RESEARCH ARTICLE

Development and Validation of Stability Indicating UV Spectrophotometric Method for Estimation of Resveratrol in Bulk and Tablet Dosage Form

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Received: 19th August, 2023; Revised: 24th October, 2023; Accepted: 24th February, 2024; Available Online: 25th March, 2024

ABSTRACT

A simple, sensitive, accurate, trustworthy, and stable UV spectrophotometric approach has been devised to quantify resveratrol in pharmaceutical formulations and bulk materials. After scanning the UV spectrum from 200 to 400 nm, the highest wavelength for absorption was determined to be 306 nm. For the medication, Beer's rule has been adhered to within a concentration range of 1 to 5 µg/mL. While the developed method was able to recover a good amount of drug (%Recovery), the precision study's percentage RSD values were ≥2%. The method demonstrated effective functionality for a pharmaceutical dosage form containing resveratrol, free from interference from the excipients. Correspondingly, the limit of detection (LoD) for resveratrol were 0.13 and 0.40 µg/mL. The results of this investigation have been confirmed in accordance with ICH standards. Research on artificial degradation has been greenlit, which investigates the impacts of several environmental factors over a broad pH spectrum, including heat, oxidation, photolysis, and hydrolysis vulnerability.

Keywords: UV spectrophotometric, Resveratrol, Forced deterioration, ICH norms

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.1.56

How to cite this article: Pathade P, Sumrao A, Sonawane B, Shirode D, Shewale S, Shinde V, Bairagi V. Development and Validation of Stability Indicating UV Spectrophotometric Method for Estimation of Resveratrol in Bulk and Tablet Dosage Form. International Journal of Drug Delivery Technology. 2024;14(1):385-388.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Many plant species create phytoalexin in reaction to harmful pathogens, such as bacteria, fungi, or damage. It is a member of the stilbenoid class of natural phenols. It can be found in fruits that people often eat, such as peanuts, blackberries, and blueberries. A wide range of biological properties of resveratrol are evident, such as its anti-inflammatory, neuroprotective, cardio-protective, and anticancer effects. Cellular reactions like cell cycle arrest, differentiation, apoptosis, and the inhibition of cancer cell proliferation are brought on by these activities. Figure 1 shows the structure of resveratrol.[1]

Simple, reliable, sensitive, UV spectrophotometric method for determining resveratrol from bulk and tablet formulation as per resveratrol's capacity to inhibit the phosphatidylinositol 3-kinase (PI3K)/Akt pathway is one notable mode of action, controlling cellular processes such as cell differentiation, growth, and proliferation.[2]

It is essential to find the most stable dosage form as soon as possible in the development of novel drugs. The International Commission for Harmonisation (ICH) has established regulations governing stability testing for registration applications. These regulations center on stress testing conditions, which assess the drug's reaction to extreme conditions, such as pH and temperature fluctuations. In the early stages of pharmaceutical research, these studies are essential for calculating the shelf life of a medicinal product.[3] Literature survey revealed that few spectrophotometers[4], For the purpose of developing analytical procedures, high-performance liquid chromatography (HPLC) techniques were documented for resveratrol in both its pure and tablet forms.[5-7]

There has been no known method for assessing this medication in pharmaceutical formulations using UV stability indicators. Because of its affordability, specificity, and ease of use, spectrophotometry continues to be a favorite for drug determination. A reliable, cost-effective, and validated analytical approach for resveratrol quantification in pharmaceutical dosage forms and pure form is presented in this paper. Resveratrol in bulk and formulations can be routinely and accurately analyzed using this technology because it complies with ICH criteria.[8-10]

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MATERIALS AND METHODS

Lupin Laboratories, located in Aurangabad, Bihar, gave a complimentary sample of resveratrol. Research Lab, Mumbai, supplied the chemicals and solvents of analytical reagent quality. The spectra were recorded using a Jasco UV/VIS double-beam spectrophotometer (Model No.: V-730) equipped with identical quartz cells that were 1-cm in diameter. Methanol was consistently utilized in all experimental procedures.

Standard Stock Solution

After carefully measuring out 10 mg of resveratrol, it was put to a volumetric flask of 100 mL. After that, about 100 mL of methanol was added to dissolve it. A 100 µg/mL stock solution was produced by adjusting the volume with methanol until it reached the mark.

Calibration Curve for Resveratrol

Through scanning an appropriate solution in UV-vis spectrophotometer between 200 and 400 nm in wavelength, λ_{max} of resveratrol was 306 nm (Figure 2). Then, aliquots (1, 2, 5 mL) were pipetted into each of five volumetric flasks from the standard resveratrol solution, and the volume was adjusted with methanol up to 10 mL and concentration were prepared 1 to 5 ppm respectively. At next, the absorbance at 306 nm was calculated using a blank for the reagent. Overlain spectra were found in Figure 3. Figure 4 shows the results of creating a calibration curve by graphing absorbance against concentration (µg/mL). We also calculated the correlation coefficient. Table 1 summarizes the analytical parameters in detail, whereas Table 2 provides the data from the calibration curve.

Method Validation

Parameters for validation. The process has been evaluated in terms of ruggedness, accuracy, and precision. This approach aligns with the ICH Q2B guidelines, emphasizing the importance of thorough method validation in analytical chemistry. The linearity of the suggested approach was determined by taking absorbance readings from standard solutions by concentrations reaching from 1 to 5 µg/mL and analyzing results using least square regression. The conventional addition method confirmed the suggested approach's accuracy, and recovery experiments were conducted at 80, 100, and 120% of the objective concentration. The percentage of analytical recovery was determined by comparing the concentration achieved after adding spiked samples to the actual predicted theoretical concentration increase. By doing the study for six concentrations at two distinct times during the day, the intra-day precision was obtained. Analysis on two consecutive days was used to determine inter-day precision similarly. The proposed methods' limit of detection (LoD) and limit of quantitation (LoQ) were determined. One indicator of the method's precision or bias is the success rate of analyte recovery from a given matrix.[11]

Stability Studies of Resveratrol

Forced degradation of resveratrol was performed to carry out stability tests. This study examined the effects of oxidation, temperature, photolysis, and hydrolysis all over a broad pH range. 0.1, 1 and 3% H_2O_2 were utilized for the oxidation study, 0.1, 0.5, 1 N HCl for acidic hydrolysis, 0.1, 0.5, 1 N NaOH for basic hydrolysis, and methanol for neutral hydrolysis. The sample solution was exposed to sunlight for three days to undergo photolysis, and it was also heated to 60 to 70°C for one hour in order to apply thermal stress.[12]

RESULT AND DISCUSSION

Creating a quick, sensitive, accurate, and affordable analytical technique for routine quantitative sample analysis can lessen

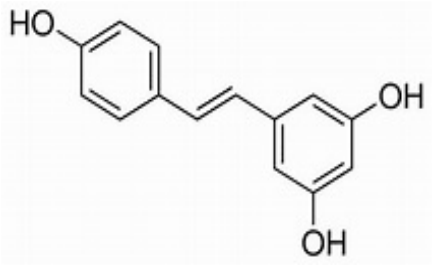

Figure 1: Structure of resveratrol

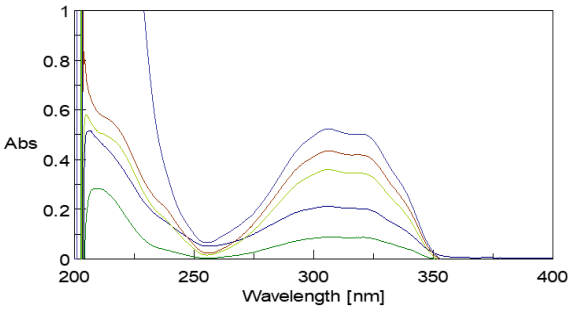

Figure 3: Overlain spectra of resveratrol

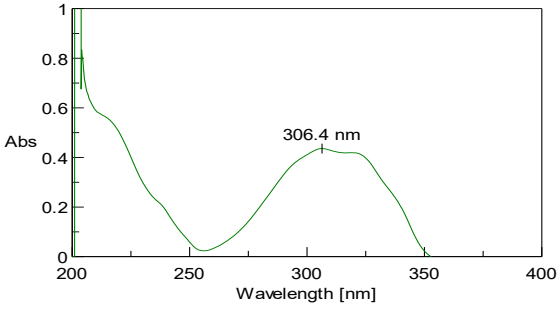

Figure 2 : Absorption maxima of resveratrol

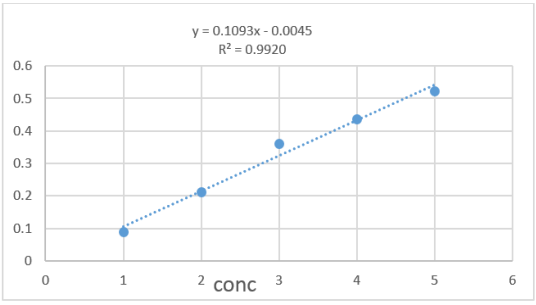

Figure 4: Calibration curve of resveratrol

the need for labor-intensive sample preparations and lower overall labor and material costs. Figure 2 shows the resveratrol absorption spectra in methanol.

Through scans across the whole UV area, the drug's Amax for analysis was determined to be 306 nm. The data from the calibration curve, which were set up within the expected concentration range of 1 to 5 µg/mL, obeyed Beer's law. Results from regression equation Y = 0.1093x+0.0045 showed a correlation coefficient (r) of 0.9920. Table 2 presents specifics of calibration plot properties. Repeat studies of standard solutions were used to evaluate recommended trials' accuracy and precision (Tables 3 and 4). Determined boundaries of it was found that the computed LoQ and LoD were 0.404 and 0.132 µg/mL, correspondingly.

Table 1: Optical characteristics of proposed method

Factors	*Outcome*
λ_{max}	306 nm
Beer's law limit (µg/mL)	1–5
Regressionequation (y = m x + c)	Y = 0.1093x+0.0045
Correlation coefficient (r)	0.9920
Slope	0.1093
LoQ (µg/mL)	0.404
Intercept	0.0045
LoD (µg/mL)	0.132

Table 2: Calibration curve data for resveratrol

Concentration (µg/mL)	*Absorbance*
1	0.088
2	0.212
3	0.359
4	0.435
5	0.523

Table 3: Outcomes of recovery studies

Level of %recovery	*Amount of standard drug Added (µg/mL)*	*Amount of drug taken from tablet (µg/mL)*	*%Mean recovery**	*Standard deviation*	*%RSD*
80	2.4	3	98.40	0.041	0.52
100	3	3	99.49	0.087	0.82
120	3.6	3	101.02	0.032	0.06

*Average of three estimations

Table 4: Arithmetical Validation for inter-intraday precision

Parameters	*Statistical estimation*
Intraday*	1.02%
%RSD	99.87
%Mean ± S.D	0.00442
Interday*	1.32%
%RSD	99.82
%Mean ±S.D	0.00454

%RSD = Relative Standard Deviation, *Denotes average of six determination S D. = Standard Deviation

Table 5: Outcome of forced degradation of resveratrol

Condition applied	*Conc. Taken (µg/mL)*	*Average conc. found*	*Observation*
Acid hydrolysis (0.1, 0.5, 1N HCl)	3	66.57	Degraded
Base hydrolysis (0.1, 0.5, 1N NaOH)	3	58.21	Degraded
Thermal stress (70°C, 1 hour)	3	50.97	Degraded
H_2O_2 (6% w/v)	3	83.84	Degraded
Sunlight treatment day 1	3	28.96	Degraded
Sunlight treatment day 2	3	32.03	Degraded
Sunlight treatment day 3	3	23.95	Degraded

These results demonstrate that the created approach was easy to use, affordable, quick, accurate, and precise. As a result, it may be used to determine the amount of resveratrol in pharmaceutical tablets without the excipients interfering. Regular and quality control analyses of resveratrol in pharmaceutical formulations and raw materials based on forced degradation tests (Table 5) that meet ICH criteria can be conducted using this technology.

CONCLUSION

Resveratrol in bulk and pharmaceutical formulations can be quantitatively determined using an established, reliable UV spectrophotometric method. The following advantages of this method stand out: reproducibility, sensitivity, accuracy, and precision. It minimizes the need for solvents and thus cuts down on the time needed to establish a generic approach. We have demonstrated the stability-indicating nature of the technique through forced degradation studies conducted under a number of ailments such as temperature, oxidation, neutral conditions, photolysis, and hydrolysis susceptibility throughout a wide pH range. The protocols followed by these investigations were those established by the ICH. The suggested approach works well for both bulk and pharmaceutical formulation resveratrol regular analysis and quality.

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