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Optimization and Validation of Resveratrol Using Analytical UV Method Development

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Abstract

Objective-The objective of this work is to develop and validate UV method using Resveratrol.

Method-Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenolic phytoalexin. Resveratrol suppresses NF-kappaB (NF-kappaB) activation in HSV infected cells. Reports have indicated that HSV activates NF-kappaB during productive infection and this may be an essential aspect of its replication scheme. A novel, safe and sensitive method of spectrophotometric estimation in UV-region has been developed for the Resveratrol (trans-3,4',5,-trihydroxystilbene) using Methanol, phosphate buffer pH 6.8, 0.1 N hydrochloric acid and water.

Results-Among the different solvents methanol showed better results, hence methanol was selected as a solvent for the proposed method. Resveratrol showed maximum absorbance at 303nm. Method was quantitatively evaluated in terms of linearity, accuracy and precision.

Conclusion-The method was simple, convenient and suitable for the determination of Resveratrol.

Keywords : Resveratrol, UV-visible spectrophotometer, 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. 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.

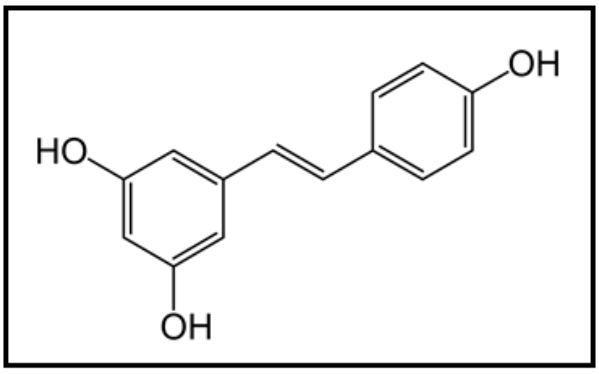

Fig.No.1 : Structure of Resveratrol

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. [1]

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. [2]

MATERIAL AND METHODS: [3-7]

Chemical :

Trans-Resveratrol was procured from Biotivia Pvt Ltd. (Italia). Hydrochloric acid and methanol were purchased from S.D. Fine chemicals (Mumbai, India). All these solvents were of HPLC grade. Water used in all experiments was passed through a Milli-Q water purification system (Millipore, USA).

Equipment :

1. UV-VISIBLE SPECTROPHOTOMETER-Make-Jasco V-500 UV.
2. ANALYTICAL BALANCE. Make-Winsor India.
3. ULTRA SONICATOR. Make-Winsor, WUC Series.

Method development :

ANALYTICAL METHOD DEVELOPMENT BY U.V. SPECTROPHOTOMETRY

Preparation of standard plot in methanol

For preparation of stock solution (1000 µg/ml), 10 mg drug was dissolved into 10 ml of methanol. 0.1 ml Stock solution was further diluted with methanol to get 10µg/ml. Similarly further dilutions were made ranging from 0.8µg/ml to 5.6µg/ml. The absorbance of these solutions was read at 303 nm and the standard graph was plotted.

Preparation of standard plot Phosphate buffer pH 6.8

10mg drug was dissolved into 10ml of phosphate buffer pH 6.8 to get 1000 µg/ml stock solution. 0.1ml of this solution was diluted to10ml of phosphate buffer pH 6.8 to give a solution of concentration 10µg/ml. Similarly further dilutions were made ranging from 0.4µg/ml to 6.4µg/ml with phosphate buffer pH 6.8.

Preparation of standard plot in 0.1N Hydrochloric acid

For preparation of stock solution (1000 µg/ml), 10 mg drug was dissolved into 10 ml of 0.1N HCl. 0.1ml of this solution was diluted to 10ml with 0.1N HCl to give a solution of concentration 10µg/ml. Similarly further dilutions were made ranging from 0.4µg/ml to 8µg/ml.

Preparation of standard plot in water

10mg drug was dissolved into 10ml of water to get 1000 µg/ml stock solution. 0.1ml of this solution was diluted to 10ml with water to give a solution of concentration 10µg/ml. Similarly further dilutions were made ranging from 0.4µg/ml to 5.6µg/ml. The absorbance of this solution was taken and the standard graph was plotted.

Method optimization

Selection and Optimization of Solvent

It is well known that the solvent do exerts a profound influence on the quality and shape of the peak [3]. The choices of solvents for UV method development are: Methanol, Phosphate buffer pH 6.8, 0.1 N hydrochloric acid, water etc. Different of solvents were optimized. Out of which methanol satisfied all the conditions relative to Peak quality & non-interference at the specified wavelength.

Selection of WavelengthThe wavelength at which maximum absorption takes place in UV detector is selected for further analysis i.e. 303 nm.

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result, or a product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision and Specificity.

Linearity

The linearity was evaluated by analysing the different concentration of standard solution of Resveratrol. The Beer-Lamberts concentration range was found to be 0.8-5.6µg/ml for resveratrol. The linearity was determined by plotting the graph of absorbance and concentration as shown in figure 3 and table no.1.

Accuracy (% recovery)

The accuracy study was performed using the standard addition method at 80%, 100% and 120% resveratrol solution. Absorbance was measured at 303nm and the concentration of drug was determined. The SD and %RSD were calculated at each concentration level as shown in table 2.

Precision :

Repeatability of the method was established by analysing various samples of Resveratrol. Precision was carried out by performing interday and intraday variation. In inter day variation the sample was analysed on three consecutive days. In an intraday variation the absorbance was measured three times in a day. Inter and intraday precision was determined using 10 ppm concentration.

Intraday Precision

In the intraday variation study was determined for a solution (10ppm) and was analysed three times for the consecutive days (i.e. morning, afternoon, evening). Mean, standard deviation and % RSD was calculated and shown in table 3.

Interday Precision

The interday precision was determined for a solution (10ppm) and was analysed for the three times on a different day. % RSD was calculated shown in Table 4.

RESULT AND DISCUSSION:

Determination of λmax in U.V.:

A solution of 10µg/ml of resveratrol in methanol was scanned in the range 200-400 nm. The UV scan of the drug was shown below in figure 2. λmax of the drug was found to be 303 nm in methanol.

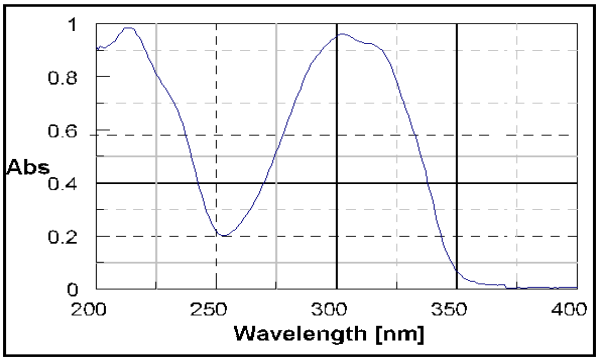

Fig.No.2 : UV spectrum of Resveratrol

ANALYTICAL METHOD DEVELOPMENT BY U.V. SPECTROPHOTOMETRY

Standard plot of resveratrol in methanol

The data for the standard plot of resveratrol in methanol is as shown in Table-1 and Figure-3. Beer Lamberts law was obeyed over the range of 0.8-5.6µg/ml and the data was found to fit the equation given below:

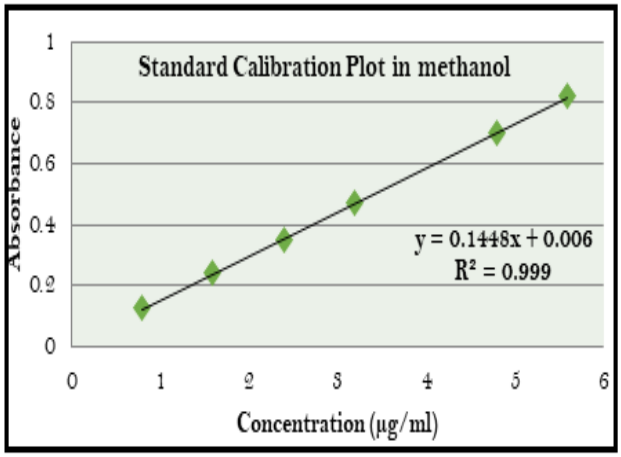

Fig.No.3 : Standard plot of Resveratrol in methanol

Standard plot of resveratrol in Phosphate buffer pH 6.8

The standard plot is shown in Figure-4. Beer Lamberts law was obeyed over the range of 0.4-6.4µg/ml and the data was found to fit the equation given below:

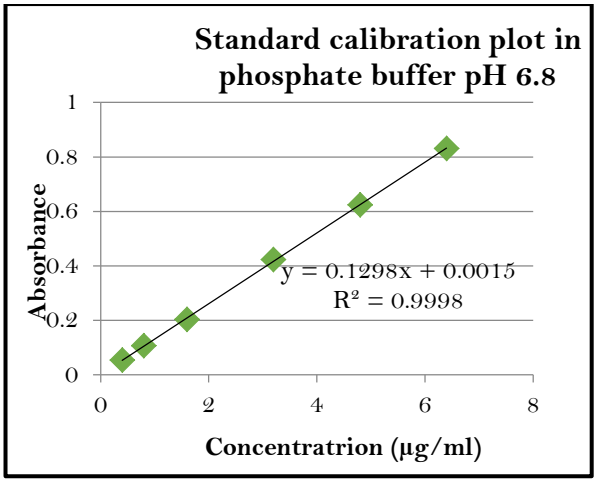

Fig.No.4 : Standard Plot for Resveratrol in phosphate buffer pH 6.8

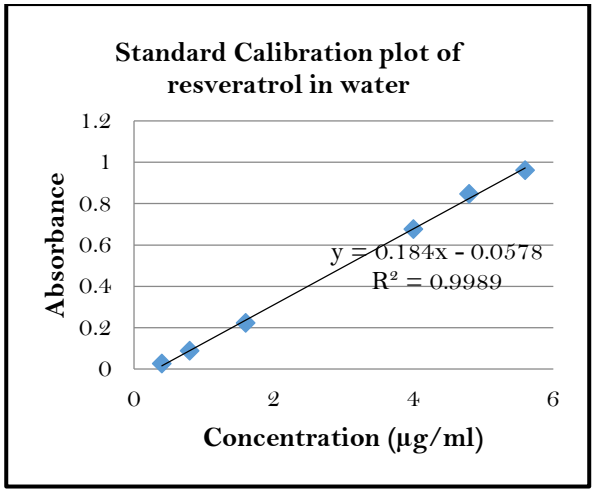

Fig.No. 6 : Standard Curve for resveratrol in water

Standard plot of resveratrol in 0.1N HCl:

The standard plot is shown in Figure-5. Beer Lamberts law was obeyed over the range of 0.4-8µg/ml and the data was found to fit the equation given below:

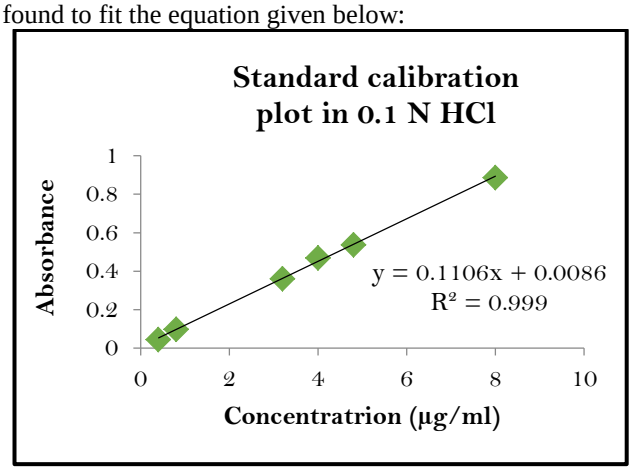

Fig.No.5 : Standard plot for resveratrol in 0.1N HCl

Standard plot of resveratrol in water

The standard plot is shown in Figure-6. Beer Lamberts law was obeyed over the range of 0.4-5.6µg/ml and the data was found to fit the equation

Method validation

Linearity

The linearity was evaluated by analysing the different concentration of standard solution of Resveratrol.

Table No.1: Linearity study of Resveratrol in methanol

Eq. of Line: y = 0.1448x + 0.0069		R² = 0.9999
Sr. No.	**Concentration (µg/ml)**	**Mean absorbance**
1	0.8	0.1249
2	1.6	0.2405
3	2.4	0.3499
4	3.2	0.4694
5	4.8	0.7008
6	5.6	0.8206
Mean		0.451017
SD		0.268408
%SD		26.84084
RSD		0.595119
%RSD		59.51187
Slope		0.1448

Accuracy (%recovery)

The accuracy study was performed using the standard addition method at 80%, 100% and 120% resveratrol solution.

Precision :

Precision was carried out by performing interday and intraday variation.

Intraday Precision

In the intraday variation study was determined for a solution (10ppm) and was analysed three times for the consecutive days (i.e. morning, afternoon, evening).

Table No. 2 : Statistical validation of accuracy (%recovery) studies of Resveratrol (n=3)

S.N.	Concentration (ppm)	Absorbance	Mean	SD	RSD	%RSD
1	**80%**	0.3256	0.3315	0.0225	0.0679	6.7984
		0.3564				
		0.3125				
2	**100%**	0.4568	0.4714	0.0234	0.0498	4.9836
		0.4589				
		0.4985				
3	**120%**	0.5632	0.5445	0.0193	0.0354	3.5493
		0.5246				
		0.5456				

Table No.3 : Intraday Precision For Resveratrol

S. N.	Concentration (ppm)	Absorbance-I	Absorbance-II	Absorbance-III
1	10	0.231	0.221	0.214
2	10	0.235	0.224	0.201
3	10	0.239	0.215	0.207
4	10	0.229	0.226	0.219
5	10	0.238	0.215	0.204
6	10	0.229	0.219	0.209
Average		0.2335	0.22	0.209
SD		0.004461	0.004561	0.006603
RSD		0.019105	0.02073	0.031593
%RSD		1.910467	2.073046	3.159344
Average % RSD		2.380953		

Interday Precision

The interday precision was determined for a solution (10ppm) and was analysed for the three times on a different day.

Table No.4 : Interday Precision For Resveratrol

S. N.	Concentration (ppm)	Absorbance-I	Absorbance-II	Absorbance-III
1	10	0.231	0.245	0.284
2	10	0.235	0.256	0.298
3	10	0.239	0.275	0.294
4	10	0.229	0.265	0.310
5	10	0.238	0.284	0.308
6	10	0.229	0.276	0.325
Average		0.2335	0.266833	0.303167
SD		0.004461	0.014442	0.014317
RSD		0.019105	0.054123	0.047224
%RSD		1.910467	5.412306	4.722372
Average % RSD		4.015049		

DISCUSSION :

The results obtained in the preliminary studies were satisfactory. After optimizing all the parameters, the method was checked for quality control purpose successfully.

Abbereviations :

UV-Ultra Violet, %-Percent, ppm-part per million, SD-standard deviation, RSD-relative standard deviation, µg-micro gram, ml- mili litre, HCL-Hydrochloric acid.

CONCLUSION :

The proposed method quantitatively evaluated in terms of linearity, accuracy and precision. All these factors lead to the conclusion that the proposed UV-spectrophotometric method is simple, accurate, precise, sensitive and cost effective. Out of the different solvents, methanol shows excellent results for the Resveratrol and this method was adopted for the use of economical and easily available mobile phase and for the UV detector.

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Authors Contribution

All authors contributed equally.

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