Ind. J. Pharm. Edu. Res., 2025; 59(2):841-848.
https://www.ijper.org

Original Article

Quality by Design-Driven HPLC Method Development for Quantification of Resveratrol in Bulk and Pharmaceutical Dosage Form and its Validation

Swati Devendra Raysing*, Ashish Prakash Gorle

Department of Quality Assurance, R.C. Patel Institute of Pharmaceutical Education and Research, Near Karwand Naka, Shirpur, Dhule, Maharashtra, INDIA.

ABSTRACT

Background: The purpose of the recent research was to establish a simple, efficient, sensitive and accurate High-Performance Liquid Chromatography (HPLC) method for estimating Resveratrol (RVT) in bulk and pharmaceutical dosage form based on Quality by Design (QbD). As risk assessment and statistics are not used in a conventional approach, the Analytical Quality by Design (AQbD) was employed with scientific techniques including Analytical Target Profile (ATP), Critical Quality Attributes (CQA), Design of Experiment (DoE), method validation and continuous method monitoring for RVT quantification. **Materials and Methods:** To execute this work, a Central Composite Design (CCD) was employed to make the method robust and effective in developing a chromatographic database. Using a fractional factorial design, the factors were screened. **Results:** The optimized chromatographic conditions were achieved using a C_{18} (250x4.6 mm, 2.5m) column at 25ºC with methanol and 0.05% Ortho-Phthalaldehyde (OPA) buffer pH 2.8 in a 50:50, v/v ratio as the mobile phase and a flow rate of 0.7 mL/min, at 319 nm of detection wavelength. The RVT's retention time was observed to be 3.64 min. The precision and accuracy results were within the specified limits (<2% RSD). **Conclusion:** The proposed QbD-based method proved to be helpful for critical analysis is RVT in the bulk and pharmaceutical dosage forms. Hence, it can be employed for routine analysis in quality control laboratories.

Keywords: Resveratrol, High-Performance Liquid Chromatography, Central Composite Design, Quality by Design, Validation.

Correspondence:
Mrs. Swati Devendra Raysing
Department of Quality Assurance, R.C. Patel Institute of Pharmaceutical Education and Research, Near Karwand Naka, Shirpur-425405, Dhule, Maharashtra, INDIA.
Email: swatiraysing123@gmail.com

Received: 10-08-2023;
Revised: 31-07-2024;
Accepted: 24-01-2025.

INTRODUCTION

Resveratrol (RVT) also known as 3,5,40-trihydroxy-trans -stilbene, is a type of polyphenol stilbenoid characterized by the connection of two phenyl rings through an ethylene bridge. It is abundantly present in nature and has been identified in more than 70 plant species. Among its primary sources are grape seeds and skin, along with small quantities found in red wines and various food items.[1] It is used to treat atherosclerosis, dermatitis, fever, gonorrhoea, hyperlipidaemia and inflammation.[2] Macrophages get triggered with Lipopolysaccharides (LPS), or Phorbol esters (PMA), forming superoxide radicals (O_2•-) and Hydrogen Peroxide (H_2O_2), which RVT inhibits. It also reduces arachidonic acid [3H] release produced by LPS, PMA, or O_2•- / H_2O_2 exposure.[3] RSV's antioxidant activity was determined by its ability to inhibit Fe^{2+}-induced lipid peroxidation in microsomes and Cu^{2+}-induced Low-Density Lipoprotein (LDL) oxidation in rat liver microsomes,[4] as well as prevention of iron- or UV-irradiation-catalyzed lipid peroxidation (IC_{50}=4.8 and 3.9 M, respectively).[5] The basic structure is shown in Figure 1. Both the cis and trans isoforms of RVT can be glucosylated, with the predominant trans-isomer being the physiologically active form.

It is also synthesized chemically[6] and biotechnologically[7] and following its derivation from the Itadori plant, it is now marketed as a nutritional supplement.[8] The drug has high absorption and a strong affinity toward protein binding but has very low bioavailability. It works by inhibiting the activation of NF-kappaB (NF-kappaB) in HSV-infected cells. It has been observed that, during productive infection, HSV activates NF-kappaB, which could be a crucial aspect of the virus's replication.[9] The literature reports methods for estimating RVT in various samples by the HPLC method.[10-16] Apart from the HPLC method, Ultra-High-Performance Liquid Chromatography (UHPLC), UV spectroscopy, Gas Chromatography (GC), Mass Spectroscopy (MS) and Capillary Electrophoresis (CE) techniques are also developed for the estimation and quantification of RVT. Common limitations associated with analytical techniques for

DOI: 10.5530/ijper.20254922

the estimation of RVT include challenges in achieving sufficient sensitivity for trace amounts, matrix interference in complex samples, time-consuming sample preparation, derivatization requirements for some techniques, limited availability and high cost of specialized instrumentation, difficulties in method transferability and lack of standardization leading to inconsistent results. Careful consideration of these limitations is essential for selecting and optimizing the appropriate analytical method for accurate Resveratrol estimation. Despite the available literature on RVT estimation using liquid chromatography methods, the efficiency and reliability of these approaches may give rise to obstacles due to significant variability and inconsistent method performance. Multiple factors contribute to the variability, including buffer solutions, expensive solvents, guard columns and chromatographic factors such as flow rate, mobile phase composition, injection volume, pH, flow gradient and column temperature.[17,18]

In this regard, the method validation according to ICH standards does not provide much assurance in terms of minimizing method variability over traditional robust testing. As a result, the QbD technique has been widely adopted in developing analytical methods to produce high robustness and better method operation.[19] AQbD allows for a scientific and risk-based comprehension of the significant deviance sources, followed by the identification of Critical Method Parameters (CMPs) utilizing risk evaluation and factor screening studies to recognize high-risk variables having a significantly affecting analytical performance and finally, their optimization using appropriate experimental designs to improve method performance.[20] Literature research on various drugs has revealed the great help of the AQbD technique for building analytical procedures for drug substances during the previous few decades.[21,22] Analytical method development of RVT using the QbD approach is necessary to ensure robustness, efficiency, method understanding, risk mitigation and regulatory compliance. It helps optimize the method's performance, leading to reliable and accurate results for Resveratrol analysis.

To address the limitations, a study was conducted to develop a simple and reliable method for estimating RVT in bulk and pharmaceutical dosage forms. The research aimed to establish a cost-effective, user-friendly and highly sensitive HPLC technique, utilizing Quality by Design (QbD) principles for optimization. The method was subsequently evaluated for accuracy, precision, linearity, specificity and robustness to ensure its reliability and performance.

MATERIALS AND METHODS

Materials

Sami Labs Limited, Karnataka, India, provided RVT as a gift sample and Casproh Restage 500 mg tablets were purchased from a local pharmacy. HPLC grade Methanol was purchased from Merck Specialties Pvt. Ltd., Mumbai, India and Ortho-Phthalaldehyde was purchased from Chemtex Speciality Limited., Mumbai, India. The ultra-pure water of HPLC grade was available through the in-house Mili-Q water system.

Instrumentation

The method development and validation were performed using HPLC (AGILENT 1100) with a G1310A ISOPUMP with degasser, G-13148 (DAD) detector in the wavelength range 200-400 nm. The C18 (250x4.6 mm, 2.5 µm) column was used for the analysis. For all samples, the mobile phase was methanol: 0.05% OPA buffer (51:49, v/v), with a flow rate of 0.8 mL/min in an isocratic mode and the 20 L of injection volume. Agilent ChemStation 4.03 software was used to record and process data, while QbD parameters were implemented using the design expert software version 13.

Methods

The selection and optimization of the HPLC conditions for RVT estimation are discussed below.

Preparation of standard stock solution

Accurately weighed RVT (5 mg) was dissolved in a 5 mL solution of methanol: 0.05% O-Phthalaldehyde (OPA) buffer pH 2.8 (26:74, v/v) in a 10 mL volumetric flask. The solution was sonicated for 10 min and the final volume was adjusted to 10 mL with a diluent mixture

Preparation of working solution

In a 10 mL volumetric flask, a 0.5 mL standard stock solution was pipette out and diluted with methanol:0.05% OPA buffer pH 2.8 (26:74, v/v) as diluent mixture up to 10 mL to give a concentration of 25 µg/mL.

Preparation of mobile phase

The mobile phase was prepared by using a mixture of methanol and 0.05% OPA buffer pH 2.8 at a ratio of 51:49 v/v. Both solutions were mixed properly and passed through a 0.45 µm nylon filter and degassed for 10 min. The mobile phase was sonicated before use.

Initial chromatographic condition

Various trials were performed with Columns (C8 and C18) and mobile phases (methanol, acetone and phosphate buffer) to estimate and quantify the RVT. The successful chromatographic separation was done by injecting 20 µL sample solution in the HPLC system at a flow rate of 0.8 mL/min and column temperature of 25°C. The analyte detection was performed using G-13148 (DAD) detector at a wavelength of 319 nm. Methanol:0.05% OPA buffer pH 2.8 (51:49, v/v) was used as the mobile phase.

Design of Experiments (DOE)

Quality by Design refers to systematic product development and process design techniques. To better comprehend the process of product development, it is vital to establish a systematic objective in product development.[23,24] Here, it was planned to develop an HPLC method for RVT that is robust, accurate, with short analysis time (i.e., <10 min) and has a sufficient number of theoretical plates as per QbD requirements. The crucial variables that affect retention time were identified using the software-generated results. The effect of methanol concentration, flow rate and the detection wavelength on RT was found to be crucial. Apart from this, an important parameter was the selection of the stationary phase. On the C-18 column, the drug's nature was more retentive than on the C-8 column.

Central Composite Design (CCD) was used to develop and optimize the analytical method of RVT. Flow rate (A, mL/min), Methanol concentration (B, %) and detection wavelength (C, nm) were considered as independent variables and RT of RVT was considered as the dependent variable. The independent variables were varied at 5 levels and their effect on the dependent variable was determined. The flow rate was varied from 0.6 to 1 mL/min, methanol concentration was varied from 49 to 53% and wavelength was varied from 317 to 321 nm (Table 1). The effect of buffer pH 2.8 and column temperature 25°C was less significant on retention time, hence were kept constant. The Optimisation trials considering CCD are presented in Table 1.

Analysis of RVT tablets

RVT tablets (n=10) were weighed and triturated to a fine powder using a pestle and mortar. Powder equivalent to 0.1 mg was weighed accurately and transferred to a 10 mL volumetric flask. The volume was adjusted to 10 mL by adding methanol: 0.05% OPA buffer pH 2.8 (26:74, v/v) as diluent. The solution was sonicated for 5 min to dissolve the content uniformly. 1 mL solution was pipette out and transferred to a 10 mL volumetric flask and volume was adjusted with diluent to get the final solution of 1000 µg/mL concentration. The resulting solution was filtered through a 0.45-micron nylon syringe filter and injected into an HPLC column for analysis.

System suitability

The system performance study was verified by examining the system appropriateness parameters. When evaluating the applicability of the system, a number of factors were taken into account, including the theoretical plate count, tailing factor and retention/capacity factor.

Validation of analytical method

The developed analytical method was validated as per the International Conference on Harmonization (ICH) Q2 (R1) guideline s. Accuracy, precision and linearity parameters were validated.[25-28]

Accuracy

The proposed method's accuracy was determined using the spiking method. In this study, RVT was spiked at 3 different levels of 80%, 100% and 120% and recovery was calculated using the linearity equation. To establish the mean and % RSD, duplicate determinations of these 3 levels were recorded.

Precision

To assess the precision of the method, variation studies were conducted both intra-day and inter-day.

Inter-day precision

For the inter-day variation studies, 3 replicates of the 3 different concentrations (10, 15 and 20 µg/mL) were analyzed over two consecutive days and % RSD was determined.

Intra-day precision

In the intra-day study, 3 replicates of the 3 different concentrations (10, 15 and 20 µg/mL) were analysed within a single day (morning and evening) and % RSD was calculated.

Linearity

A standard stock solution of RVT with a concentration of 1000 µg/mL was prepared. From this stock solution, a solution of 100 µg/mL was prepared with proper dilution using a diluent. The serial dilutions were made to prepare the solutions ranging from concentrations of 5-25 µg/mL. The samples were analyzed and a calibration curve was plotted between peak area and concentration. The regression equation with slope and the correlation coefficient was determined from the calibration curve.

RESULTS

Optimisation of initial chromatographic conditions

A Symmetry C18 column (250 mm×4.6 mm, 2.5 µm) and a mobile phase composed of methanol and 0.05% OPA buffer at pH 2.8 (51:49, v/v) were employed, resulting in an excellent chromatogram (Figure 2). The flow rate was set at 0.8 mL/min, with an injection volume of 20 µL and the column temperature was maintained at 25°C. The detailed optimised chromatographic conditions are presented in Table 2.

Optimization trials using CCD

The results of all optimization trials are presented in Table 3.

Effect of independent variables on dependent variables and statistical analysis

Based on the p-value (0.0047), the entire model was found to be statistically significant (Table 4). As a result, the model's

significance for response prediction was determined. For optimization, the complete model was well-fitted. All 3 independent variables showed a significant effect on the RT of the RVT based on the *p*-value observed (Table 4). Figure 3 shows the Pareto chart of the independent variable having a significant impact on the method.

Figure 4 demonstrates the influence of methanol concentration and flow rate on the retention time, with a constant detection wavelength of 319 nm. The graph reveals that the retention time was optimized when the methanol concentration ranged from 50 to 51.5% and the flow rate was set between 0.7 and 0.75 mL/min. Additionally, the retention time decreases when the flow rate exceeds 0.75 mL/min, within the range of 0.75 to 0.9 mL/min.

Composite desirability parameters were employed to determine the optimal conditions for achieving the desired result. Based on the obtained experimental runs, conditions with high desirability were selected, including a flow rate of 0.7 mL/min, a mobile phase composition of methanol: 0.05% OPA buffer at pH 2.8 (50:50, v/v), a detection wavelength of 319 nm, an injection volume of 20 µL, a column temperature of 25°C and a recorded peak at a retention time of 3.64 min. The predicted response was then compared to the actual response under a set of conditions for further evaluation.

Method validation

System suitability

The developed HPLC method for RVT has produced theoretical plate above 2000 for resveratrol with tailing factor less than 2. The retention time of resveratrol was less than 2, which ensure the suitability of the developed method. The results of system suitability study were summarised in Table 5.

Accuracy

The accuracy of the proposed method was evaluated at 3 different concentration levels (80%, 100% and 120%). The obtained data reveal % RSD values of 0.09%, 0.15% and 0.21% for RVT, demonstrating that the method exhibits acceptable accuracy within a 2% range.

Precision

Inter-day precision

Inter-day precision is also known as intermediate precision used to assess the variation in results obtained on different days, with different analysts and using different equipment. It accounts for the day-to-day variability and other factors that may affect the method's performance. Intermediate precision is also expressed as % RSD. The method precision, evaluated across 3 sample preparations, yielded a 2% variation, indicating excellent precision of the developed method.

Intra-day precision

Intra-day precision, also known as repeatability measures the variation in results obtained when multiple measurements of the same sample are performed within a short timeframe. It provides information about the method's precision under controlled and consistent conditions. Repeatability is typically expressed as the percent Relative Standard Deviation (% RSD). Intra-day precision for 3 sample preparation was found to be <2%.

Table 1: CCD-based independent variables and their effect on the dependent variable.

Run	Independent variables		
	A (Flow rate)	B (Methanol Conc.)	C (Wavelength)
	mL/min	%	nm
1	1	51	319
2	0.6	51	319
3	0.9	52	318
4	0.9	52	320
5	0.9	50	320
6	0.7	52	318
7	0.8	51	317
8	0.7	50	320
9	0.8	51	321
10	0.7	50	318
11	0.7	52	320
12	0.8	49	319
13	0.9	50	318
14	0.8	53	319

Linearity

A linear relationship was observed and calculated using linear regression analysis for concentration v/s peak area within 5-25 µg/mL. The calibration curve's correlation coefficient and slope were 0.9995 and 108.35, respectively as shown in Figure 5.

Analysis of dosage form

The developed and validated analytical method of RVT was applied in the determination of the assay of marketed tablet formulations to check their workability. The % assay of RVT was determined to be 98.7%, with the amount of drug obtained being 19.74 µg and the peak area of 2225.51 which is the standard and acceptance level in any pharmaceutical quality control laboratory

DISCUSSION

During the development of an HPLC method for the analysis of RVT, several parameter changes were made. The most significant alteration involved the mobile phase ratio, which had a notable impact on the peak purity of RVT. The optimal wavelength for RVT detection was determined to be 319 nm. The C8 columns are valuable tools in HPLC analysis, offering advantages in terms of speed, selectivity and versatility, hence, C8 and C18, were evaluated during the development phase, with the C18 column exhibiting greater retention for the drug compared to the C8 column. CCD was used to optimize the analytical method for RVT. Flow rate (A, mL/min), Methanol concentration (B, %) and detection wavelength (C, nm) were considered as independent variables and RT of RVT was considered as the dependent variable. The effects of the independent variables and interaction terms on the dependent variable, i.e., retention time, were determined using Analysis of Variance (ANOVA). The p-value <0.05 is the cut-off point beyond which we assert that the results are statistically significant, according to the convention.[29,30] Methanol concentration (p=0.0001), flow rate (p=0.0001) and detection wavelength (0.0047) were seen to be significant. To establish a robust design space for the technique, response surfaces and contour plots were utilized to illustrate the effects and interactions of variables. The accompanying 3D graph provides valuable insights into the variables' impacts and interactions on the response. To ascertain the suitability of the developed method for its intended purpose, a validation was undertaken in accordance with the validation guidelines outlined by the International Council for Harmonisation (ICH). The method underwent validation across multiple parameters, encompassing, accuracy, precision and linearity. These comprehensive validations were conducted to ensure the method's reliability and effectiveness in consistently generating accurate and precise results. The accuracy of the method is typically expressed as percent recovery or percent relative error. Percent recovery indicates how close the measured values are to the expected values, while percent relative error quantifies the deviation of the measured values from the expected values. The accuracy study provides important information about the ability of the analytical method to provide reliable and accurate results for RVT. It helps ensure that the method is capable of correctly quantifying RVT in samples and provides confidence in the accuracy of the reported results. The precision

Table 2: Optimised Chromatographic Conditions.

Sl. No.	Parameter	Condition
1	HPLC Instrument	AGILENT 1100
2	Detector	G-13148 (DAD) detector
3	Column	C18 RP (250 mm x 4.6 mm, 2.5 µm).
4	Wavelength	319 nm
5	Mobile Phase	Methanol: 0.05% OPA buffer at pH 2.8 (51:49, v/v).
6	Diluent	Methanol: 0.05% OPA buffer at pH 2.8 (51:49, v/v).
7	RT	3.64 min
8	Run time	10 min
9	Injection Volume	20 µL
10	Column Oven temperature	25°C
11	Flow Rate	0.8 mL/min

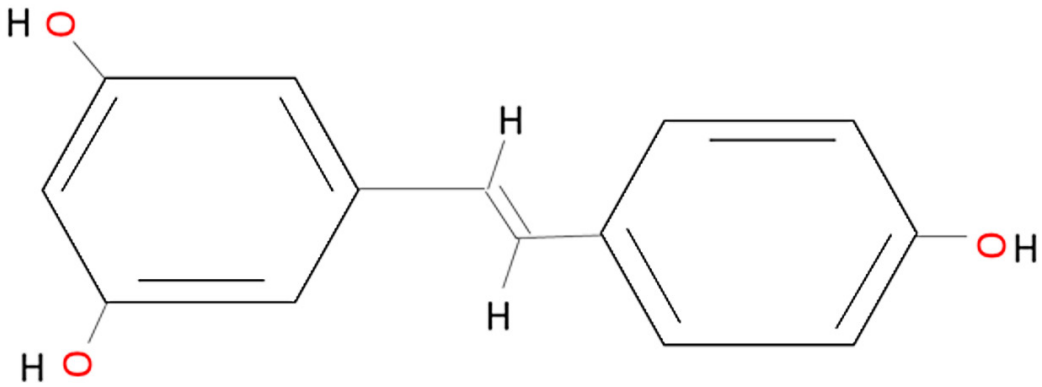

Figure 1: Basic structure of RVT.

Table 3: Optimisation trial results of CCD statistical design.

Run	Independent variables			Dependent variable
	A (Flow rate)	B (Methanol Conc)	C (Wavelength)	Retention Time
	mL/min	%	nm	min
1	1	51	319	3.267
2	0.6	51	319	5.518
3	0.9	52	318	3.18
4	0.9	52	320	3.134
5	0.9	50	320	3.511
6	0.7	52	318	3.94
7	0.8	51	317	3.657
8	0.7	50	320	4.397
9	0.8	51	321	4.349
10	0.7	50	318	3.642
11	0.7	52	320	3.991
12	0.8	49	319	3.927
13	0.9	50	318	3.304
14	0.8	53	319	3.239

Table 4: Regression coefficients and corresponding *p*-values and showing the model used for RVT was significant.

Source	Sum of Squares	D_f	Mean Square	F-value	*p*-value	
Model	0.0018	1	0.0018	11.30	0.0047	Significant
A (Flow rate)	1.09	1	1.09	167.02	< 0.0001	
B (Methanol conc	1.87	1	1.87	286.60	< 0.0001	
C (Wavelength)	0.0018	1	0.0018	11.30	0.0047	
Residual	0.0022	14	0.0002			
Cor Total	0.0040	15				

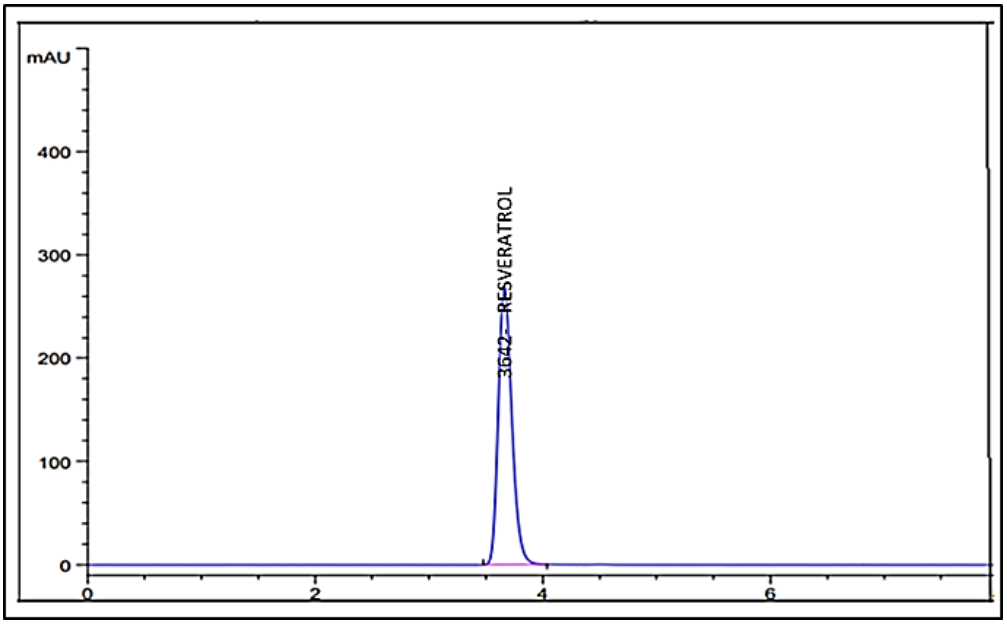

Figure 2: Chromatogram of the initial method developed for estimation of RVT in bulk drug.

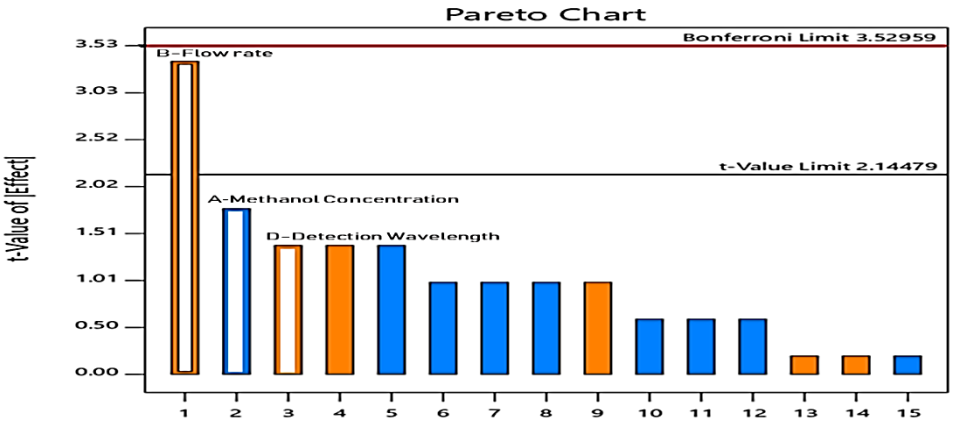

Figure 3: Pareto chart of the independent variable having a significant impact on the method.

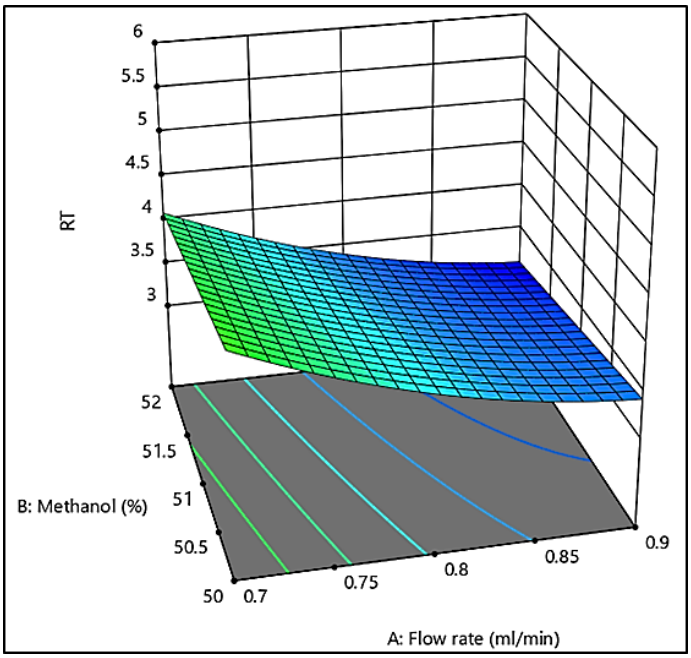

Figure 4: Response surface (3D) and a contour plot representing the effects of methanol concentration and flow rate on RVT's retention time.

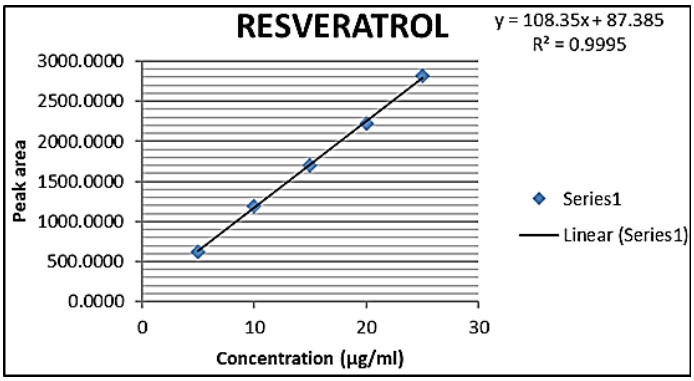

Figure 5: Calibration curve of RVT for linearity.

Table 5: System suitability study.

Parameters	Resveratrol	Acceptance criteria
Retention time, min	3.64 min	-
Theoretical plates [N]	6772	>2000
Asymmetry factor	0.81	<2

study provided valuable information about the method's ability to produce consistent and reliable results over time and across different operational conditions. It demonstrated the robustness and reproducibility of the method, enhancing confidence in the precision of the reported analytical data.

This study introduced a straightforward and cost-effective HPLC method that excels in sensitivity, precision, accuracy, speed and robustness for quantifying Resveratrol in both bulk materials and pharmaceutical dosage forms. Developed through a Quality by Design (QbD) framework, this method offers significant potential for enhancing routine quantitative analysis within the pharmaceutical sector. Its streamlined approach promises improved efficiency and cost-effectiveness, marking a noteworthy advancement in Resveratrol analysis.

CONCLUSION

The method emphasized in this research work determines RVT in bulk and pharmaceutical dosage form was simple, sensitive, accurate, precise, rapid, robust and economical, as the initial investment in time and resources required in this method are less, the long-term benefits outweigh the costs. The HPLC method employed methanol: 0.05% OPA buffer pH 2.8 in a 50:50 (v/v) ratio as the mobile phase, enabling efficient estimation of RVT. The developed method exhibited excellent linearity with a correlation coefficient of 0.9995. The accuracy study conducted at 80%, 100% and 120% concentrations yielded % RSD values of 0.09, 0.15 and 0.21%, respectively, demonstrating high accuracy. Additionally, the precision studies demonstrated the method's precision. This Quality by Design (QbD) based HPLC method overcomes the limitations of previously reported methods by eliminating the

need for complex estimation, achieving shorter retention times and utilizing a cost-effective isocratic method. The proposed method can be readily applied for routine quantitative analysis of RVT in bulk and formulation samples using HPLC.

ACKNOWLEDGEMENT

We would like to thank to R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur., for providing the facility to perform the research work.

CONFLICT OF INTEREST

The author declares that there is no competing interest.

ABBREVIATIONS

RVT: Resveratrol; **HPLC:** High-performance liquid chromatography; **QbD:** Quality by Design; **AQbD:** Analytical Quality by Design; **ATP:** Analytical Target Profile; **CQA:** Critical Quality Attributes; **DoE:** Design of Experiment; **CCD:** Central composite design; **LPS:** Lipopolysaccharides; **LDL:** Low-density lipoprotein; **OPA:** O-phthalaldehyde; **RT:** Retention time; **ICH:** International Conference on Harmonization; **RSD:** Relative standard deviation.

SUMMARY

The research presents a simple, sensitive, accurate, precise, rapid, robust and economical HPLC method for the quantification of Resveratrol in bulk and pharmaceutical dosage forms. This method, developed with a Quality by Design (QbD) approach, holds great promise for routine quantitative analysis in the pharmaceutical industry, offering improved efficiency and cost-effectiveness.

REFERENCES

1. Salehi B, Mishra AP, Nigam M, Sener B, Kilic M, Sharifi-Rad M, *et al.* Resveratrol: A double-edged sword in health benefits. Biomedicines. 2018;6(3):91. doi: 10.3390/biomedicines6030091
2. Careri M, Corradini C, Elviri L, Nicoletti I, Zagnoni I. Direct HPLC analysis of quercetin and trans-resveratrol in red wine, grape and winemaking byproducts. J. Agric. Food Chem. 2003;51(18):5226-31. doi:10.1021/jf034149g
3. Juran JM. Juran on quality by design: the new steps for planning quality into goods and services. Simon and Schuster; 1992 May 4.
4. Habib NM, Ali NW, Abdelwhab NS, Abdelrahman MM. Different spectrophotometric and TLC-densitometric methods for determination of pyrazinamide in presence of its impurity. Bull. Fac. Pharm. Cairo Univ. 2017;55(1):185-94. doi:10.1016/j.bfopcu.2017.02.004
5. Kalariya PD, Kumar Talluri MV, Gaitonde VD, Devrukhakar PS, Srinivas R. Quality by design: A systematic and rapid liquid chromatography and mass spectrometry method for eprosartan mesylate and its related impurities using a superficially porous particle column. J Sep Sci. 2014;37(16):2160-71. doi: 10.1002/jssc.201301364
6. Martinez J, Moreno JJ. Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. Biochem Pharmacol. 2000;59(7):865-70. doi: 10.1016/s0006-2952(99)00380-9.
7. Fauconneau B, Waffo-Teguo P, Huguet F, Barrier L, Decendit A, Merillon JM. Comparative study of radical scavenger and antioxidant properties of phenolic compounds from Vitis vinifera cell cultures using *in vitro* tests. Life Sci. 1997;61(21):2103-10. doi: 10.1016/s0024-3205(97)00883-7
8. Miura T, Muraoka S, Ikeda N, Watanabe M, Fujimoto Y. Antioxidative and prooxidative action of stilbene derivatives. Pharmacol Toxicol. 2000;86(5):203-8. doi: 10.1034/j.1600-0773. 2000.d01-36.x
9. Drugbank.com, https://go.drugbank.com/drugs/DB02709 (last accessed on 20-04-2022.)
10. Xu S, Luo H, Chen H, Guo J, Yu B, Zhang H, *et al.* Optimization of extraction of total trans-resveratrol from peanut seeds and its determination by HPLC. J Sep Sci. 2020;43(6): 1024-31.doi:10.1002/jssc.201900915
11. Zupančič Š, Lavrič Z, Kristl J. Stability and solubility of trans-resveratrol are strongly influenced by pH and temperature. Eur J Pharm Biopharm. 2015;93:196-204. doi: 10.1016/j.ejpb.2015.04.002
12. Kurangi B, Jalalpure S, Jagwani S. A validated stability-indicating HPLC method for simultaneous estimation of resveratrol and piperine in cubosome and human plasma. J. Chromatogr. B. 2019; 1122:39-48. doi: 10.1016/j.jchromb.2019.05.017
13. Blanchard OL, Friesenhahn G, Javors MA, Smoliga JM. Development of a lozenge for oral transmucosal delivery of trans-resveratrol in humans: proof of concept. PloS one. 2014;9(2):e90131. doi: 10.1371/journal.pone.0090131
14. Chen X, He J, Tan G, Liang J, Hou Y, Wang M, *et al.* Development of an enzyme-linked immunosorbent assay and a dipstick assay for the rapid analysis of trans-resveratrol in grape berries. Food Chem. 2019;291:132-8. doi: 10.1016/j.foodchem.2019.04.023
15. El-Lakany SA, Elzoghby AO, Elgindy NA, Hamdy DA. HPLC methods for quantitation of exemestane-luteolin and exemestane-resveratrol mixtures in nanoformulations. J Chromatogr Sci. 2016;54(8):1282-9. doi: 10.1093/chromsci/bmw063.
16. Han WC, Shi N, Wang XY, Wang ZH, Wang KL, Gao M, *et al.* Application of natural cotton fibers as an extraction sorbent for the detection of trans-resveratrol in adulterated peanut oils. Food Chem. 2021;339:127885. doi: 10.1016/j.foodchem.2020.127885
17. Shankar VK, Wang M, Ajjarapu S, Kolimi P, Avula B, Murthy R, *et al.* Analysis of docosanol using GC/MS: Method development, validation and application to *ex vivo* human skin permeation studies. J Pharm Anal. 2022;12(2):287-92. doi: 10.1016/j.jpha.2021.08.004
18. Orlandini S, Pinzauti S, Furlanetto S. Application of quality by design to the development of analytical separation methods. Anal Bioanal Chem. 2013;405:443-50. doi: 10.1007/s00216-012-6302-2
19. Monks K, Molnár I, Rieger HJ, Bogáti B, Szabó E. Quality by design: multidimensional exploration of the design space in high performance liquid chromatography method development for better robustness before validation. J Chromatogr A. 2012;1232:218-30. doi: 10.1016/j.chroma.2011.12.041.
20. Rozet E, Ziemons E, Marini RD, Boulanger B, Hubert P. Quality by design compliant analytical method validation. Anal Chem. 2012;84(1):106-12. doi: 10.1021/ac202664s.
21. Kolimi P, Shankar VK, Shettar A, Rangappa S, Repka MA, Murthy SN. Development and validation of HPLC method for efinaconazole: application to human nail permeation studies. AAPS PharmSciTech. 2022;23(1):63. doi: 10.1208/s12249-021-02196-3.
22. Beg S, Sharma G, Katare OP, Lohan S, Singh B. Development and validation of a stability-indicating liquid chromatographic method for estimating olmesartan medoxomil using quality by design. J Chromatogr Sci. 2015;53(7):1048-59. doi: 10.1093/chromsci/bmu165
23. Pramod K, Tahir MA, Charoo NA, Ansari SH, Ali J. Pharmaceutical product development: A quality by design approach. Int J Pharm Investig. 2016;6(3):129. doi :10.4103/2230-973X.187350
24. Gkountanas K, Malenović A, Dotsikas Y. Determination of Bupropion and Its Impurities via a Chaotropic Chromatography Method Following Analytical Quality-by-Design Principles for Method Development. Pharmaceuticals (Basel). 2022;15(10):1196. doi: 10.3390/ph15101196.
25. Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005;1(20):05.
26. Awotwe-Otoo D, Agarabi C, Faustino PJ, Habib MJ, Lee S, Khan MA, *et al.* Application of quality by design elements for the development and optimization of an analytical method for protamine sulfate. J Pharm Biomed Anal. 2012;62:61-7. doi: 10.1016/j.jpba.2012.01.002
27. Schmidt AH, Stanic M, Molnár I. *In silico* robustness testing of a compendial HPLC purity method by using of a multidimensional design space build by chromatography modeling-Case study pramipexole. J Pharm Biomed Anal. 2014;91:97-107. doi: 10.1016/j.jpba.2013.12.023.
28. Somasekhar V, Sankar DG. Development and Validation of a Rapid RP-HPLC Method for the Estimation of Esmolol Hydrochloride in Bulk and Pharmaceutical Dosage Forms. J. Chem 2010;7(3):807-12. doi:10.1155/2010/470785
29. Shrikant P, Vijayakrishna K, Jaiprakash S. Quality by Design (QbD) approach towards the development and validation of HPLC method for Gentamicin content in biodegradable implants. Der Pharma Chemica. 2016;8(2):340-6.
30. Kalariya PD, Kumar Talluri MV, Gaitonde VD, Devrukhakar PS, Srinivas R. Quality by design: A systematic and rapid liquid chromatography and mass spectrometry method for eprosartan mesylate and its related impurities using a superficially porous particle column. J Sep Sci. 2014;37(16):2160-71. doi: 10.1002/jssc.201301364.

Cite this article: Raysing SD, Gorle AP. Quality by Design-Driven HPLC Method Development for Quantification of Resveratrol in Bulk and Pharmaceutical Dosage Form and its Validation. Indian J of Pharmaceutical Education and Research. 2025;59(2):841-8.