A NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF RESVERATROL IN PURE AND PHARMACEUTICAL DOSAGE FORM

CHAITANYA MITTA, ADLA PRATHYUSHA, BOMMANA SAHITHI, SHAIK KHASIM, JULAPALLY SHREYA, SONIA AHMED*
Department of Pharmaceutical Analysis and Quality Assurance, Bojjam Narasimhulu Pharmacy College for Womens, Saroor Nagar West, Saidabad, Hyderabad, Telangana 500059, India

Abstract : *The objective of the present research work was to develop an innovative, simple, and economic method for estimation of Resveratrol in bulk and dosage form by RP-HPLC.The chromatographic conditions were performed on Symmetry C18, 250 mm x 4.6 mm and 5µm Column, Mobile Phase: Acetonitrile: Water (60:40), flow 1.0 ml/min with Injection Volume 20µl, at detection wavelength 275 nm, run time at 10 mins and Retention time was 5.776mins. The analytical method is valid for estimation of Resveratrol over a range of 0-35 µg/ml. The results of system suitability test, linearity, precision and accuracy, robustness, specificity, LOD and LOQ and stabilities presented in this report are within the acceptance range. A specific, sensitive, economic method estimation of Resveratrol has been developed based on ICH Guidelines with bulk and dosage forms.*
Key Words: *Resveratrol, HPLC, Method Development, ICH, Validation, Accuracy, Precision.*

I.INTRODUCTION

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.
The IUPAC Name: 5-[(E)-2-(4-hydroxyphenyl) ethynyl] benzene-1,3-diol.

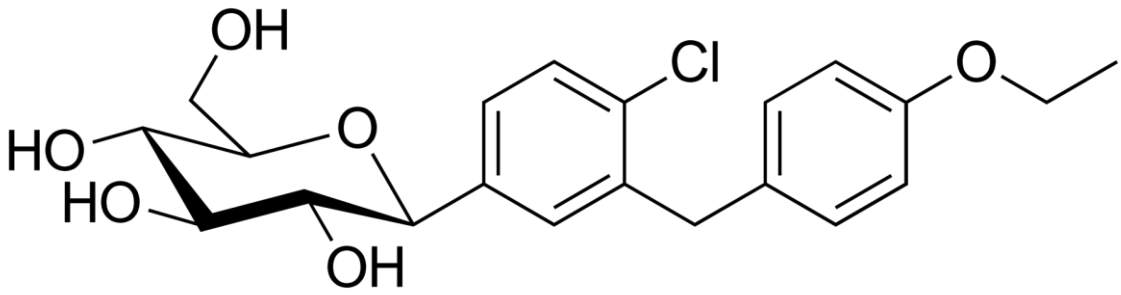


Fig-1: Structure of Resveratrol

II.EXPERIMENTAL

2.1 Materials and Methods:

Pharmaceutical grade working standard Resveratrol were obtained from Orchid Pharma from India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt Ltd, Mumbai, India.

2.2 Instrumentation:

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system EMPOWER2 software, UV-Visible double beam spectrophotometer (T60-LAB INDIA), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Symmetry C18 Column, 250 mm x 4.6 mm and 5µm (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

2.3 Sample & Standard Preparation for the Analysis

10 mg of Resveratrol standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilutions was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 100ppm concentration. Then, the final concentration was made by

transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration. It is scanned in the UV spectrum in the range of 200 to 400nm.

2.4 Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in methanol diluting with the same solvent. (After optimization of all conditions) for UV analysis. It is scanned in the UV spectrum in the range of 200 to 400nm. While scanning the Resveratrol solution we observed the maxima at 235 nm.

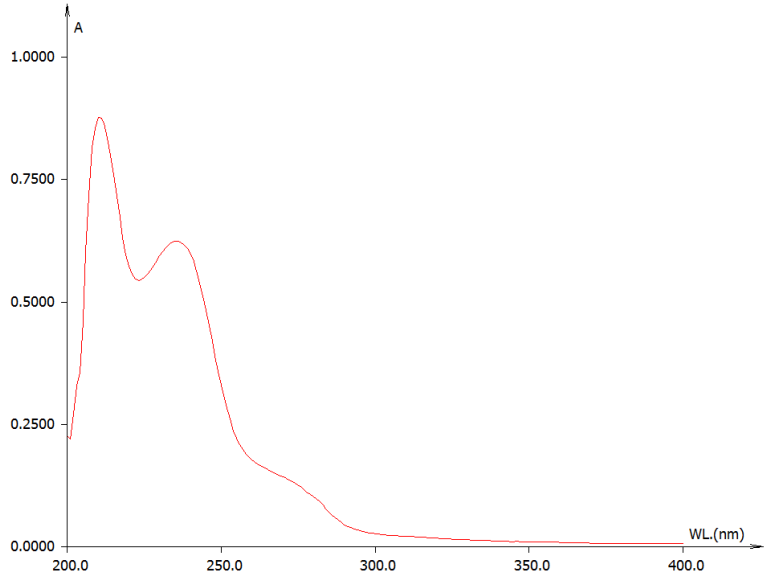


Fig-2: UV Spectrum for Resveratrol

2.5 Method Development

2.5.1 Preparation of Mobile Phase:

420ml of HPLC Grade Acetonitrile and 580ml of HPLC Grade Methanol were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

2.5.2 Summary of Optimized Chromatographic Conditions:

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-1: Summary of Optimized Chromatographic Conditions

Mobile phase	Acetonitrile: Methanol = 42:58
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	235nm
Flow rate	1.0 ml/ min.
Run time	08 min.
Temperature of Auto sampler	Ambient
Diluent	Methanol

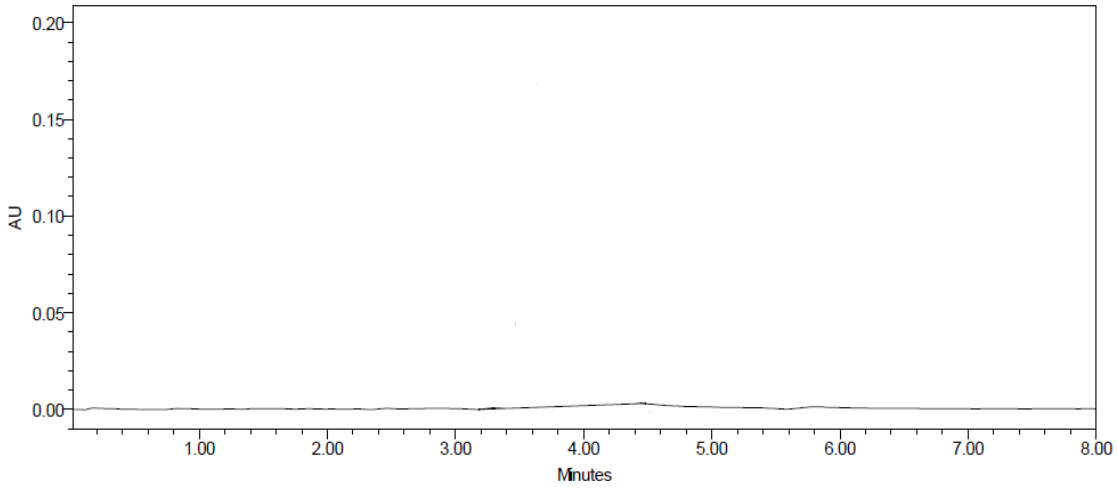
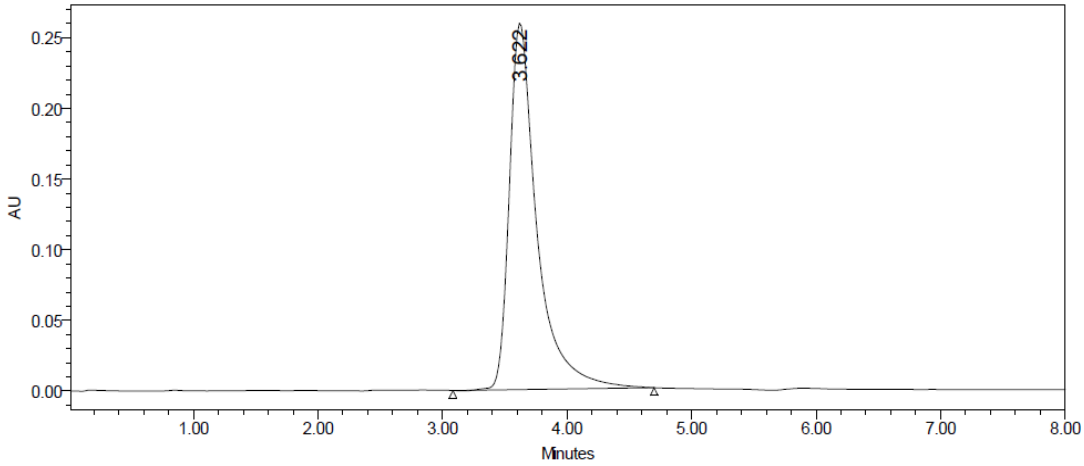


Fig-3: Chromatogram for Blank Preparation



Fig–4: Chromatogram of Resveratrol in Optimized Condition

2.6 Method validation:

2.6.1 Linearity & Range:

Calibration standards at five levels were prepared by appropriately mixed and further diluted standard stock solutions in the concentration ranges from 0-12µg/mL for Resveratrol. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the linearity graphs. Chromatograms of each solution were recorded.

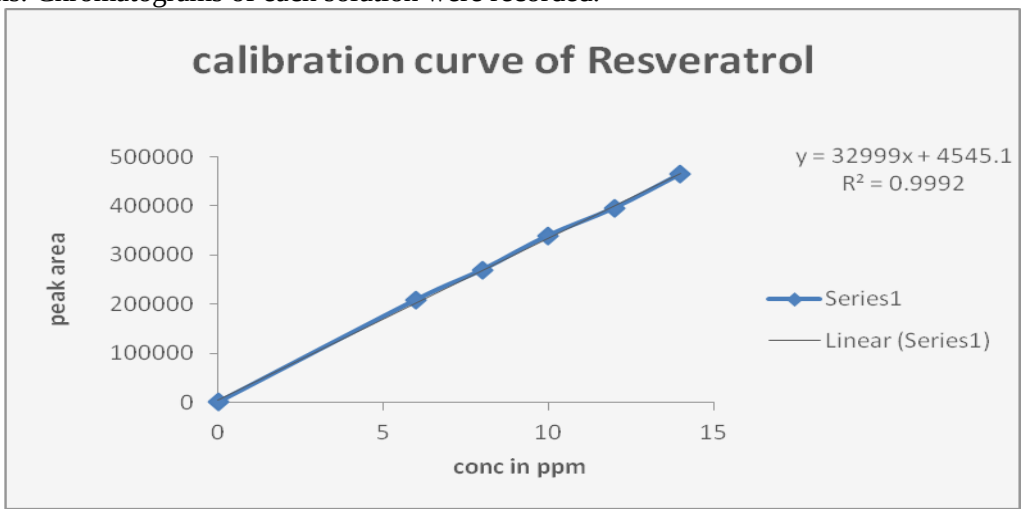


Fig-5: Calibration Curve of Resveratrol (API)

Table-2: Concentration of Resveratrol

S. No.	Conc. (µg/ml)	Mean Peak Area
1	0	0
2	6	208668
3	8	269412
4	10	339421
5	12	395426
6	14	464287

2.6.2. Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Resveratrol were taken and added to the pre-analysed formulation of concentration 20µg/ml. From that percentage recovery values were calculated. The results were shown in table-3.

Table-3: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	7.872346	259825	98.40433	Mean= 99.9174% S.D. = 1.156494 % R.S.D.=1.569779
S_2 : 80 %	8	8.051412	265734	100.6427	
S_3 : 80 %	8	8.002259	264112	100.0282	
S_4 : 100 %	10	9.969682	329035	99.69682	Mean= 100.4315% S.D. = 0.773152 % R.S.D.= 0.7698301
S_5 : 100 %	10	10.12381	334121	101.2381	
S_6 : 100 %	10	10.03596	331222	100.3596	
S_7 : 120 %	12	11.76628	388321	98.05235	Mean= 99.16214% S.D. = 1599115 % R.S.D.= 1.612628
S_8 : 120 %	12	12.11941	399974	100.9951	
S_9 : 120 %	12	11.81268	389852	98.43898	

2.6.3. Precision:

2.6.3.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Resveratrol (API). The percent relative standard deviation was calculated for Resveratrol are presented in the Table-4.

Table-4: Repeatability Readings of Resveratrol

HPLC Injection Replicates of Resveratrol	Peak Area
Replicate – 1	2948323

Replicate – 2	2935751
Replicate – 3	2979135
Replicate – 4	2971013
Replicate – 5	2919463
Replicate – 6	2974741
Average	2954738
Standard Deviation	24108.89
% RSD	**0.815940**

2.6.3.2. Intermediate precision:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Resveratrol revealed that the proposed method is precise.

Table-5: Results of Intra-Assay & inter-assay

Conc. Of Resveratrol (API) (µg/ml)	Observed Conc. Of Resveratrol (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.97	0.59	8.09	0.89
10	9.88	0.43	10.05	0.76
12	12.03	0.52	11.79	0.63

2.6.4. Method Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection (±2nm) & organic phase content in mobile phase (±5%) studied to determine the robustness of the method are also in favour of (Table-6, % RSD < 2%) the developed RP-HPLC method for the analysis of Resveratrol (API).

Table-6: Results of Method Robustness Test

Change in parameter	**% RSD**
Flow (1.1 ml/min)	0.60
Flow (0.9 ml/min)	0.74
More Organic	0.68
Less Organic	0.80
Wavelength of Detection (237 nm)	0.88
Wavelength of detection (233 nm)	0.98

2.6.5. LOD & LOQ:

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 0.04(SD/S).

L.O.Q. = 0.12(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.08 & 0.24µg/ml respectively.

2.6.6. System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-7.

Table-7: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.34
2	Asymmetry	T ≤ 2	Resveratrol =0.16
3	Theoretical plate	N > 2000	Resveratrol =1865
4	Tailing Factor	T<2	Resveratrol =1.55

2.6.7 Estimation of Resveratrol in Tablet Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 10 mg of drug were transferred to 10 ml volumetric flask, and 8 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 10 ml with mobile phase. Then 1ml of the above solution was diluted to 10 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.

Table-8: Assay of Resveratrol Tablets

Brand name of Resveratrol	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Bestvite	500mg	499.9 (± 0.498)	99.96 (± 0.343)

The amount of drugs in Bestvite was found to be 499.9 (± 0.546) mg/tab for Resveratrol & % assay was 99.96 %.

III. RESULTS

The selected and optimized mobile phase was Acetonitrile: Methanol = 42:58 and conditions optimized were flow rate (1.0 ml/minute), wavelength (235nm), Run time was 08 mins. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

The results obtained in method validation were:

Linearity: The calibration curve showed good linearity in the range of 0 – 12µg/ml, for Resveratrol (API) with correlation coefficient (r^2) of 0.999. A typical calibration curve has the regression equation of y = 32999x + 4545 for Resveratrol.
Accuracy: The mean recoveries were found to be 99.9174%, 100.4315%and 99.16214%for Resveratrol. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.
Repeatability: The repeatability study which was conducted on the solution having the concentration of about 20 µg/ml for Resveratrol (n =6) showed %RSD. It was concluded that the analytical technique showed good repeatability.
LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.08 & 0.24µg/ml respectively.
Assay: The number of drugs in Bestvite was found to be 499.9 (± 0.546) mg/tab for Resveratrol & % assay was 99.96 %.

IV. DISCUSSION

To develop a precise, linear, specific RP-HPLC method for analysis of Resveratrol, different chromatographic conditions were applied & the results observed were compared with the methods available in literatures.
Gurinder Singh*et, al, A rapid reversed-phase high performance liquid chromatography (RP-HPLC) method was developed for the determination of trans-resveratrol (t-RVT) in PLGA nanoparticle formulation. A new formulation of t-RVT loaded PLGA nanoparticles (NPs) with potential stealth properties was prepared by nanoprecipitation method in our laboratory. The desired chromatographic separation was achieved on a Phenomenex C18 column under isocratic conditions using UV detection at 306 nm. The optimized mobile phase consisted of a mixture of methanol: 10 mM potassium dihydrogen phosphate buffer (pH 6.8): acetonitrile (63 : 30 : 7, v/v/v) at a flow rate of 1 mL/min.
The result shows the developed method is yet another suitable method for assay which can help in the analysis of Resveratrol in formulations.

V. CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Resveratrol API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Resveratrol indicated that the developed method is specific for the estimation of Resveratrol. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

REFERANCE

[1] Farina A, Ferranti C, Marra C: An improved synthesis of resveratrol. Nat Prod Res. 2006 Mar;20(3):247-52.
[2] Renaud S, Ruf JC: The French paradox: vegetables or wine. Circulation. 1994 Dec;90(6):3118-9.
[3] Wang Y, Catana F, Yang Y, Roderick R, van Breemen RB: An LC-MS method for analyzing total resveratrol in grape juice, cranberry juice, and in wine. J Agric Food Chem. 2002 Jan 30;50(3):431-5.
[4] Lyons MM, Yu C, Toma RB, Cho SY, Reiboldt W, Lee J, van Breemen RB: Resveratrol in raw and baked blueberries and bilberries. J Agric Food Chem. 2003 Sep 24;51(20):5867-70.
[5] Walle T, Hsieh F, DeLegge MH, Oatis JE Jr, Walle UK: High absorption but very low bioavailability of oral resveratrol in humans. Drug Metab Dispos. 2004 Dec;32(12):1377-82. Epub 2004 Aug 27.
[6] Csaki C, Keshishzadeh N, Fischer K, Shakibaei M: Regulation of inflammation signalling by resveratrol in human chondrocytes in vitro. Biochem Pharmacol. 2008 Feb 1;75(3):677-87. Epub 2007 Sep 18. [PubMed:17959154]10. Walls D, Loughran ST. Protein chromatography: Methods and protocols, methods in molecular biology. 2011;681.
[7] https://en.wikipedia.org/wiki/Resveratrol.
[8] Gurinder Singh et, al 2014, A rapid reversed-phase HPLC method for analysis trans-resveratrol (t-RVT) in PLGA nanoparticle formulation, https://www.hindawi.com/journals/isrn/2014.