

Development and Validation of RP-HPLC Method for Estimation of Resveratrol Using AQBD Approach

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ABSTRACT

RP-HPLC technique for assessment of Resveratrol (RVT) in API & pharmaceutical formulation was established, which is accurate and reliable. The movable phase was acetate buffer & methanol (58:42 v/v) at a flow rate of 1.0 mL/min, column temperature of 30°C & detection at 306 nm & run time of 15 minutes. The retention time for Resveratrol was 5.29 minutes. The technique was validated as per ICH guidelines. The % relative standard deviation values for instrument precision, technique precision and intermediate precision were 0.18%, 1.19% and 1.43% respectively and intra & inter-day precision was less than 2%. The technique was linear, accurate & was found to be commercially acceptable with Limit of Detection is 0.31 µg/mL and LOQ is 0.94 µg/mL ($r^2 = 0.9997$). Stress studies confirmed degradation under oxidative, acidic, basic, dry heat and under the influence of light.

Keywords: Resveratrol, RP-HPLC, Validation, Precision, Forced Degradation.

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INTRODUCTION

Resveratrol (RVT) also known as three, four, five-transtrihydroxy-stilbene, otherwise five-[(E)-two-(four-hydroxy phenyl)-ethyl benzene-one, three-diol; C₁₄H₁₂O₃; M.W. 228.25], is an in naturally obtaining powerful antioxidant that belongs to the Stilbene family of phenolic constituents (**Fig. 1**). It was initially separated since the roots of white hellebore. In commercial settings, it has been used since the roots of Japanese Knotweed.

Additionally, it has been claimed that it can be found in a mixture of foods & food goods, such as mulberries, grapes, wine, cranberries & peanuts. RVT is heavily utilized as an anti-inflammatory, anti-aging, antioxidant & cardio-protective agent. Not only does it boost the body's metabolism, but it is also utilized in chemotherapy. Research has provided evidence that RVT has neuro-protective properties, meaning that it protects neurones from oxidative stress & cellular death^{1,2}.

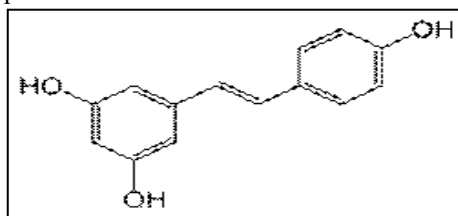


Fig. 1: Structure of RVT².

The pharmaceutical industry widely uses HPLC for the estimation and quality control of pharmaceutical substances and formulations, while the traditional trial-and-error approach to method development may not consistently ensure robustness or regulatory compliance³⁻⁵. Analytical Quality by Design (AQbD) gives a systematic, risk-based framework for developing robust and reliable HPLC methods by identifying critical method parameters, optimizing them through experimental designs, and validating the method for linearity, accuracy, specificity, precision, and robustness^{6,7}.

MATERIALS & METHODS

Chemicals & Reagents: A kind sample of RVT was acquired from Aadhaar Life Sciences Pvt. Ltd., while glacial acetic acid, sodium acetate, and HPLC-grade methyl alcohol were acquired from Merck Life Sciences Pvt. Ltd., Loba Chemie Pvt. Ltd., and Qualigen, Thermo Fisher Scientific India Pvt. Ltd., respectively, with deionized HPLC-grade water got from an in-house Milli-Q purification system, and all chemicals and solvents were of analytical or HPLC grade, with NABL-calibrated analytical balances & Type-borosilicate glassware used to ensure accuracy, traceability, consistency, and precision. The novelty lies in the systematic AQbD/DoE-driven

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development and optimization of the RVT RP-HPLC method, followed by comprehensive validation, robustness evaluation and forced-degradation assessment.

Development & Optimization of Method by A.Q.b.D. Design

Analytical Target Profile (A.T.P.): The A.T.P. is a predefined set of criterion that defines what the analytical method must achieve to ensure reliable, consistent, and scientifically sound results for its intended purpose. ATP set for design of experiment/ AQbD are Method Validation parameters (Specificity, Precision, Accuracy, Linearity, etc.), serving as the foundation for method development, risk assessment, and control strategy.

Critical Analytical Attributes (CAA): CAA is the key performance characteristic of an analytical method that must be measured and controlled to confirm approach is dependable & fit for its proposed persistence. The essential measurable attributes were defined as method quality and reliability in AQbD. The critical analytical attributes to achieve ATP were retention time, theoretical plates, asymmetry, and peak purity⁸⁻¹¹.

C.M.P. viz., Critical Method Parameters: The CMPs are the input variables or parameters of an analytical method that have a significant impact on its critical analytical attributes (CAAs). The CMP chosen were wavelength, movable phase ratio, buffer strength, flow rate, and column temperature.

HPLC Method Development: Multiple trials with results and data collection were optimized utilizing AQbD design during development¹²⁻¹⁴.

Composition of Buffer: In 1000 ml HPLC water, 3.402 gm of Sodium Acetate Trihydrate was accurately dissolved, and the pH was adjusted to 4.50 by 10% Acetic acid.

Composition of Mobile Phase: In a solvent bottle, 580 ml of Buffer and 420 ml of methanol were separately measured and mixed together, filtered twice over a 0.45-micron nylon membrane filter & sonicated to degas for 15 mins.

Composing of Standard Stock Solution

a. Composing of RVT stock solution: 10.00 mg of standard was weighed & shifted in 10.00 ml volumetric flask, as well as about 8.00 ml of diluents was taken &

mixed well & brought to the required diluents. Further, 1.00 ml of this solution was diluted to 10.00 ml & mixed well. Label it as SS-I (Concentration of RVT = 100 µg/ml).

b. Composing of working standard: In a 10.00 ml volumetric flask, 1.0 ml of SS-I was transferred & brought to volume with diluents & mixed well¹⁵⁻¹⁸.

Method Validation¹⁹⁻²¹

Specificity & Assay: i. An individual sample of Blank and RVTWS was prepared, and the analyzed peak was identified from the retention time.

ii. Peak area was recorded: Acceptance Criteria: There should be no blank peak that interferes with the major analysis peak.

a. Repeatability as well as System Appropriateness: i. A sole operational standard was made as elaborated in sections 2.00 & 6.00. Injections were manufactured since a similar solution & estimated for system appropriateness. ii. System appropriateness aspects are as follows: 1. Asymmetry (Tailing factor), 2. Theoretical plates, 3. Peak Purity Index, 4. Retention Time^{22, 23}.

b. Approval criterion: The % R.S.D. for peak area & Retention period should be minimal 2.0. System appropriateness criteria should be met as follows: The theoretical plates would be not less than 2000.00, Asymmetry would be within 0.5-1.5 and Peak purity index should be not less than 0.99²⁴⁻²⁶.

c. Linearity & Range: i. 5.00 solution of altering amounts range since 80.00-120.00 % of the test concentration was prepared. ii. The linearity of RVT method was checked at 5 concentration levels from 80% to 120%. Corresponding RVT concentrations were 8, 9, 10, 11, and 12 µg/mL at 80%, 90%, 100%, 110%, and 120% levels, respectively. iii. The sample provision is illustrated as under; iv. Five aliquots of Standard Solution-I of volumes 0.8, 0.9, 1.0, 1.1 and 1.2 mL were prepared. Aliquots were diluted to obtain the appropriate concentration of each aliquot for analysis.

Acceptance criteria: The r^2 should be not less than 0.99.

d. L.O.D. as well as L.O.Q.:

i. Was estimated by using the ANOVA method.

ii. Formula:

$$LOD = \frac{3.3 \times \text{Std. Error of Intercept}}{\text{Coefficients of X Variable 1}}$$

$$LOQ = \frac{10 \times \text{Std. Error of Intercept}}{\text{Coefficients of X Variable 1}}$$

e. Accuracy: i. Samples were made of 80.00 %, 100.00 % & 120.00 % amounts by spiking the X ml of SS-I solution, in a 10.00 ml volumetric flask, & produce the mark using diluents²⁷. Standard Solution-I (0.8, 1.0, 1.2 mL) were diluted to 10.0 mL to obtain RVT concentration levels of

8.0, 10.0 and 12.0 µg/mL. The method was tested for accuracy and analytical performance at these concentrations. ii. Substances were injected in three times to estimate percent R.S.D. iii. Amount Recovered was estimated by using the following formula;

$$\text{Amount Recovered} = \frac{Au}{As} \times \text{Spiked conc. of 100\%}$$

Where,

Au = Peak area of analysis at respective level, As = Peak area of Standard solution,

iv. % Recovery was also estimated by employing below formula;

$$\% \text{ Recovery} = \frac{\text{Amount Recovered}}{\text{Spiked amount}} \times 100$$

f. Acceptance criteria: The % Recovery should be within 95-105%. The % RSD of recoveries at each level should not be more than 2.0.

g. Robustness: i. The Robustness was conducted by altering the pH of the buffer & the wavelength by ± 0.1 & ± 2.00 nm. ii. Working standard solution was injected & percent R.S.D. of Retention time & Peak region was estimated at every circumstance^{27, 28}. The robustness of the method was tested by regulating pH of buffer from 4.4 to 4.6, around nominal value of 4.5 and the wavelength of detection from 304 to 308 nm around the reference wavelength of 306 nm. These variations were added to test the reliability of the method when there are small operational variations.

h. Recognition criterion: The percent R.S.D. for retention time, as well as the peak region, should be a minimum of 2.0. System appropriateness criteria should be met as follows: The theoretical plates would be not less than 2000.00, Asymmetry would be between 0.5 and 1.5. Peak Purity index should be not less than 0.99.

i. Intra & Inter-day Precision: i. Standard solution was made & injected two times in a day at various period intervals to estimate intra-day precision. ii. The standard solution was estimated on next day to assess the inter-day precision. iii. The percent R.S.D. of Retention time as well as Peak region was estimated at every interval & stability of solutions was determined²⁹⁻³¹.

j. Acceptance criteria: The % RSD for retention time & peak area was minimal 02.00.

k. Method Precision: Six standard solutions were prepared and injected.

l. Recognition criterion: The percent R.S.D. for peak area & Retention time is minimal 2.0.

m. Intermediate Precision: Six standard solutions were made by various analysts on various days & injected into the HPLC system.

RESULTS

HPLC Method Development: To ensure robust chromatographic performance, numerous critical choices were made before method development. Analyte polarity determined the stationary phase selection. Through literature review, physical characteristics like pKa value, solubility, and the nature of the drug were determined. The following is the data obtained from the literature review. For the best elution and peak form, buffer and methanol were used. The Phenomenex Kinetex XB-C18 (150.00 x 4.6 millimetre, 5 μ m) was selected for its immobile stage chemistry and compatibility with polar analytics. To maintain repeatability, column temp. was placed at 30.00 °C as well as injection quantity was controlled at 10.00 μ L. The 50:50 v/v Buffer-Methanol diluents used for standard and sample preparation matched the mobile phase's polarity and minimized peak distortion (**Table 1**).

Table 1: Results of Method Development.

Trial Number	Mobile Phase	Ratio	Wavelength	R.T.	T.P.	A.S.Y.	P.P.
1	Buffer A: MeOH	50-50	250 nm	2.95	8145	0.99	1.00
2	Buffer A: MeOH	55-45	250 nm	4.12	9124	1.00	1.00
3	Buffer A: MeOH	60-40	250 nm	6.29	9706	1.02	1.00
4	Buffer A: MeOH	60-40	306 nm	6.33	9930	1.03	1.00
5	Buffer A: MeOH	60-40	306 nm	6.41	9768	0.98	1.00
6	Buffer A: MeOH	60-40	306 nm	7.56	10508	0.97	0.79
7	Buffer B: MeOH	60-40	306 nm	6.58	9934	0.97	1.00
8	Buffer B: MeOH	60-40	306 nm	7.39	10256	0.95	1.00

R.T. – Retention Time, T.P. – Theoretical Plates, A.S.Y. – Asymmetry, PP – Peak Purity

Improving using AQbD design:

To develop a scientifically rational and solid RP-HPLC method for estimation of RVT, the Analytical Quality by Design (AQbD) approach was used. The Analytical Target Profile (ATP) was created with the goal of getting a method that could yield reliable, precise, accurate, specific, and robust estimation of RVT. The critical

analytical attributes (CAAs) were determined based on the ATP and were retention time, theoretical plate number, asymmetry (tailing factor), and peak purity as these parameters directly reflect the ability to chromatograph the material, peak shape, separation performance and method specificity.

As per the quality target outline that had been recognized in proceed for the expansion work, AQbD design was used to optimize the consequences.

The chromatograms for AQbD trials are given below (**Fig. 2**).

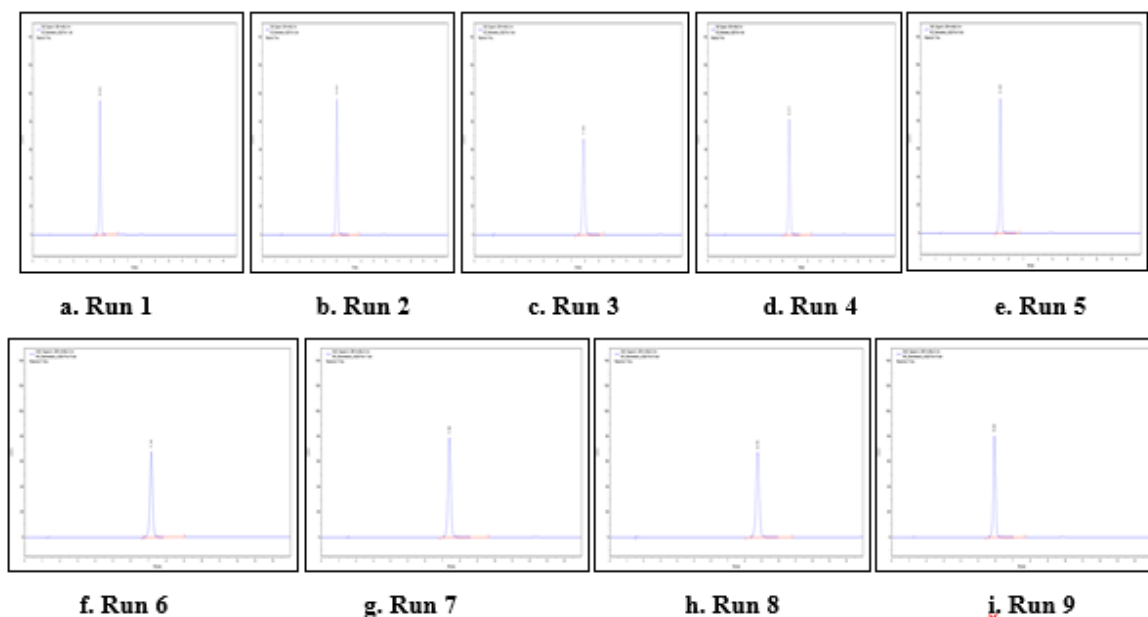


Fig. 2: (a) to (i). AQbD trials for RVT.

ANOVA spectrums for RVT from Design Expert software:

Response 1: Theoretical Plates: The actual and predicted theoretical plate values for the nine RVT runs ranged from 10017.00–10699.00 and 10066.44–10586.44, respectively, **Contour Plot:**

demonstrating good model reliability, with Factor 1 showing the most significant positive influence (+2599.77778), followed by Factor 2 (+129.08333), while Factor 3 had a weak negative effect (−18.33333) on theoretical plate values [**Fig. 3(a), (b), and (c)**].

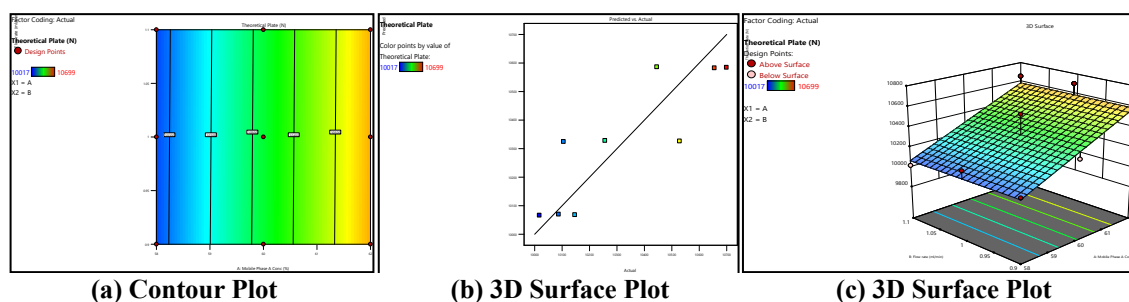


Fig. 3: (a), (b) and (c). Contour Plot and 3D Surface Plot.

Response 2: Retention Time: The close agreement between actual and predicted retention times across all nine RVT experimental runs, along with small and randomly distributed residuals and a maximum deviation

of −0.1589 in run 4, confirms the model's good fit, absence of systematic error, and high predictive reliability [**Fig. 4(a), (b), and (c)**].

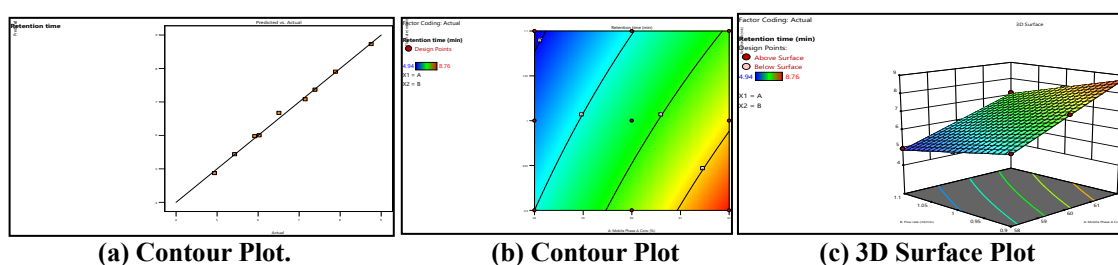


Fig. 4: (a), (b) and (c). Contour Plot and 3D Surface Plot.

In the process of preliminary method development experiments and literature evaluation, potential critical method parameters (CMPs) that may affect the selected CAAs were identified. The chromatographic variables studied were: mobile phase composition, wavelength, buffer strength/pH, flow rate, and column temperature. Initial trials were done to develop appropriate chromatographic conditions. Experimental optimization was then carried out by an AQbD/DoE approach to assess the influence of the method variables selected on the chromatographic responses.

Experimental responses from the AQbD trials were analysed using the Design-Expert software. Theoretical plate number and retention time were the main responses obtained that were optimized, and chromatographic peak shape and purity were also taken into account when optimizing the condition. There was good agreement between experimental and predicted values from the nine runs of the AQbD indicating good model performance. The experimental values for the theoretical plates were from about 10017 to 10699, while the predicted values were from about 10066 to 10586. According to the analysis, Factor 1, Factor 2 and Factor 3 had the most significant positive influence on the theoretical plate number, while Factor 3 had the least significant negative influence.

The same was true for the retention-time response, which was in good agreement with the calculated values for the 9 experimental runs. Residual analysis revealed that the deviations were small and randomly distributed, the maximum deviation being around -0.1589 min in Run 4 which did not suggest any noticeable systematic error. The effects of the investigated factors on the chromatographic responses were presented in the form of contour and three-dimensional response-surface plots and the region that gives the desired chromatographic performance was identified.

After the evaluation of the responses predicted and obtained, the peak characteristics and overall analytical performance, it was decided to run Condition #4 as the optimized condition for AQbD. Optimized method: under this conditions, RVT was found to have an adequate retention time of about 5.29 min, and the method was optimized on Phenomenex XB-C18 column (150×4.6 mm, $5 \mu\text{m}$), buffer/methanol (58:42 v/v), flow rate of 1.0 mL/min, detection at 306 nm, column temperature of 30°C , injection volume of $10 \mu\text{L}$, and total run time of 15 min.

Using AQbD thus gave an “educated” way to identify and optimize method variables, as opposed to just trial-and-error experimentation. The optimized method was then validated with regard to specificity, linearity, accuracy, precision, robustness, sensitivity and forced degradation studies, to confirm its suitability for reliable pharmaceutical analysis of RVT.

Final Method of Analysis: Hence, AQbD run 4 was selected as the final technique. The chromatographic estimation was performed with an Agilent 1260.00 Infinity II HPLC system and a Phenomenex XB-C18.00 column (150.00×4.6 mm, $5.00 \mu\text{m}$). The separation was carried out with a 100% buffer/58% methanol mobile phase, flow rate 1.0 mL/min, injection volume of $10 \mu\text{L}$ & a total runtime of 15 minutes at 306 nm detection. The diluents were buffer/methanol (50:50 v/v) & column oven temp. was kept at 30°C with a robustness allowance of $\pm 2^\circ\text{C}$.

Method Validation: Specificity: All peaks in the chromatogram were well-resolved, with no intrusion found at the preservation time of RVT in the blank sample. The API exhibited a consistent retention time of 5.29 minutes. The absence of interfering peaks confirms the method's specificity and reliable identification of RVT (**Fig. 5 and Fig. 6**).

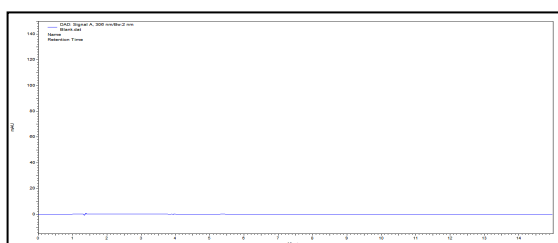


Fig. 5: Specificity of RVT for Blank Solution

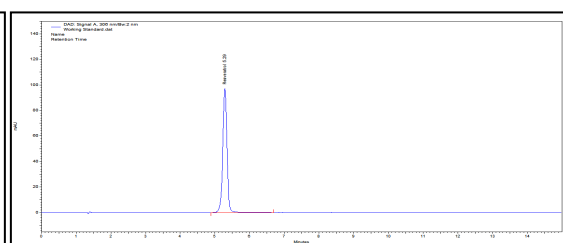


Fig. 6: Specificity of RVT for Working Std. Solution

Linearity: Multiple concentrations were tested for linearity. A calibration curve exposed a significant linear association between peak area & quantity (**Fig. 7**). The method Resveratrol was validated within the range of 8.0

– $12.0 \mu\text{g/mL}$ or 80 – 120% of the Target concentration. The peak area changed in a proportional manner from 1,374,294 to 2,057,703 with increase in concentration, indicating good linearity of the analytical method.

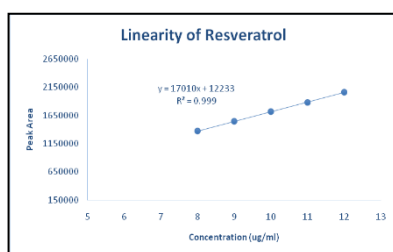


Fig. 7: Linearity graph of RVT

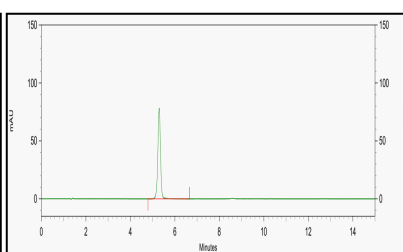
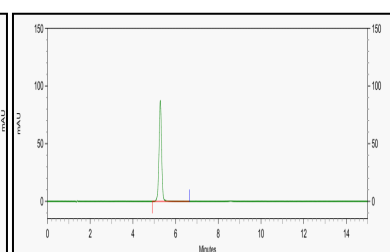
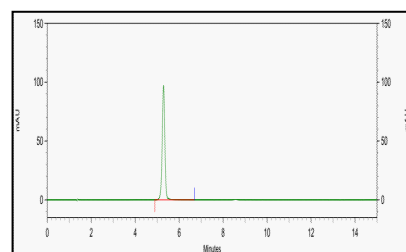


Fig. 8: (a) 80.00 %

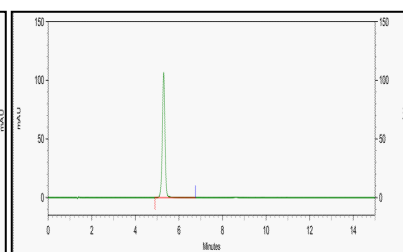


(b) 90.00 %

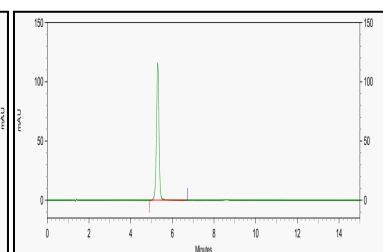
As of the above data, it was found that the correlation coefficient of RVT was 0.9997. The chromatograms for Linearity are given below (Fig. 8).



(c) 100.00 %



(d) 110.00 %



(e) 120.00 %

Fig. 8: (a), (b), (c), (d), (e). Linearity Chromatograms for Resveratrol.

LOD & LOQ: The LOD & LOQ was estimated for RVT. This approach showed good sensitivity for RVT with a LOD of 0.31 µg/mL & a LOQ of 0.94 µg/mL. The approach was effective in determining low drug concentrations because RVT had a low LOD and LOQ.

recovery % were calculated. % RSD must be below 2% for replicate injections (Table 2). The stock solution concentration for 99.74 µg/mL and peak area of 1,711,196 was obtained from standard weight of 10 mg and 99.74% potency of RVT for the accuracy study.

Accuracy: Accuracy was assessed at three concentrations. Three replicate injections' relative standard deviation and

Table 2: % Recovery for RVT.

% Level	Responses (Rep.)	Spiked amount (µg/ml)	Peak Region	Amount Recovered (µg/ml)	Percent Recovery	Average	S.D.	Percent R.S.D.
80.00	Rep. 1	7.98	1374294	8.01	100.39	99.57	0.71	0.72
	Rep. 2	7.98	1358256	7.92	99.22			
	Rep. 3	7.98	1356674	7.91	99.10			
100	Rep. 1	9.97	1715635	10.00	100.26	100.05	0.19	0.19
	Rep. 2	9.97	1709335	9.96	99.89			
	Rep. 3	9.97	1711254	9.97	100.00			
120	Rep. 1	11.97	2057703	11.99	100.21	99.98	0.21	0.21
	Rep. 2	11.97	2049334	11.94	99.80			
	Rep. 3	11.97	2052051	11.96	99.93			

RVT three replicates at 80%, 100%, and 120% accuracy had 0.72%, 0.19%, and 0.21% relative standard deviation (% RSD). At accuracy levels of 80%, 100%, and 120%, recoveries were 99.57%, 100.05%, and 99.98%.

Robustness Study: The robustness study showed that the buffer pH (4.4–4.6), detection wavelength (304–308 nm), and column oven temperature (28–32°C) had no significant impact on the RVT retention time (5.29 min) and acceptable peak area precision (%RSD 1.43–1.58%), theoretical plates (>8000), asymmetry (<2.0), and peak purity (1.00). It was overall very robust and reliable, as no significant changes occurred of the chromatographic performance or peak area when the analytical conditions were slightly changed.

Intra & Inter-day precision: Intra-day as well as inter-day precision studies examined RVT peak area % RSD changes over time. % RSD readings below 2.00 % met acceptance requirements and confirmed the method's consistency and dependability over time. RVT intra- & inter-day % RSD was 0.39%. Hence, working standard solution of RVT is 1.32 for 2 days based on the % Assay of the APIs peak.

Forced Degradation: A number of forced degradation experiments were carried out with these products, and the results demonstrated that RVT was degraded under different stresses ranging between 0.04–8.19%. The maximum degradation occurred under basic conditions (8.19%), followed by peroxide (5.32%) and dry heat

(2.67%). The obtained % assay values were in the range of 91.81–99.96% which exhibited stability representative ability of developed method & confirmed its ability to

detect the degradation of RVT under acidic, basic, oxidative, thermal and photolytic conditions [Fig. 9(a–g)].

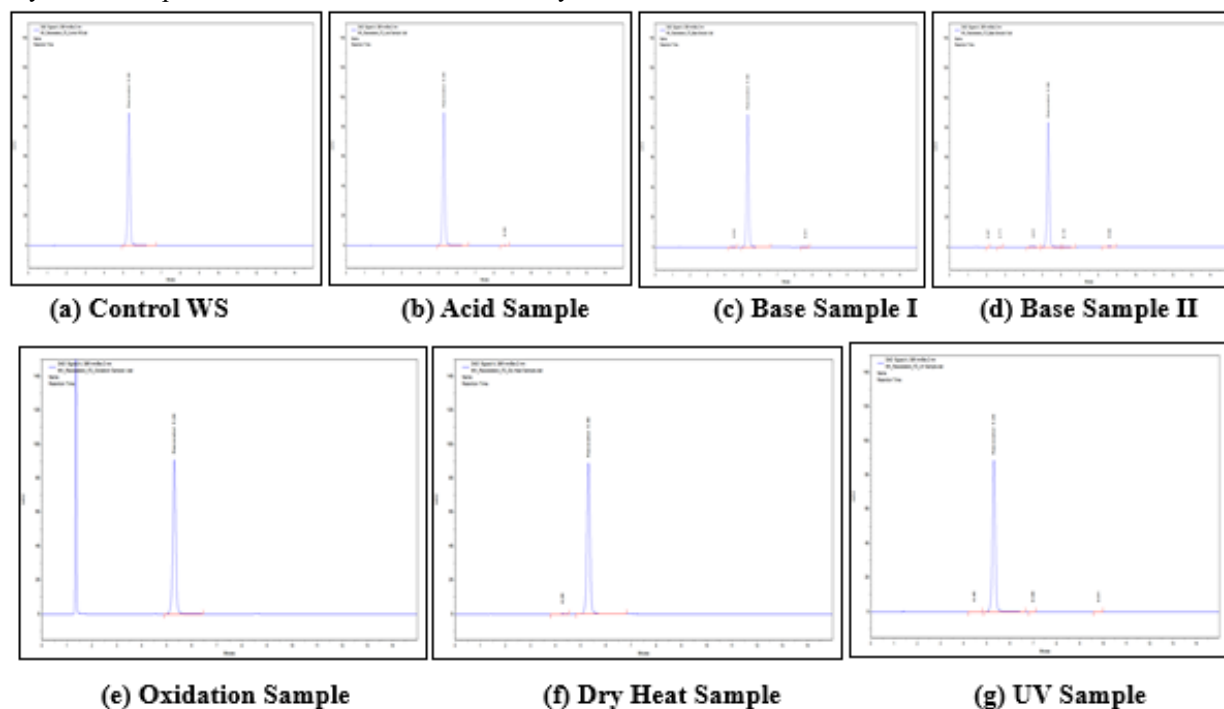


Fig. 9: (a) to (g). Forced degradation Chromatograms of RVT.

DISCUSSION

HPLC method development with a systematic approach for optimization of chromatographic conditions and application of AQbD method gave a reliable and robust analytical procedure for resveratrol (RVT). The best chromatographic performance was achieved by selecting the Phenomenex Kinetex XB-C18 column and optimizing the buffer–methanol composition as well as the wavelength, flow rate, and column temperature. AQbD trials demonstrated a good level of agreement between the actual and predicted responses for theoretical plate numbers and for retention time. The optimized method demonstrated good specificity, with no peak of interference at the RVT retention time (5.29 min); the precision studies revealed the low level of variability for instrument, method and intermediate precision (% RSD 0.18%, 1.19% and 1.43%, respectively). The low LOD (0.31 µg/mL), LOQ (0.94 µg/mL), high recoveries (99.57–100.05%) and %RSD (less than 1%) values indicate the high sensitivity and reliability of the method and its accuracy to ensure the reliability of the results. Moreover, deliberate changes in pH, wavelength, and column temperature resulted in consistent chromatographic

responses, thus proving the method to be robust. The forced degradation results showed that the method was able to differentiate the drug from its degradation products and the observed degradation products and assay profile showed the RVT was susceptible to acidic, basic, oxidative, thermal and photolytic stress. In conclusion, the developed AQbD based HPLC method was found to be sensitive, specific, precise, accurate, robust & stability representative which can be useful to routine quality control estimation of RVT in bulk drug and pharmaceutical formulations.

Compared with the reported approaches, the present study provides a more comprehensive analytical strategy by integrating AQbD/DoE-driven optimization, systematic method validation, robustness assessment, and forced-degradation studies within a single RP-HPLC method for RVT (**Table 3**). Thus, the novelty and scientific strength of the present work lie not merely in RP-HPLC estimation of RVT, but in establishing a rational, robust, sensitive, and stability-indicating analytical method through an AQbD-based framework, thereby providing greater confidence for routine pharmaceutical quality control.

Table 3: Comparison of the developed AQbD-based RP-HPLC method with previously reported analytical approaches.

Sr. No.	Reference / Method	Analytical approach	Key characteristics reported in literature	AQbD/DoE approach	Forced-degradation / stability-indicating evaluation	Distinguishing feature compared with present study
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01	Sung & Leo, 2021 [32]	HPLC-UV	Validated HPLC-UV method for quantification of formononetin and biochanin A in <i>Trifolium pratense</i> extract	Not reported in the cited article	Not the primary focus	Demonstrates conventional HPLC-UV validation, whereas the present study applies AQbD-based optimization specifically to RVT
02	Blessy et al., 2014 [32]	Review of forced-degradation methodologies	Comprehensive discussion of forced degradation and stability-indicating methods	Not applicable	Yes; review of acidic, basic, oxidative, thermal and photolytic stress approaches	Provides the scientific basis for forced-degradation studies; the present work experimentally applies these stress conditions to RVT
03	Beg et al., 2015 [34]	Stability-indicating LC	QbD-based development and validation of an LC method for olmesartan medoxomil	Yes – QbD	Yes	Demonstrates the value of QbD for chromatographic method development; the present study applies the AQbD concept to RVT
04	Kolimi et al., 2022 [35]	HPLC	Development and validation of HPLC method for efinaconazole with application to permeation studies	Not primarily presented as AQbD	Application-oriented	Shows the utility of validated HPLC methods in pharmaceutical analysis, while the present study combines systematic AQbD optimization with forced degradation
05	Monks et al., 2012 [36]	HPLC method development	Multidimensional exploration of design space for improved chromatographic robustness	Yes – QbD/DoE	Not the primary focus	Establishes the scientific principle of design-space-based chromatographic optimization; the present method implements this concept for RVT
06	Rozet et al., 2012 [37]	Analytical method validation	QbD-compliant analytical method validation and assessment of analytical performance	Yes – QbD	Not the primary focus	Supports the QbD philosophy used in the present study to establish method reliability
07	Present study	RP-HPLC-UV	Phenomenex Kinetex XB-C18 (150 × 4.6 mm, 5 µm); buffer: methanol 58:42 v/v; 1.0 mL/min; 306 nm; 30°C; RT 5.29 min; 15-min run time	Yes – AQbD with experimental optimization of critical method parameters and evaluation of critical analytical attributes	Yes – acidic, basic, oxidative, dry-heat and photolytic stress	Integrated AQbD-driven optimization, validation, robustness assessment and forced-degradation evaluation for RVT; linearity $r^2 = 0.9997$ and LOD/LOQ = 0.31/0.94 µg/mL

The comparative assessment demonstrates that the present analytical procedure offers a comprehensive AQbD-based strategy for resveratrol analysis (Table 3). Although previously reported studies have established the usefulness of HPLC and quality-by-design principles for pharmaceutical analysis, the present work integrates systematic AQbD-driven optimization with method validation, robustness evaluation and forced-degradation

assessment in a single analytical workflow. The optimized method provided satisfactory chromatographic performance with a retention time of 5.29 min, excellent linearity ($r^2 = 0.9997$), low LOD and LOQ values of 0.31 and 0.94 µg/mL, respectively, and recoveries close to 100%. In addition, deliberate variations in critical chromatographic conditions demonstrated the robustness of the method, while acidic, basic, oxidative, thermal and

photolytic stress studies demonstrated its potential for stability-indicating analysis. Therefore, the principal contribution of the present work is not merely the development of another RP-HPLC method for resveratrol, but the application of an AQbD-driven and scientifically controlled methodology that provides a rational basis for optimization, robustness and reliable routine quality-control analysis.

CONCLUSION

This study presents an accurate, precise & reliable RP-HPLC technique for RVT measurement in bulk & pharmaceutical preparations. The technique was tested for correctness, exactness & resilience using the AQbD approach and applied to commercial dosage forms. Its simplicity, sensitivity, selectivity, and fast analysis make it excellent for low-concentration quality control. Validation showed the method's dependability and cost-effectiveness. Forced degradation experiments showed RVT susceptibility to acidic, basic, and oxidative stress (1-8% degradation), emphasizing the necessity for light and air protection. This approach outperforms others in peak symmetry, run time, and deterioration profiling.

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