Research Article

Analytical Method Development and Validation for Estimation of Resveratrol in Bulk Dosage Forms by HPLC

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Received: 18-05-2020; **Revised:** 24-07-2020; **Accepted:** 30-07-2020.

ABSTRACT

As a natural compound, Resveratrol has been extensively researched for its use as a Nutraceuticals and therapeutic agent for many diseases. A new selective and sensitive high performance liquid chromatography (HPLC) method was developed for the quantification of Resveratrol in pharmaceutical dosage forms. The chromatographic separation was achieved on a xterra column (150*4.6,5µ) with Auto Sampler and DAD or UV detector using 0.05 M orthophosphoric acid (pH 2.0) 30% and Methanol HPLC grade as 70%, as the mobile phase at a flow rate of 1.0 mL/min and monitored at 306 nm. The run time was 7 min. The methods were validated as per International Conference on Harmonization (ICH) requirements and this included linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness. The calibration curve was linear over the concentration range from 20 to 60 µg/ml. The accuracy and precision of the method were found within the acceptable limit. All the results were acceptable and this suggested that the method is suitable for its intended use in routine quality control and assay of drugs.

Keywords: Resveratrol, HPLC, Accuracy, Precision, Robust.

INTRODUCTION

Resveratrol is a stilbenoid polyphenol, possessing two phenol rings linked to each other by an ethylene bridge. IUPAC name of resveratrol is E-5-(4-hydroxystyryl) benzene-1, 3-diol. The chemical structure of resveratrol (trans-3, 5, 4'-trihydroxystilbene) is identified in two isomeric forms, cis and transresveratrol that coexist in plants and in wine. However, cis-resveratrol has never been found in grape extract [1, 2]. The trans-isomer appears to be the more predominant and stable natural form. Cis-isomerisation can occur when the trans-isoform is exposed to solar [3] or artificial light or ultraviolet radiation [4] at a wavelength of 254 [5] or 366 nm [6]. Resveratrol can be found in some fruits, which are part of the human diet, such as blueberries, blackberries and peanuts [7, 8]. Resveratrol possesses a wide range of biological properties, among them antioxidant, cardio protective, neuroprotective, anti-inflammatory and anticancer activities [9, 10] inducing cellular responses such as cell cycle arrest, differentiation, apoptosis and to enhance cancer cells anti-proliferation [11-13].

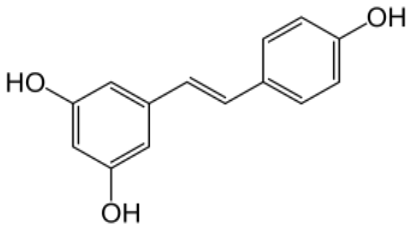

Figure 1: Structure of Resveratrol

MATERIALS AND METHODS

Instrumentation

Isocratic HPLC system (CYBER LAB 22 HPLC Model-LC-100 with Chrom work station Software) containing Luna column (150*4.6) 5µ & xterra column (150*4.6,5µ) with Auto Sampler and DAD or UV detector.

Chemicals

Resveratrol test and Working standards was purchased from the Vital herbs. Water, methanol, Orthophosphoric acid of HPLC Grade were used for the method development. All the chemicals used were analytical grade.

Chromatographic conditions

The mobile phase was prepared by weighing 2.72 grams of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. The pH was adjusted to 2.8 with Orthophosphoric acid. Mix a mixture of above buffer 300 ml (30%) and 700 ml of Methanol HPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration. The analysis was carried out on Isocratic HPLC system (CYBER LAB 22 HPLC Model-LC-100 with Chrom work station Software). The analytes were conducted on an Luna column (150*4.6) 5µ & xterra column (150*4.6,5µ) with Auto Sampler and DAD or UV detector with 306 nm. Ambient operating temperature of the column was set at 30°C. The injection volume was 15 µL, and the flow rate was maintained at 1.0 mL/min. The run time was 7 minutes.

Method development for the estimation of Resveratrol by using HPLC

Preparation of the Resveratrol Standard & Sample Solution

Standard Solution Preparation

The stock solution was prepared by weighing 10 mg of Resveratrol into a 100ml clean dry volumetric flask about 70ml of diluent was added and sonicated to dissolve it completely, the volume was made upto the mark with the same solvent. Further 4ml of above stock solution was pipetted into a 10ml volumetric flask and diluted upto the mark with diluent.

Sample Solution Preparation

The stock solution was prepared by weighing 10 mg of Resveratrol sample into a 100ml clean dry volumetric flask, about 70ml of diluent was added and sonicated to dissolve it completely and the volume was made upto the mark with the same solvent. Further 4ml of above stock solution was pipetted into a 10ml volumetric flask and diluted upto the mark with diluent.

Procedure

Inject 15 µL of the standard and sample solution of Resveratrol into the chromatographic system and measure the areas for the Resveratrol peaks and calculate the % assay.

Analytical Method Validation

Method was validated as per ICH guidelines and the validation parameters include linearity, accuracy, precision, robustness, limit of detection and limit of quantification [14, 15].

Precision

Precision of a measurable technique is the degree of agreement among individual tests, when the technique is applied repetitively to analyze multiple replicates in three different occasions. The standard solution was injected for five times and measured the area for all six injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The intraday precision was assessed by analyzing the calibration curves of six replicates of different concentrations of Resveratrol within the same day. The inter-day precision was determined by analyzing of six replicates of different concentrations of Resveratrol on three different days. The total precision of the method was expressed as the relative standard deviation (%RSD). In the current method development and validation protocol, precision was determined by six replicate analyses at a concentration of 40 µg/mL of standard Resveratrol solution using the developed method and % RSD ≤ 2% was accepted.

Accuracy

Inject the standard solution, at a concentration of (50%, 100% and 150%) 20, 40, 60 µg/mL solutions. The percentage recovery of added Resveratrol and RSD were calculated for each of the replicate samples.

Linearity

Inject each level into the chromatographic system and measure the peak area. Plot a graph with peak area on Y-axis versus concentration on X-axis and the correlation coefficient was calculated. Then linearity was evaluated using the calibration curve to calculate coefficient of correlation, slope and intercept. In general, a value of correlation coefficient (r2) > 0.998 is considered as the evidence of an acceptable fit for the data to the regression line.

Limit of Detection (LOD) & 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 precision and accuracy[16]. The limit of detection (LOD) is the concentration that gives a signal-to-noise ratio of approximately 3 : 1, while the limit of quantification (LOQ) is the concentration that gives a signal-to-noise ratio of approximately 10 : 1 with %RSD (n=3) of less than 10%.

Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions.

RESULTS AND DISCUSSION

Precision

The precision refers to the variability of the results in repeated analyses of the sample under identical experimental conditions. The methods were validated by evaluating the intra and inter day precision. The precision was calculated from an average of five determinations of a homogeneous sample. The intra and inter-day precision assays were expressed as %RSD 0.24 and 0.15 respectively, indicating that the method exhibits a good precision.

Table 1: Intraday precision of Resveratrol

S.No.	Name	RT	Area	Height (µV)
1	Resveratrol	2.423	693877	117760
2	Resveratrol	2.424	696531	117366
3	Resveratrol	2.424	693977	117612
4	Resveratrol	2.424	695278	117573
5	Resveratrol	2.423	697676	117829
Mean			**695468**	
Std. Dev			**1642.7**	
% RSD			**0.24**	

Table 2: Interday precision of Resveratrol

	Name	RT	Area	Height (µV)
1.	Resveratrol	2.423	693078	117646
2.	Resveratrol	2.424	693338	117177
3.	Resveratrol	2.424	695080	117534
4.	Resveratrol	2.424	694843	117535
5.	Resveratrol	2.423	695336	117665
Mean			**694335**	
Std. Dev			**1047.5**	
% RSD			**0.15**	

Accuracy

Accuracy is one of the most important parameters of an analytical methodology and can be expressed as the percent recovery of known amounts of drug added to a sample. The recoveries were determined by adding known amounts of the Resveratrol standard substance (20.0 µg.mL-1, 40.0 µg.mL-1 and 60.0 µg.mL-1).

Linearity

The peak areas obtained from the HPLC were plotted against concentrations to obtain the calibration graph. The result of linearity study has given a linear relationship over the concentration range of 20 - 60 µg/ml for Resveratrol. From the regression analysis, a linear equation was obtained: goodness-of-fit (r2) was found to be 0.999, indicating a linear relationship between the concentration of analyte and area under the peak.

Table 3: Accuracy results for Resveratrol

S. No.	Name	50%			100%			150%		
		RT	Area	Height (µV)	RT	Area	Height (µV)	RT	Area	Height (µV)
1.	Resveratrol	2.431	726104	158416	2.433	1376694	179922	2.439	2114604	189510
2.	Resveratrol	2.429	729450	157801	2.433	1377029	180126	2.439	2114196	189758
3.	Resveratrol	2.430	729306	157865	2.436	1380876	181017	2.441	2117641	189550
Mean			**728287**			**1378200**			**2115480**	
Std. Dev			**1891.6**			**2324.1**			**1882.2**	
% RSD			**0.25**			**0.17**			**0.08**	

Table 4: Linearity results for Resveratrol

S. No.	Name	Conc. (µg/ml)	RT	Area	Height (µV)
1	Resveratrol	20	2.422	264840	47778
2	Resveratrol	30	2.426	491451	86412
3	Resveratrol	40	2.426	677620	116697
4	Resveratrol	50	2.430	873311	139497
5	Resveratrol	60	2.433	1048958	157990

Limit of Detection (LOD) & Limit of Quantification (LOQ)

The results showed an LOD and LOQ for Resveratrol as 2.94 and 9.8 µg respectively and found within the specified limits.

Robustness

The results of robustness testing showed that a minor change of method conditions, such as the composition of the mobile phase, temperature, flow rate and wavelength is robust within the acceptable limits. It was found that variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates, the method is robust even by change in the Mobile phase ±1.

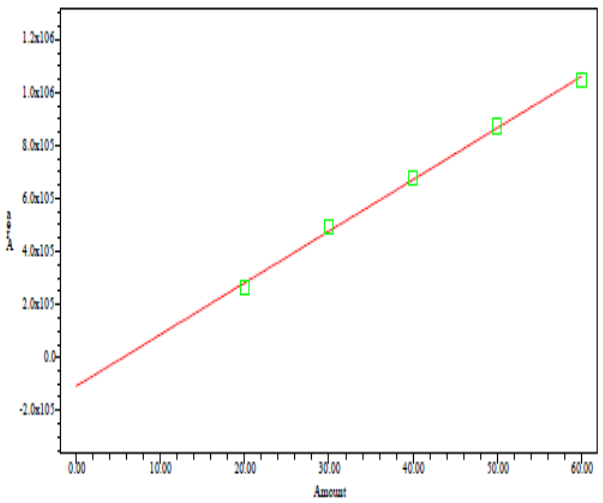

Figure 2: Standard Calibration curve of Resveratrol with a concentration range from 20 µg/ml to 60 µg/ml

Table 5: LOQ, LOD data for Resveratrol

Parameter	Name	Retention Time (Min)	Area (V*sec)	Height (V)	µg
LOD	Resveratrol	2.422	842	152	2.94
LOQ	Resveratrol	2.422	2810	507	9.8

Table 6: Robustness data for Resveratrol

S. No.	Name	Retention Time (Min)	Height (µV)	USP Plate count	USP Tailing
1	Resveratrol	2.646	118897	4187.6	1.5
2	Resveratrol	2.646	764483	4194.5	1.5
3	Resveratrol	2.230	637152	4084.7	1.4
4	Resveratrol	1.951	124442	3097.0	1.4

CONCLUSION

Resveratrol is a Nutraceuticals that has gained a lot of research attention due to its potential pharmacological effects found in many plants including grapes, peanuts and berries. In the present analytical research, a fast, simple, accurate, precise, and linear stability indicating HPLC method has been developed and validated for Resveratrol and hence it can be executed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for Resveratrol. In addition, the developed method has good retention time around 7 min. The method was validated in accordance with ICH guidelines. The method was robust enough to reproduce accurate and precise results under different chromatographic conditions. It may be extended to study the kinetics of Resveratrol and also for its concentration estimation in plasma and other biological fluids.

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Source of Support: None declared.

Conflict of Interest: None declared.