0.89. Results revealed that the proposed UV method was rugged as it showed % RSD values less than 2 as shown in Table 6.

Table 6: Ruggedness data of UV method for Resveratrol.

Ruggedness Study of Resveratrol					
Concentration=6µg/ml					
Instrument	**I**	**II**	**III**	**Mean**	**%RSD**
Jasco	2.97	2.99	3.01	2.99	0.46
Bioage	2.98	2.96	2.95	2.96	0.89

LOD and LOQ

LOD and LOQ of proposed HPLC method was found to be 0.0752and 0.2280µg/ml. Lower LOQ value indicated that proposed method would be sensitive enough to quantify the resveratrol content of samples at its lower level.

Estimation of resveratrol content in pharmaceutical formulation

The developed UV method was successfully applied for estimation of resveratrol content in Marketed tablets formulation. By proposed UV method, resveratrol content in the tablets was found to be 100.45 ± %.Further, it was found that proposed UV method is specific for the resveratrol No interference of the excipients of pharmaceutical formulation was observed during the UV analysis.

Conclusion

A simple, accurate and precise UV-Visible spectrophotometric method for the estimation of resveratrol was developed and validated. The Proposed method was found to be robust and rugged in nature

and was successfully used for the estimation of resveratrol.

Acknowledgement

The extra-mural grant support of DST-DPRP, Govt. of India (Ref:-VI-D&P/626/2018-19/TDT) sanctioned to P.I. Dr. Sachin S. Bhusari for the proposed research work is highly acknowledged.

References

1. Bahare Salehi, Abhay Prakash Mishra and Javad Sharifi-Rad. Resveratrol: A Double-Edged Sword in Health Benefits. Biomedicines. 2018; 6(3):1-20.
2. J. A. Baur and D. A. Sinclair. Therapeutic potential of resveratrol: the in vivo evidence. Nature Reviews Drug Discovery. 2006; 5(6): 493–506.
3. P. Langcake and R. J. Pryce. The production of resveratrol by Vitis vinifera and other members of the Vitaceae as a response to infection or injury. Physiological Plant Pathology.1976; 9(1): 77–86.
4. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, et al. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science. 1997; 275:218–20.
5. Burns J, Yokota T, Ashihara H, Lean MEJ, Crozier A. Plant foods and herbal sources of Resveratrol. Journal of Agricultural and Food Chemistry. 2002; 50(11): 3337–3340.
6. Juan ME, Maijo M, Planas JM. Quantification of trans-resveratrol and its metabolites in rat plasma and tissues by HPLC. Journal of Pharmaceutical and Biomedical Analysis. 2010; 51:391–8.
7. H. He, X. Chen, G. Wang, J. Wang, and A. K. Davey. High-performance liquid chromatography spectrometric analysis of trans-resveratrol in rat plasma. Journal of Chromatography B. 2006; 832(2): 177–180.
8. C. D. Bernardes, R. J. Poppi, and M. M. Sena,. Direct determination of trans-resveratrol in human plasma by spectrofluorimetric and second-order standard addition. Talanta. 2010; 82(2): 640–645.
9. M. A. Vian, V. Tomao, S. Gallet, P. O. Coulomb, and J. M. Lacombe. Simple and rapid method for cis- and trans-resveratrol and piceid isomers determination in wine by high-performance liquid chromatography using chromolith columns. Journal of Chromatography A. 2005; 1085(2): 224–229.
10. S. Das and K.-Y. Ng. Quantification of trans-resveratrol in rat plasma by a simple and sensitive high performance liquid chromatography method and its application in pre-clinical study. Journal of Liquid Chromatography and Related Technologies. 2011; 34(14):1399–1414.
11. G. Singh, R. S. Pai, and V. Pandit. Development and validation of a HPLC method for the determination of trans-resveratrol in spiked human plasma. Journal of Advanced Pharmaceutical Technology & Research. 2012; 3(1): 130–135.
12. G. Singh, R. S. Pai, and V. Pandit. In vivo pharmacokinetic applicability of a simple and validated HPLC method for orally administered trans-resveratrol loaded polymeric nanoparticles to rats. Journal of Pharmaceutical Investigation. 2014; 44: 69-78.
13. Note for guidance on validation of analytical procedures: text and methodology. European Medicines Agency: 1995; 1-15.
14. Validation of analytical procedures: text and methodology q2 (r1). ICH harmonised tripartite guideline.1994