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Extraction of resveratrol from grape skin and determination by HPLC method

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ABSTRACT:
Resveratrol is a major polyphenol with many biological effects, including fighting cardiovascular disease, preventing cancer, and treating diabetes. Extra resveratrol is extracted from grape skins. This study presents a method to detect resveratrol species in grape skins using a high-performance liquid chromatography (HPLC) system.This method is capable of drug selectivity and quantification. The method was validated on a passive Cosmosil C18 analytical column (4.6 X 250 mm, 5 µm) with an equilibration phase consisting of methanol: water (70:30% v/v) at a pH of 3. The flow rate was 0.8 mL/min and the column was maintained at ambient temperature. The photodiode array detector was set at a wavelength of 306 nm. The calibration curve is linear in the concentration range of 10-50 ppm and the correlation coefficient (r2) is 0.999. The interday and intraday precision % RSD values were 0.1480% and 0.09095 %, respectively. The limit of detection and detection were determined to be 0.1329 µg/ml and 0.4028 µg/ml, respectively. This method is validated in terms of precision, accuracy, linearity, limit of detection, quantification of the method. Due to its accuracy, this method can be used for the estimation and analysis of resveratrol in active pharmaceutical products and drugs.
KEYWORDS: Resveratrol, Grape Skin, RP-HPLC, Method development, Validation.

INTRODUCTION:

Resveratrol (3,5,40-trihydroxystilbene) is a major polyphenol with many biological effects, including fighting cardiovascular disease, preventing cancer, and treating diabetes (Szkudelska and Szkudelski. 2010, Ginkel et al., 2007, Thirunavukkarasu, et al., 2001). It can be obtained

from several plants such as Arachis hypogea (peanut), Fellopia japonica and Vitisvinifera (grape) (Xiong Q et al., 2014, Zhang D, et al., 2009, Pascual-Marti 2001). Grapes are an everyday crop in China and are widely known for their edible fruits. Although much is known about the extraction of antioxidant polyphenols from grapes, seeds, and skins, little is known about the uses of grape leaves, which are usually discarded. Using grape leaves saves resources (Sun H. et al., 2018).

Various methods have been used to determine resveratrol in liquid samples, including gas chromatography-mass spectrometry, GC-MS, capillary electrophoresis, or the most common reverse phase liquid chromatography with UV detection, RP-HPLC (Sagratini G. et al., 2012, Ballus C. et al., 2012), by fluorescence detection (1) or mass spectrometry. These compounds can be easily measured by direct injection into alcohol and other beverages (Feij O. et al., 2008, Geana E, et al., 2015).

Although many studies have focused on the concentration of resveratrol in red wine, some studies have included trans-resveratrol content in the skin of the berry and some authors (Gurbuz O. et al., 2007, Inal E, et al., 2013). Resveratrol is determined by HPLC (mainly reverse phase) using UV detection, mass spectrometric detection, fluorescence detection or electrochemical detection. Thin layer chromatography has also been used. Gas chromatography, gas chromatography-mass spectrometry and capillary electrophoresis can also be used (Inal E, et al., 2013).

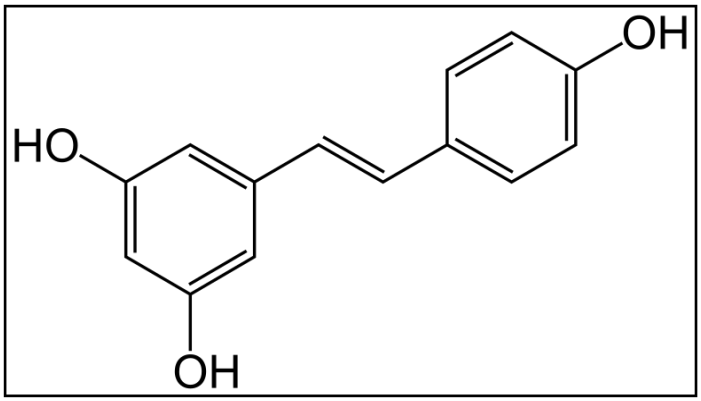

Figure 1: **Chemical structure of Resveratrol**

The most common sample treatment includes alcohol filtration (usually the guard column is placed before the separation column), liquid-liquid extraction (Lorrain B. et al., 2013) (the extract is usually dry and then concentrated), solid or liquid extraction of followed by separation, liquid-liquid extraction or solid-to-solid extraction (Lorrain B. et al., 2013, Cho Y. et al., 2006, Careri M. et al., 2003). Studies have been conducted on the superior extraction of polyphenol compounds in a sterile matrix, ethyl acetate or methanol modifier (30%), extraction temperature 35 °C and dynamic extraction time 15 min (Lee H. et al., 2012). In another study, CO2 was extracted with 5% methanol and the extraction parameters were 50 °C, 350 bar and the extraction time was 15 min. Phenolic compounds are also extracted from natural sources such as olive leaves (Tian Y. et al., 2017, Kalogiouri N. et al., 2022). However, to our knowledge, there are no articles on the extraction of these compounds from grape skin samples and from natural sources.

This report describes an experimental method to measure resveratrol in grape skins by drying the sample using HPLC measurement with CO2 and UV ultraviolet detection (Kalogiouri N. et al., 2022).

The aim of this study is to: (a) develop a suitable method for the determination of resveratrol in grape skins; (b) Quality control of excess water extraction (SFE) for a potential production

process for the extraction of resveratrol from grape skins. (c) Determine the amount of resveratrol in different grape varieties to establish a correlation between grape varieties and resveratrol content.

MATERIAL AND METHODS:

Chemicals and reagents:

All the solvents used for research work were of 'HPLC grade' and were of 'Merk' company.

Instrumentation:

The HPLC binary gradient system used for method development and validation consisted of an HPLC 3000 series (Analytical Technologies Ltd.), UV visible detector. 2012 (Analytical Technologies Limited), Weighing Balance (PGB 100) for sample weighing, Vansor Ultra Sonicator (WUC-4L) used for sample preparation.

Preparation of stock Solution:

Standard Stock solution preparation of 1000 ppm of individual drug.

10mg of pure drug dissolved in 10ml of solvent (solvent was used as your mobile phase only); this gives 1000ppm solution. The 12.272 mg was weighed and dissolved it into 10 ml to get 1000 ppm of solution and further dilutions were prepared as above.

Table 1: Preparation of standard stock solution

Sr. No.	Name of Drugs (conc.)	Volume of stock solution taken (ml)	Final Solution Volume (ml)
1	Resveratrol 10 ppm	0.1	10
2	Resveratrol 20 ppm	0.2	10
3	Resveratrol 30 ppm	0.3	10
4	Resveratrol 40 ppm	0.4	10
5	Resveratrol 50 ppm	0.5	10

Preparation of sample solution:

Accordingly weigh 20 tablets, crushed with a mortar and pestle in a clean, dry 10 ml volumetric flask equal to 10 mg Resveratrol sample (marketed formulation) and add approximately 7 ml of mobile phase until completely dissolved and achieve the desired volume. Label with the same solvent. Another 1 mL of the above solution was pipetted into a 10 mL volumetric flask and diluted to volume with the mobile phase.

Table 2: Recovery studies sample preparation

Sr. No.	Recovery	Conc. Of formulation (ppm)	Conc. Of Std. (ppm)	Combined conc. (ppm)
1	50% Recovery	20	10	30
2	100% Recovery	20	20	40
3	150% Recovery	20	30	50

Chromatographic (HPLC) condition:

Resveratrol was separated on anCosmosil C18 (4.6 X 150 mm) 5 µm particle size analytical column. Samples were extracted in a mobile phase of methanol and water pH 3 solution (70:30% v/v) at a flow rate of 0.8 ml/min. The vehicle model and column temperature are set to ambient

conditions. An injection volume of 20 µl was used and the detector was set at a wavelength of 306 nm, and pressure is 12-13 MPa.

Method Validation:

The optimized chromatographic conditions were evaluated for specificity, range, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system compatibility parameters according to ICH Q2 (R1) guidelines (Maithani M. et al., 2023).

RESULTS AND DISCUSSION:

Here, we developed a simple and reproducible method to measure Resveratrol using reverse-phase high-performance liquid chromatography. Using this method is very cheap and environmentally friendly.

Selection of wavelength:

Typical HPLC chromatograms of grape skins and red wines are presented in Figs. 1 and 2, respectively.The UV spectrum of resveratrol solution shows absorption maxima at 250 and 306 nm (Figure 2). Chromatograms of probes at wavelengths of 250 and 306 nm are shown in Figure 3. The wavelength of 306 nm was chosen as a suitable wavelength to avoid interference due to TFA with a symmetrical peak response due to its clear and flat base

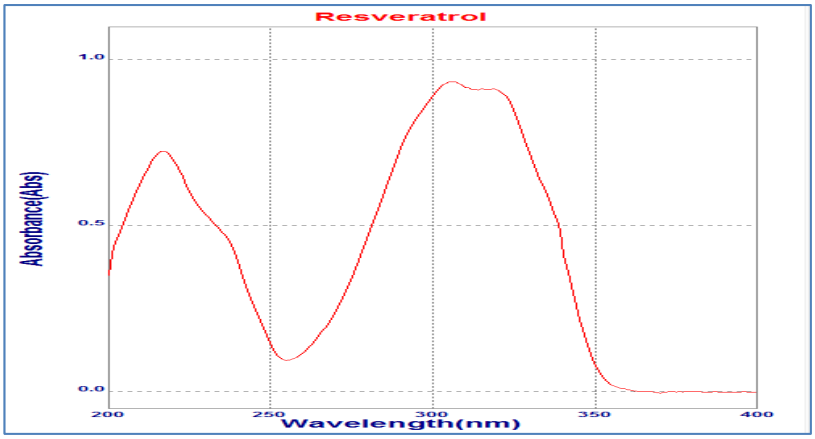

Figure 2: UV Spectrum of Resveratrol

Sample Name : Resveratrol
Mobile Phase : Methanol : Water (70 : 30)
Flow rate : 0.8ml/min
Wavelength : 306nm
Injection Volume : 20ul
Pressure : 12-13MPa

Method validation:

The optimized chromatographic conditions were evaluated for specificity, range, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system compatibility parameters according to ICH Q2 (R1) guidelines (Maithani M. et al., 2023).

Percentage Assay study:

20 microliters of Resveratrol sample and standard solutions were injected into three chromatographic systems. The peak area of each injection was measured. The concentration is calculated by comparing the peak area of the standard chromatogram with the sample chromatogram using the following formula:Consider the standard areas from linearity of

reported concentration and get the sample areas from assay folder.The obtained results are shown in the table 3.

Table 3: Results of % of assay

Sr. No.	Conc.	Area of standard	Area of sample	% assay
1	30 ppm	1047865	1050072	100.21062

System suitability parameters:

System Suitability Parameters are the standards to compare your results with the aproximate standard values. They includes as follows:

1. Resolution: Resolution value should be greater than 1.75. This parameter is applicable only when there is a combination of two samples. In case of single sample it will show zero '0' value.

2. Theoretical Plates: Number of theoretical plates should be greater than 2000. It indicates the efficiency of column.

3. Tailing/Asymmetry Factor: Value of asymmetry factor should be less than 2.

All the above required values are already reported in the individual spectra, no need to calculate them.

Linearity:

Resveratrol calibration standard solutions at concentrations of 10, 20, 30, 40 and 50 µg/ml were prepared and injected into the chromatographic system. Linear regression was used to plot the calibration curve of Resveratrol peak area (y-axis) versus concentration (x-axis). Each peak area is used to calculate the correlation coefficient (r2) 8.9. The linear results are shown in Table 4 and Figure 3.

Table 4: Linearity levels preparation assay of Resveratrol.

Concentration	Area
10	316207
20	666577
30	1047865
40	1398361
50	1737610

The values of Conc. and Area in the given column and get the Linearity graph with R sq. value.
Limit: The 'R'sq. value should be near to 1
y=mx+c

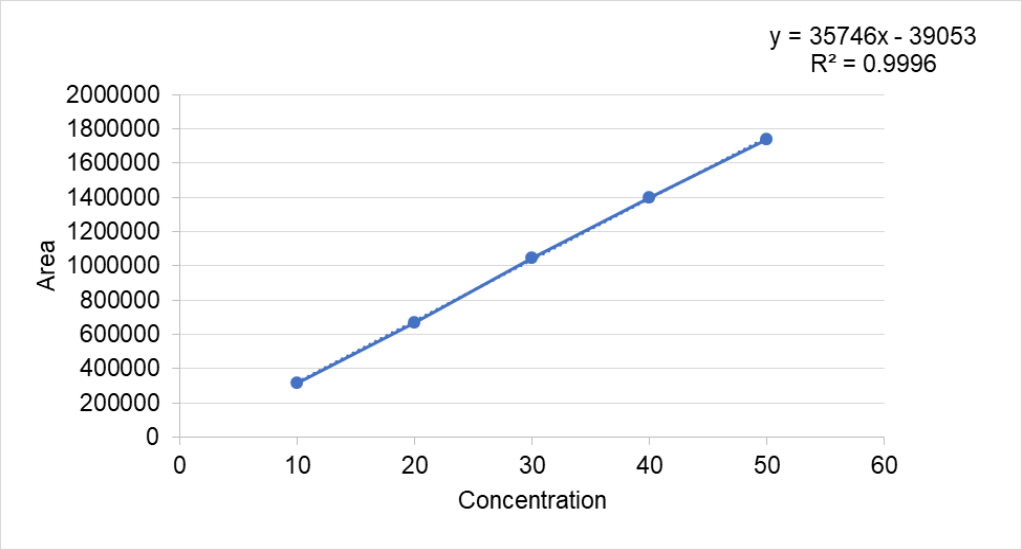

Figure 3: Calibration plot obtained for assay of Resveratrol

Accuracy (recovery):

The accuracy of this method was determined by calculating the recovery value of Resveratrol by the standard addition method. Specific volumes of 50, 100, and 150% Resveratrol standard solution were added to the predetermined Resveratrol sample solution and injected into the chromatographic system. Each standard solution was prepared and analyzed in triplicate. The peak area of each point was used to calculate the recovery rate. The results were done according to ICH guidelines. According to ICH guidelines, the recovery rate should be between 96-101%. The results are summarized in Table 5.

Table 5: Accuracy Data for Resveratrol

Resveratrol			**Standard Deviation**		**Accuracy**	**Precision**
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**% SD**	**%RSD**
1	10	316207	315004.00	1596.3386	0.5067677	
	10	313193				
	10	315612				
2	30	1047865	1046175.333	1534.0437	0.1466335	0.22855187
	30	1045791				
	30	1044870				
3	50	1737610	1736945.333	1440.047337	0.0829069	
	50	1735293				
	50	1737933				

Limit: %SD and %RSD value should be less than 2%

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

The limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 3.3 × SD/S and 10 × SD/S, respectively, according to ICH guidelines, where SD is the standard deviation of the response (Y-intercept) and S is the slope of the calibration curve. The LOD is the lowest analyte concentration that gives a measurable response (signal-to-noise ratio of 3). The LOQ is the lowest analyte concentration that gives a definite and measurable response (signal-to-noise

ratio of 10). The calculated LOD and LOQ values are shown in Table 6. Take minimum Std. Deviation calculated from accuracy and slope from linearity.

LOD = 3.3 × S /SD

and

LOQ = 10 × S /SD

Table 6: Limit of detection (LOD) and limit of quantification (LOQ) data for Resveratrol

Sr. No.	Drug	SD	Slope	LOD	LOQ
1	Resveratrol	1440	35746	0.132937951	0.40284228

Precision:

The accuracy of the Resveratrol drug solution method was tested by increasing Six injection samples (30 ppm) containing the same concentration of 100 µg/ml Resveratrol drug solution prepared and injected into the chromatography system. The peak area of each injection was used to calculate percent RSD. To estimate the average precision, six injections with a concentration of 100 µg/ml Resveratrol were analyzed on different days by different analysts using different columns of the same parameters. Each injection area was used to calculate the %RSD is 0.14809003 % and 0.09095172 %. From the data obtained in Tables 7 and 8, the developed method was found to be accurate.

Table 7: Method precision for Resveratrol drug solution (Interday)

Interday			Standard Deviation		Accuracy	Precision
Sr. no.	Conc.	Area	Mean	SD	% SD	%RSD
1	30	1047865	1046175.333	1534.04378	0.1466335	0.14809003
	30	1045791				
	30	1044870				
2	30	1042612	1045632.333	3723.12508	0.3560645	
	30	1049792				
	30	1044493				

Table 8: Method precision for Resveratrol drug solution (Intraday)

Intraday			Standard Deviation		Accuracy	Precision
Sr. no.	Conc.	Area	Mean	SD	% SD	%RSD
1	30	1047865	1046175.333	1534.04378	0.1466335	0.09095172
	30	1045791				
	30	1044870				
2	30	1051767	1049810.000	2889.69324	0.2752587	
	30	1046491				
	30	1051172				

% Recovery:

Consider the standard areas from linearity of reported concentration and get the sample areas from % recovery folder.

Table 9: Method % recovery for Resveratrol

Sr. No.	% Composition	Area of Standard (Area Units)	Area of Sample (Area Units)	% Recovery (%)	Conc. Taken (ppm)	Conc. Found (ppm)
1	50% Recovery	1047865	1045654	99.7889	30	29.9366
2	100% Recovery	1398361	1391789	99.5300	40	39.8120
3	150% Recovery	1737610	1735276	99.8656	50	49.9328

Robustness:

The Robustness of a method is its abilityto remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phasecompositionandflowrate,wavelengthonretentiontimeandtailingfactorofdrugpeak was studied. The pH of mobile phase was changed in(±0.2) proportion and the Wavelength was changed by± 2 nm of optimized chromatographic condition.

Table 10: Method robustness for Resveratrol

	Conc.	Area	Mean	SD	%SD
Change in Wavelength	20	666577	666655.7	3088.75	0.4633204
	20	669783			
	20	663607			
Change in pH	20	666577	666348.0	386.294	0.0579718
	20	665902			
	20	666565			

Ruggedness:

Standard preparation, stock preparation and sample preparation of Resveratrol were prepared according to the methodology given in Part IV. Samples were incubated with standard solutions under different chromatographic conditions as described below

Table 11: Method of Ruggedness for Resveratrol

Concentration	Area
10	313350
20	662738
30	1052781
40	1392806
50	1740106

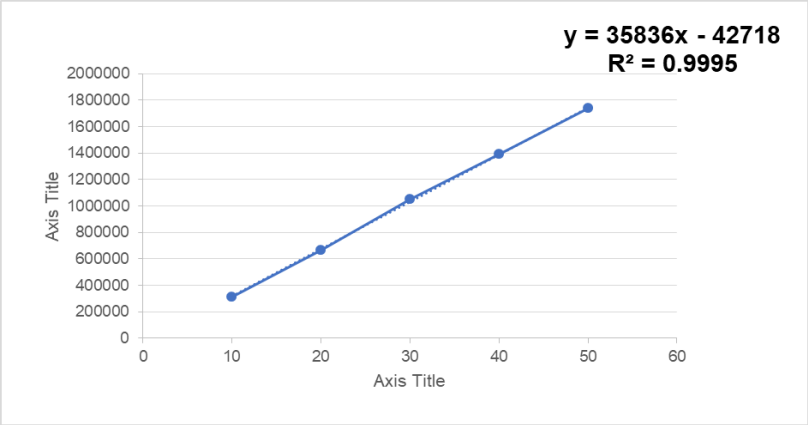

Figure 4: Calibration plot obtained for Ruggedness of Resveratrol

CONCLUSION:

Supercritical fluid extraction of resveratrol from grape skin samples results in a short purification time and sufficient precision and recovery for HPLC analysis using a C-8 column. The proposed method is suitable for continuous analysis in quality control of the production process. Analysis of the samples showed that the type of grape is an important factor in the content of resveratrol.A accurate isocratic RP-HPLC method was developed and validated for the determination of resveratrol drug dosage according to ICH guidelines. Linearity was obtained for resveratrol at 10- 50 µg/ml and the correlation coefficient (r2) was 0.999. The recovery rate with acceptance criteria is from 98 to 102 percent. The RSD percentage is less than 2%, which proves the accuracy of the developed method. The advantage of this method is the accurate retention time and high sensitivity. The effect of organic composition on mobile phase flow rate and system parameters was studied to obtain optimal chromatographic conditions. The results obtained show that the developed method is valid in terms of accuracy, precision, linearity, LOD and LOQ and robustness. Therefore, this method can be used for systematic screening of resveratrol drugs in pharmaceuticals.

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