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Spectrometric and RP-HPLC method for the determination of trans-resveratrol in vegetarian formulation

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ABSTRACT

Estimation of Trans-resveratrol was achieved by Spectrometric method. After considering the solubility studies, distilled water was selected as a solvent for the UV analysis. Trans-resveratrol 10µg/ml solution was prepared and scanned in the UV region from 180-380nm. From the spectrum, 307nm was selected as an analysing wavelength. Calibration curve was plotted by using concentration vs absorbance. From the calibration curve it was found that Trans-resveratrol obeys Beer's and lambert's law in the concentration range of 2-10µg/ml, molar absorptivitywas0.06938cm/mole, sandell's sensitivity was 0.014442µg/cm2, correlation coefficient was 0.9977, LOD: 0.0665µg/ml, LOQ: 0.2016µg/ml were calculated. The amount of Trans-resveratrol in the vegetarian formulation was found to be 123.14mg/capsule.

Trans-resveratrol was determinedby RP-HPLC method with external standard calibration method. Optimizations of chromatographic parameters were done. A wavelength of 305nm was selected as a detection wavelength. The mobile phase composition consisting of Acetonitrile: Phosphate buffer (pH-5) in the ratio of 28:72%v/v was selected for this proposed method.

1. INTRODUCTION

Trans-resveratrol is used as anti-oxidant, Anti-inflammatory, Anti-atherosclerotic, Anti-angina and Anti-cancer agent. Its solubility in water is 0.03g/l, In DMSO its solubility is 16g/l and in ethanol its solubility is 50g/l.

Trans-resveratrol is rapidly metabolized in intestine and liver into conjugated forms: glucuronate and sulfonate.

2. MATERIALS AND METHODS

Methanol, Diethyl ether, Chloroform, Acetone, Ethanol, Water, Acetonitrile, Buffer solutions of pH 5 & 3.5 were used. All the solvents and reagents used were HPLC grade, purchased from Merc.

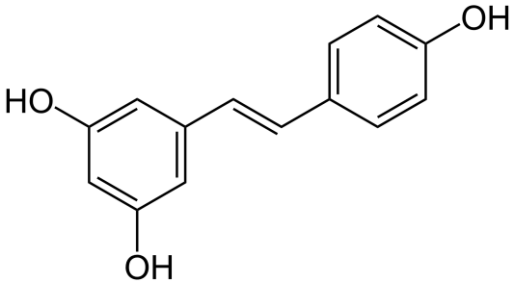

Figure.1.Trans-resveratrol

Instruments used were UV-VIS Double Beam Spectrophotometer of LabIndia, India. Model: UV 3600 and
HPLC of Shimadzu, 180(LC-10AT Vp series).

Solubility and selection of solvent: trans-resveratrol is soluble in Ethanol, Water, Acetonitrile and DMSO. Then different dilution of solvents was used to check solubility of trans-resveratrol. Finally water was selected to make dilution for assay method in the present work.

Preparation of Standard stock solution: Standard drug solution of trans-resveratrol was prepared by dissolving 5mg Trans-resveratrol in 50ml of standard flask and volume was made up using the distilled water to get a concentration of 100µg/ml.

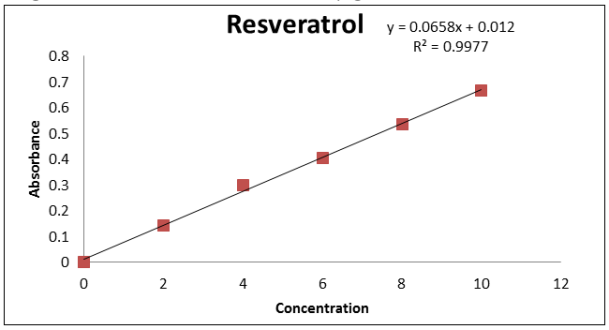

Figure.2.Calibration curve of Trans-resveratrol
The linearity range 2-10µg/ml was prepared and absorbane was determined and it obeys beer's law

Table.1.Optimized parameters Spectrometry

Sensitivity & Regression Parameters	Results
λmax(nm)	307
Colour stability	>48 hours
Beer's law limit or linearity range (µg/ml)	2-10
Molar absorptivity (1mole^{-1}cm1)	0.06938
Sandell's sensitivity, µg/cm^2/0.001	0.014442
Coefficient of Regression	0.165067
Standard deviation	0.20263
Relative standard deviation	0.4952
Correlation coefficient(R^2)	0.9977
Regression Equation (y=mx+b, m is slope, b is intercept)	Y = 0.0658x + 0.012
Limit Of Detection (µg/ml)	0.0665
Limit Of Quantitation (µg/ml)	0.2016

Preparation of solutions for HPLC:

Preparation of phosphate buffer: 6.8gm of Potassium hydrogen phosphate was dissolved in 1000ml of water and adjusted with at pH to 5 with 10M potassium hydroxide.

Standard preparation: Weigh accurately about 25 mg of Trans-resveratrol working standard working standard into 25ml volumetric flask and add 10 ml of solvent system. Shake well and make up to 25ml with solvent. Transfer 1ml of the above solution into 100ml volumetric flask and make up with mobile phase up to 100ml with mobile phase, 10 µg/ml of solution were prepared and again take 5ml from the above 10 µg/ml containing solution and made up the concentration 5 µg/ml for the analysis.

Sample preparation: Weigh 10 capsules. From the capsule powder, weigh accurately about 124.1mg of capsule powder into a 25 ml volumetric standard flask, and add 15 ml, and make up to 25 ml with mobile phase. Shake well and filter the solution. From the above filtrate pipette out1 ml into a 100 ml standard volumetric flask and make up to 100 ml with mobile phase, 10 µg/ml of solution were prepared and again take 5ml from the above 10 µg/ml containing solution and made up the concentration 5 µg/ml for the analysis.

Table.2.Optimized Chromatographic Conditions

Parameters	Information
Stationary phase Column	C_{18} phenomenex-Luna (4.6X250 mm)
Mobile phase	Acetonitrile: Phosphate buffer pH 5 (28:72)
Wave length	305nm
Flow rate	2.0ml/mint
Injector	Rheodyne
Column temperature	Ambient temperature
Volume Injection loop	20µl
Run time	6 mintes

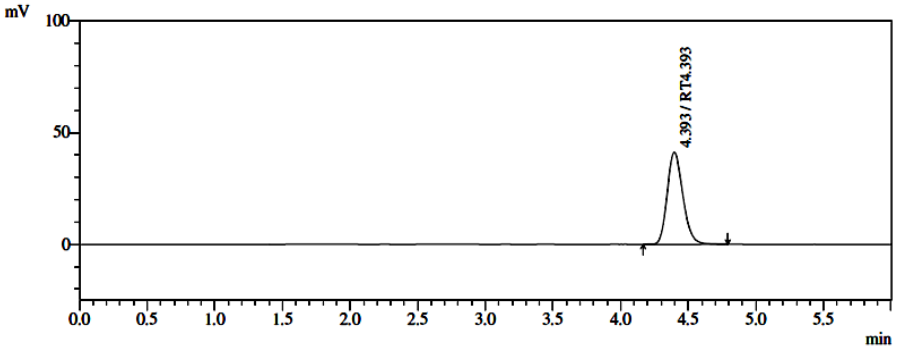

Figure.3. Chromatogram of Trans-resveratrol at optimized conditions

3. RESULTS AND DISCUSSION

The two simple, precise, accurate and reproducibility methods were developed for the quantitative and qualitative analysis of *trans-resveratrol* in standard drug and capsule solid dosage formulation. The methods are:

- Spectrometric method.
- RP-HPLC method.

Spectrometric method: The solubility of *trans-resveratrol* was determined. From the solubility distilled water was selected as a solvent for simple Spectrometric method because of solubility and availability of solvent.

Trans-resveratrol was dissolved in distilled water and diluted to get a concentration range of 8µg/ml and the solution were scanned at UV region in the wavelength range from 200-400nm against distilled water as blank with 1cm path length. The wavelength spectrum of trans-resveratrol in distilled water was recorded and from the spectrum of trans-resveratrol the wavelength maximum wavelength was found to be 307nm. Hence, this 307nm wavelength was selected for routine analysis.

Different concentrations of trans-resveratrol in distilled water were prepared and absorbance was measured at 307nm. The calibration curve was plotted using concentration Vs absorbance. The curve obtained was linear with the range of 2-10µg/ml. the correlation co-efficient value for the calibration graph was found to be 0.9977. It indicates that the concentration of trans-resveratrol has good linearity. Stability studies of the prepared solutions indicates that the prepared solutions were stable for 2 hours.

RP-HPLC method: An effort has been made to develop a simple, precise, specific and accurate HPLC method for the estimation of Trans-resveratrol in crude drug and the drug formulation.

The optimization was done by changing the mobile phase and ratio of mobile phase. The different mobile phase in various ratio were tried and chromatograms were recorded for Trans-resveratrol. The most suitable mobile phase to the analyte was selected for further analysis. The mobile phase selected for the analysis of Trans-resveratrol was Acetonitrile: phosphate buffer (pH-5) in the ratio 28:72.

Table.3.Comparative study of UV Spectrometric& RP-HPLC

Parameters	Limits	Observations		pass/Fail
		Spectrometric	RP-HPLC	
Wavelength	-------	307nm	305nm	-----
Specificity	No interference at retention time of analyte peak area or absorbance	No interference on absorbance of analyte	No interference at retention time of analyte peak.	Passes
Linearity range µg/ml	-------	2-10	0.5-1.5	------
Precision	RSD NMT 2.0%	0.1894	0.0352	passes
Linearity	Correlation co-efficient NMT 1.00	0.9977	0.9993	passes
Accuracy (%recovery range)	98-102%	98.29–99.689%	99.99 - 100.0002%	passes
Limit of detection (LOD)	Signalnoice ratio should be more than 3:1(µg/ml)	0.0665	0.01223	passes
Limit of quantitation(LOQ)	Signal noice ratio should more than 10:1(µg/ml)	0.2016	0.037068	passes

4. CONCLUSION

Two simple, rapid and accurate analytical methods spectrometricand RP-HPLC were developed and validated for the determination of trans-resveratrol and itsvegetarian formulation. Both the methods showed an excellent sensitivity, reproducibility, repeatability and accuracy, which is evidenced by percentage relative standard deviation. By comparing the results ofspectrometry and RP-HPLC methods, it was found that the values reported by RP-HPLC are more reliable and precise than Spectrometric method. Hence it is suggested that the proposed spectrometric and RP-HPLC methods can be effectively applied for the routine analysis of trans-resveratrol in crude drug and vegetarian formulation and other formulations.

REFERENCES

Abert Vian MV, Tomao S Gallet, Coulomb PO, Lacombe JM, Simple and rapid method for cis- and trans-resveratrol and piced isomers determination in wine by HPLC using Chromolith columns, J. Chromatography A, 1085 (2005) 224-229.

Adrian M, Jeandet P, Breuil AC, Levite D, Debord S, & Bessis R, Assay of resveratrol and derivative stilbenes in wines by directinjection high performance liquid chromatography, American Journalof Enology and Viticulture, 51(1), 2000, 37–41.

Alarcon de lastra and Villegas I, Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and

clinical implications. Biochemical Society Transactions. 35(5), 2007, 1156-1160.

Anupam Bishayee, Anti-tumour treatment Resveratrol in the Chemoprevention and treatment of hepatocellular carcinoma., Cancer Treatment Reviews, 36, 2010, 43–53.

Bailey JA, Mechanism of phytoalexin accumulation. In Phytoalexins, eds. J. A. Baily and J. W. Manfield, Wiley, New York, 1982, 289–318.

Beckett AH, and Stenlake JB, Text book of Pharmaceutical Chemistry, New Delhi, CBS Publishers and Distributors, 4th Edition, part 2, 1988, 157, 257-314.

Beulens JW, Alcohol consumption and risk of type 2 diabetes among older women, Diabetes Care, 28, 2005, 2933–2938.