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Development and Validation of RP-HPLC Method for Determination of Resveratrol and Curcumin

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ABSTRACT

Cancer chemopreventive agents are designed to reduce the incidence of tumorigenesis by intervening at one or more stages of carcinogenesis. Recently, resveratrol, a natural product found in the diet of humans, has been shown to function as a cancer chemopreventive agent. Resveratrol was first shown to act as an antioxidant and antimutagenic agent, thus acting as an anti-initiation agent. Resveratrol belongs to a class of polyphenolic compounds called stilbenes.(3,5,4'-trihydroxy-trans-stilbene) is a natural compound found in red grape skin, Japanese knotweed (polygonum cuspidatum), peanuts and blueberries. A naturally occurring polyphenolic phytoalexin Curcumin, [1,7-bis (4-hydroxy-3-methoxyphenyl)-1, 6- heptadiene-3, 5-dione] is the major yellow pigment extracted from turmeric, a commonly used spice, derived from the rhizome of *Curcuma longa* Linn. In animal studies, curcumin has been shown to increase the survival of tumor-bearing rodents by inhibiting tumor growth and impeding metastasis.

At present study a simple, precise, rapid and accurate reverse phase HPLC method developed for the estimation of Resveratrol and Curcumin in combination as well as individually were developed and validated. The retention time of Resveratrol and Curcumin were found to be 2.90 and 4.11 minutes. Detection was carried out at 424 and 306nm..The regression coefficient value of Resveratrol and Curcumin is 0.9904 and 0.9937 which was found to be linear in the detection range. Limit of detection and limit of quantification of Resveratrol was found to be 0.08µg/ml and 0.32 µg/ml and Curcumin is 0.05 µg/ml and 0.17 µg/ml. Analysis was performed using a C_{18} column (250 X 4.6 mm) at room temperature in isocratic mode. The mobile phase used was Citric acid (pH adjusted to 3.5): Acetonitrile (40:60) at flow rate of 1.0 ml/min.

Keywords: HPLC, Resveratrol, Curcumin, Validation.

INTRODUCTION

Resveratrol belongs to a class of polyphenolic compounds called stilbenes. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a natural compound. It is a powerful antioxidant produced by some plants to protect them against environmental stresses. Resveratrol, a naturally occurring polyphenolic phytoalexin, is present in many plants and fruits, including red grapes, eucalyptus, spruce, blueberries, mulberries, peanuts, and giant knotweed. Also, red wine contains a lot of it. Resveratrol is an effective antioxidant with strong antiinflammatory and antiproliferative properties. Resveratrol is a fat soluble compound that occurs in a trans and a cis configuration.[1]

Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (*Zingiberaceae*). Turmeric's other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are natural phenols that are responsible for the yellow color of turmeric. Curcumin can exist in several tautomeric forms, including a 1,3-diketo form and two equivalent enol forms. The enol form is more energetically stable in the solid phase and in solution. [2]

Increasing interest in the use of phytochemicals to reduce prostate cancer led to investigate two potential agents, curcumin and resveratrol as cancer chemo preventive agents. . Several *In vitro* assays studies using PTEN-CaP8 cancer cells were performed to investigate the combined effects curcumin with resveratrol on (*i*) cell growth, apoptosis and cell cycle (*ii*) impact on activated p-Akt, cyclin D1, m-TOR and androgen receptor (AR) proteins involved in tumor progression. And results of these studies indicated positive anticancer effects of these drugs[3-,4]. Thus in the present investigation simultaneous estimation method for these two drugs in combination was

developed and validated. And value was given to develop a simple, sensitive and repeatable estimation method.

EXPERIMENTAL

Preparation of standard stock solution Curcumin

For Curcumin: 100mg of Curcumin was accurately weighed and transffered to a 100ml volumetric flask and was diluted with acetonitrile (1000µg/ml) . (Stock A). Aliquots of Stock A were further diluted to get concentration of 10, 20, 30, 40 and 50 µg/ml.

Preparation of standard stock solution Resveratrol

For Resveratrol: 100mg of Resveratrol was accurately weighed and transffered to a 100ml volumetric flask and was diluted with methanol (1000µg/ml) . (Stock A). Aliquots of Stock A were further diluted to get concentration of , 20, 40, 60,80 and 100 µg/ml.

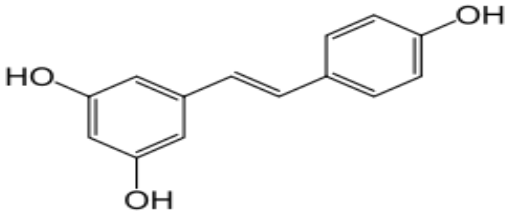

Fig 1: structure of Resveratrol

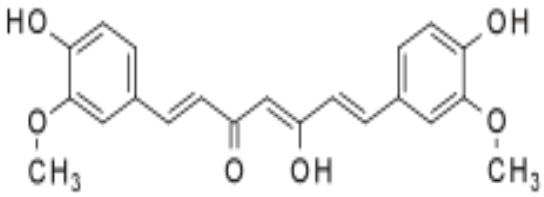

Figure 2: Curcumin

Chromatographic condition

The HPLC system included a Yung lin instrument 900' UV730D solvent delivery system (pump), injector with a 20µL loop volume. The class VP 6.01 data station software was utilized for integration. Separation was achieved using a Princeton C18 column (250 X 4.6 mm, 5µ ID), (Merck, India). The solvent system consists of Citric acid solution (pH 3.5): acetonitrile (40:60 v/v) was pumped isocratically at a flow rate of 1.0 mL/ Min. [5-7]

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solution of Resveratrol and Curcumin . Parameters that were studied to evaluate the suitability of the system are given in **Table 1**

Table 1: Results of calibration curve of Resveratrol

Sr no	Statistical analysis	Resveratrol
1	Concentration Range	20-100 µg/ml
2	Regression equation	Y=21.283x-41.619
3	Correlation Co-efficient	0.9904
4	Slope	21.28
5	Intercept	41.61

Table no.2: Results of calibration curve of Curcumin

Sr no	Statistical analysis	Curcumin
1	Concentration Range	10-50 µg/ml
2	Regression equation	Y=45.386x-13.778
3	Correlation Co-efficient	0.9937
4	Slope	45.386
5	Intercept	13.778

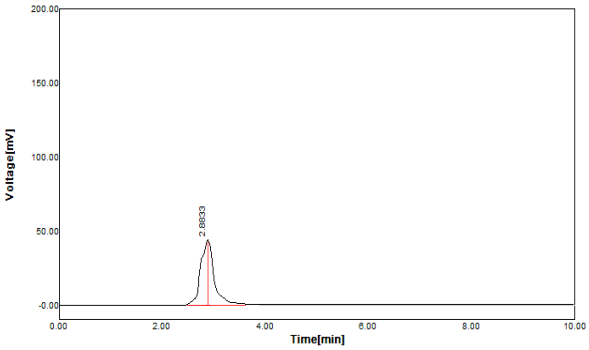

Fig 3: System suitability parameters: Resveratrol

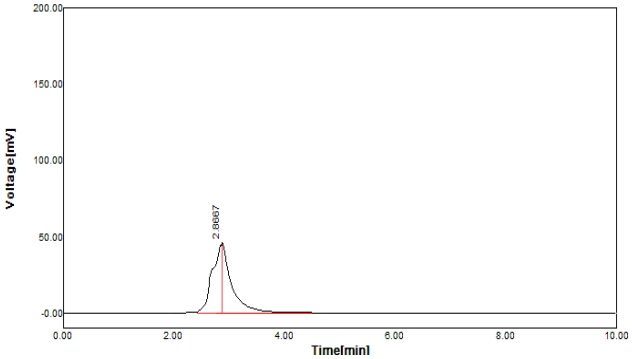

Fig 4: System suitability parameters: Curcumin

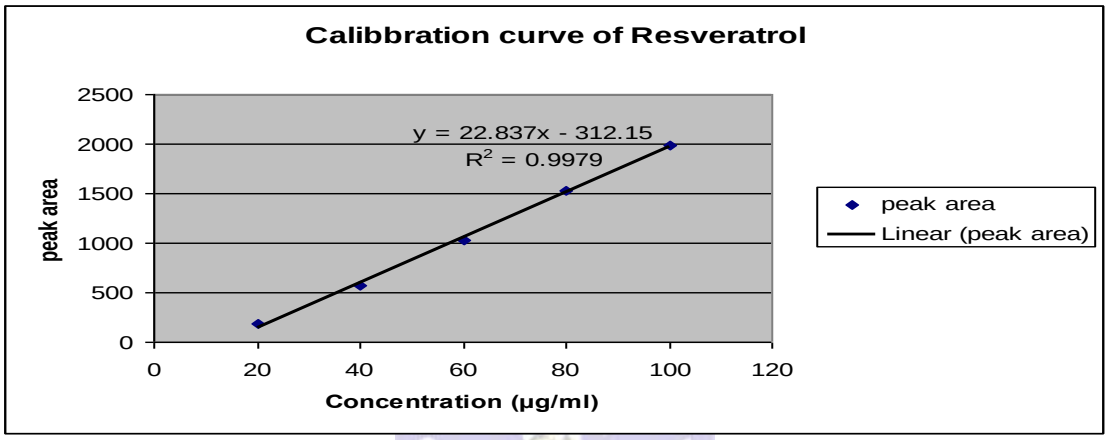

Fig :6 Calibration curve indicating linearity range of Resveratrol by HPLC

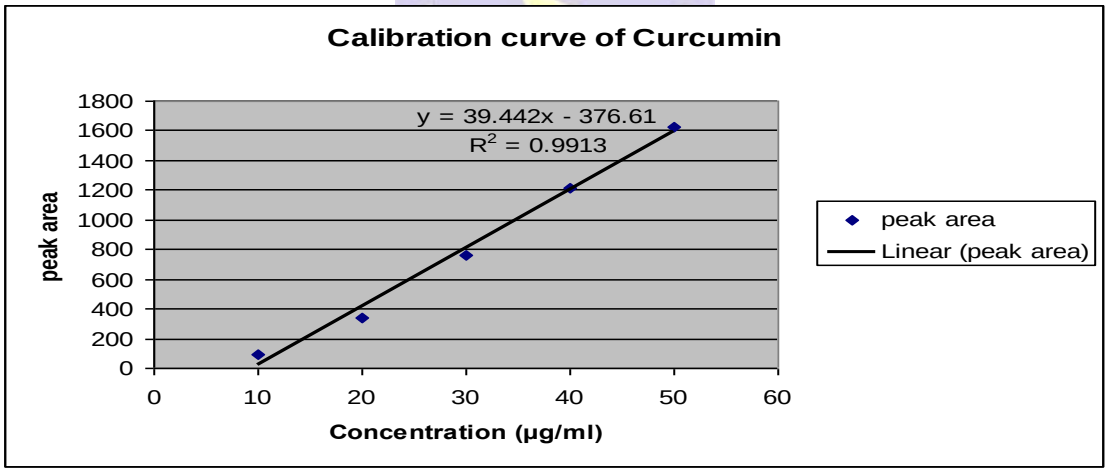

Fig 7 :Calibration curve indicating linearity range of Curcumin by HPLC

Chromatograms eluted from the developed method indicated the retention time of Resveratrol and Curcumin was 2.90 and 4.11 min respectively. A mixture of Acetonitrile and Citric acid solution (pH adjust to 3.5) in the ratio of 60:40 was found to be most suitable. The selected mobile phase was efficient in resolving two drugs to obtain well defined peaks free from tailing. In the present developed HPLC method, the sample preparation required less time . A good linear relationship for both the drugs in combination i.e for Resveratrol(r^2=0.9904) was observed between the concentration in the linear rang of 20-100µg/ml and that of Curcumin (r^2=0.9937) was between linear range of 10-50µg/ml.

Analytical Method Validation Parameters

The method was validated as per ICH guidelines (Q2 R1).

Linearity and Range

Linearity of method is its ability, within a given range, to obtain test results that are directly, or through a mathematical transformation, proportional to concentration of analyte . Good linear correlations were obtained between absorbance and concentration in the selected range of Resveratrol and Curcumin 10 –100 and 5-50µg/ml.

Precision

The precision of method expresses the degree of scatter between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Intraday precision refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment whereas Interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts . Repeatability (intraday) was assessed by analyzing these three different Concentrations of Resveratrol (20, 40, 60 µg/ml) Curcumin(10,20,30µg/ml) three times a day. Intermediate precision (Interday) was established by analyzing these three different concentrations of Resveratrol (20, 40, 60 µg/ml) Curcumin(10,20,30µg/ml), three times a day for at least three different days. The Standard Deviation, % RSD for the intra-day Resveratrol and Curcumin 0.96 and0.61.

Accuracy

Accuracy of method is the closeness of test results to true value. It was determined by the application of procedure to recovery studies, where known amount of standard is spiked in preanalyzed samples solutions .The % recovery of the Resveratrol and Curcumin 98.37% and 99.15% .

LOD and LOQ

LOD and LOQ were calculated according to the formulae:

For Resveratrol

LOD=3.3 σ / S =0.07µg/ml

LOQ=10 σ / S = 0.31µg/ml

For Curcumin

LOD=3.3 σ / S =0.04µg/ml

LOQ=10 σ / S = 0.16µg/ml

CONCLUSION

The development method of estimation indicates that it is simple sensitive and repetitive and major advantage being use of inexpensive solvents. The mixture of solvents used indicated well separation of peaks without any tailing effects. The developed method can be easily applied for routine analysis of these two drugs Resveratrol and Curcumin in free or formulation dosage forms.

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