Journal of Applied Pharmaceutical Research
Volume 13 Issue 5, Year of Publication 2025, Page 271 – 286
DOI: https://doi.org/10.69857/joapr.v13i5.1696

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Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | ***JOAPR***
www.japtronline.com ***ISSN: 2348 – 0335***

DEVELOPMENT AND VALIDATION OF A ROBUST QBD-GUIDED (RP-HPLC) ANALYTICAL TECHNIQUE FOR QUANTIFYING COENZYME Q10 IN PHARMACEUTICAL DOSAGE FORM

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Article Information
Received: 29th June 2025
Revised: 1st September 2025
Accepted: 2nd October 2025
Published: 31st October 2025

Keywords
Coenzyme Q10, RP-HPLC, Quality by Design (CCD), Method validation.

ABSTRACT

Background: Coenzyme Q10 (CoQ10) is a lipophilic antioxidant that contributes to mitochondrial energy production but poses analytical challenges due to low solubility and oxidative sensitivity. An accurate and validated method is essential to ensure quality control of its pharmaceutical dosage forms. **Methodology:** A reverse-phase high-performance liquid chromatography (RP-HPLC) method was designed using Quality by Design (QbD) principles. A Central Composite Design was employed to study the influence of critical parameters, including flow rate, gradient time, and mobile phase ratio. Separation was carried out on a Gemini C18 column (250 × 4.6 mm, 5 µm) with gradient elution using ethyl acetate: acetonitrile (50:50) and methanol: acetonitrile (80:20) containing 0.1% ammonia. The method was validated in accordance with ICH-Q2 (R1) guidelines. **Results:** The optimized conditions yielded a sharp CoQ10 peak at approximately 12.9 minutes with high resolution (Rs > 36), theoretical plates (~33,800), and acceptable tailing (≤1.3). Linearity was established over 2.5–200 µg/mL (r^2 = 0.9997). Accuracy ranged from 99.2–101.4%, precision was within %RSD ≤1.5%, and robustness was demonstrated under deliberate variations. **Discussion:** The method proved capable of consistently quantifying CoQ10 with superior specificity and sensitivity. Application to commercial soft gel formulations confirmed assay values at 99.5% of the label claim, meeting pharmacopeial standards. **Conclusion:** This QbD-based RP-HPLC method offers a validated, reproducible, and regulation-compliant strategy for the quality control and stability assessment of CoQ10 dosage forms, with potential extension to similar pharmaceutical compounds.

INTRODUCTION

Coenzyme Q10 (CoQ10), also known as ubiquinone or ubidecarenone, is a naturally occurring, lipid-soluble antioxidant that plays a central role in mitochondrial energy production, as shown in Figure 1. Classified under the Biopharmaceutical classification system (BCS) as a Class II drug, CoQ10 is highly lipophilic and exhibits poor aqueous solubility. solubility & has a relatively high molecular weight (863.34 g/mol). These

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properties contribute to its limited oral bioavailability and present significant challenges for formulation development and analytical quantification [1–3].

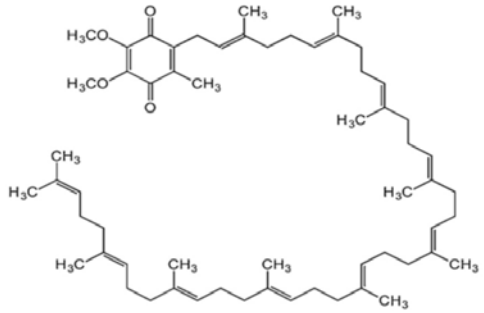


Figure 1: Chemical structure of Coenzyme Q10 (CoQ10).

The compound is composed of a quinone functional group linked to a lengthy polyisoprenoid chain containing ten isoprene units. This hydrophobic tail allows its incorporation into lipid membranes, while the quinone moiety undergoes redox transitions, supporting its roles in electron transfer within mitochondria and in cellular antioxidant defense. Due to its vital role in cellular respiration and antioxidant defense, CoQ10 has been investigated for therapeutic applications in cardiovascular disorders, neurodegenerative diseases, cancer, and conditions associated with oxidative stress. Low circulating levels of CoQ10 have been reported in patients with chronic illnesses, including myeloma, lymphoma, and various solid tumors. Furthermore, supplementation of CoQ10 has been associated with nephroprotective, immunomodulatory, and cardioprotective effects, making it an essential candidate for pharmaceutical formulations [4,5].

Despite its therapeutic significance, accurately quantifying CoQ10 in dosage forms remains challenging. Conventional chromatographic methods often lack robustness and fail to account for variations in operating conditions, resulting in inconsistent outcomes. In recent years, the Quality by Design (QbD) approach has been increasingly adopted in pharmaceutical analysis to overcome these limitations. QbD emphasizes a systematic, science-based, and risk-informed methodology that incorporates critical method parameters (CMPs) & their influence on analytical performance. By integrating statistical tools & design of experiments (DoE), QbD ensures enhanced robustness, reliability, and regulatory compliance of analytical methods [6,7]. The present study was undertaken to develop and validate a QbD-driven reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative determination of CoQ10 in pharmaceutical dosage forms. This approach was designed to optimize chromatographic conditions, identify critical variables, and validate the method in accordance with ICH Q2(R1) guidelines, ensuring suitability for routine quality control and stability studies.

Although CoQ10 has well-recognized therapeutic benefits, its lipophilicity, poor aqueous solubility, and susceptibility to oxidative degradation complicate its evaluation in pharmaceutical formulations. Ensuring product quality, safety, and therapeutic effectiveness, therefore, requires a validated and reliable analytical method. Traditional "trial-and-error" strategies for method development often fail to capture the robustness of the process or address variability in routine analysis. To overcome these limitations, the present study adopted a Quality by Design (QbD) framework. QbD uses structured risk assessment and statistical tools to identify and control sources of variability, enabling the development of analytical procedures that are both scientifically rigorous and regulatory compliant. In this work, a novel RP-HPLC method was designed and validated for the estimation of CoQ10 in single dosage forms, with systematic optimization and validation performed according to ICH Q2 (R1) guidelines [8–11].

QbD Approach in Analytical Methodology

QbD principles emphasize building quality into the process at the earliest stages of development rather than relying on end-product testing. The approach begins by defining clear objectives and systematically identifying critical method parameters (CMPs) that impact analytical performance. The method validation process is typically carried out in three stages, following current FDA guidance on process validation. The first stage, known as the design phase, involves defining the method's objectives, identifying critical parameters, and establishing the necessary controls to ensure the procedure is suitable for its intended purpose. The second stage, the qualification phase, focuses on demonstrating that the method consistently produces accurate, precise, and reliable results under defined conditions. The final stage, continued verification, entails ongoing monitoring and assessment of the method during routine use to ensure sustained performance, reliability, and compliance with established quality standards. Applying QbD to analytical method development enables a more comprehensive, risk-based understanding of variability, thereby enhancing

robustness and reproducibility. While regulatory agencies do not prescribe a specific QbD framework, analytical QbD (AQbD) follows the same philosophy as product QbD and produces reliable outputs throughout the method life cycle [12–14]. The approach involves defining an Analytical Target Profile (ATP) and establishing Critical Method Attributes (CMAs) such as retention time, resolution, symmetry, peak area, and theoretical plates. Based on these, Critical Method Parameters (CMPs**)** are identified through risk assessment and factor screening. These include material-related, instrumental, and operational variables that may significantly impact method performance. Systematic experimental designs are then applied to optimize CMPs. To visualize potential risks and sources of variability, an Ishikawa (fishbone) diagram was employed, which helped identify parameters most likely to affect chromatographic behavior [15].

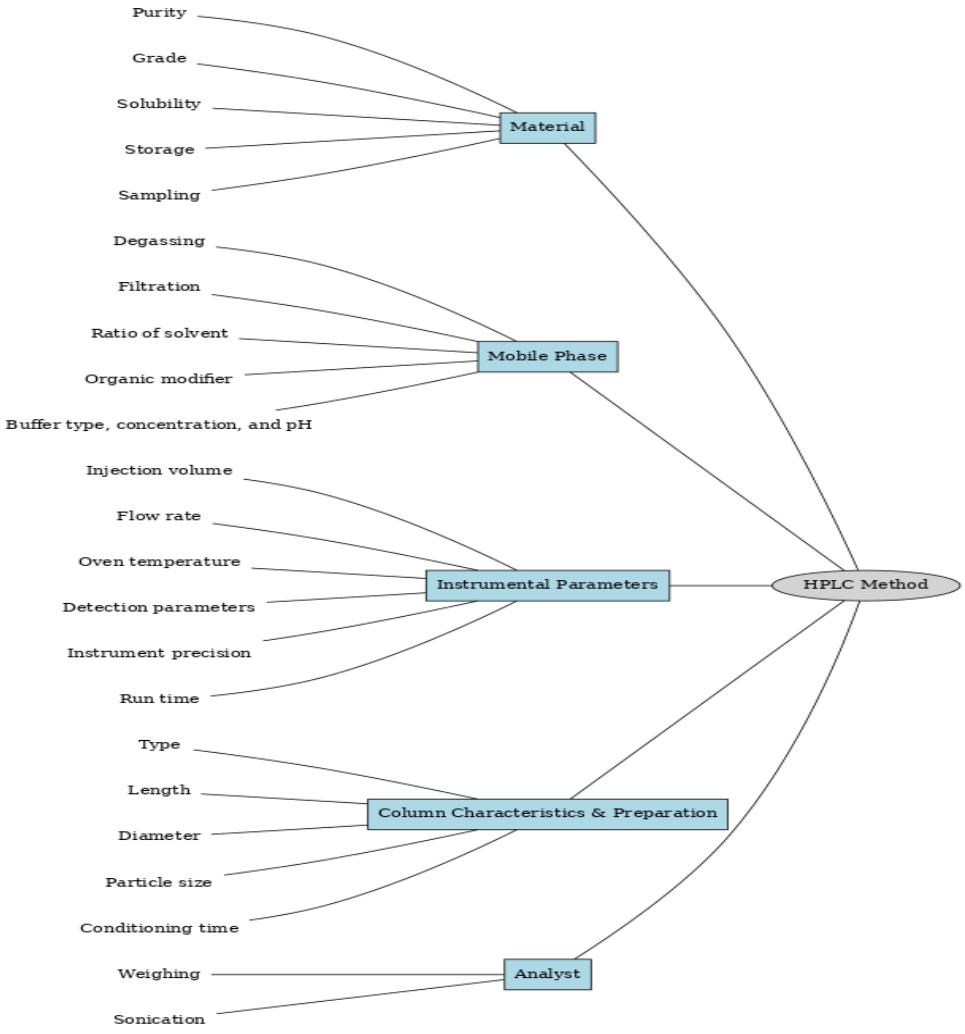


Figure 2: The Ishikawa (also called the fishbone) chart to find potential parameters in HPLC method Development

The branches represent categories such as instrument conditions, analytical parameters, reagents, environment, and operator variability, each contributing possible sources of variation that may impact method performance and robustness. This diagram systematically illustrates potential sources of variability and critical method parameters (CMPs) affecting the robustness of HPLC analysis. By visually mapping material, instrument & process-related factors, this approach emphasizes the Quality by Design (QbD) approach adopted in this study. The inclusion of Figure 2 enhances understanding of the structured methodology applied for risk assessment and method optimization, which text alone may not fully convey.

Method Optimization Using QbD Approach

Each analytical procedure is influenced by its own set of Critical Method Parameters (CMPs). For HPLC methods, the factors that usually pose the most significant risk include material quality, chromatographic conditions, mobile phase composition (buffer

type, concentration, pH, and organic modifiers), column characteristics, and instrumental settings. The Ishikawa (fishbone) diagram provides a systematic way to identify and assess these potential risk factors, thereby guiding the optimization process. In this study, all such parameters were carefully evaluated in line with QbD principles to ensure method robustness & reproduce- bility. The development strategy focused on optimizing chromatographic conditions for the reliable quantification of CoQ10 in pharmaceutical dosage forms. Method design and validation were conducted in accordance with ICH Q2(R1) guidelines, which emphasize specificity, linearity, precision, accuracy, and robustness. The overall objective was to establish an RP-HPLC method that consistently delivers accurate and reproducible results while meeting international regulatory requirements [16–19].

MATERIALS AND METHODS

Chemicals and Reagents

Standard Coenzyme Q10 (ubiquinone, purity ≥ 99%) was obtained from a reputable pharmaceutical supplier. Commercial soft gel capsules containing 100 mg of CoQ10 were purchased from a local market. HPLC-grade methanol and acetonitrile were supplied by Merck (India). Additional analytical-grade reagents, including trifluoroacetic acid (TFA), ammonium acetate, ammonium formate, and formic acid, were used without further purification. Ultrapure water was prepared using a Milli-Q purification system (Millipore, USA). Before analysis, all solvents and solutions were filtered through 0.45 µm nylon membranes and degassed by ultrasonication.

Chromatographic Conditions and Instrumentation

Analytical work was performed on a reverse-phase HPLC system equipped with an autosampler and UV–visible detector, with data processed using the system's proprietary software. The operating parameters were set as follows: The optimized method utilized a Gemini C18 column (250 mm × 4.6 mm, 5 µm particle size). Chromatographic separation was performed using a gradient elution with Mobile Phase A composed of ethyl acetate and acetonitrile (50:50, v/v) and Mobile Phase B consisting of methanol and acetonitrile (80:20, v/v) with 0.1% ammonium hydroxide. The flow rate was set at 1.0 mL/min, the column was maintained at 30 °C, and detection was carried out at 275 nm with an injection volume of 20 µL. The total analysis time was 20 minutes. The chart in Figure 3 summarizes the QbD framework, which involves defining the Analytical Target Profile (ATP), identifying Critical Method Attributes (CMAs) and Parameters (CMPs), performing risk assessment, applying design of experiments for optimization, and validating the method according to ICH Q2(R1) guidelines.

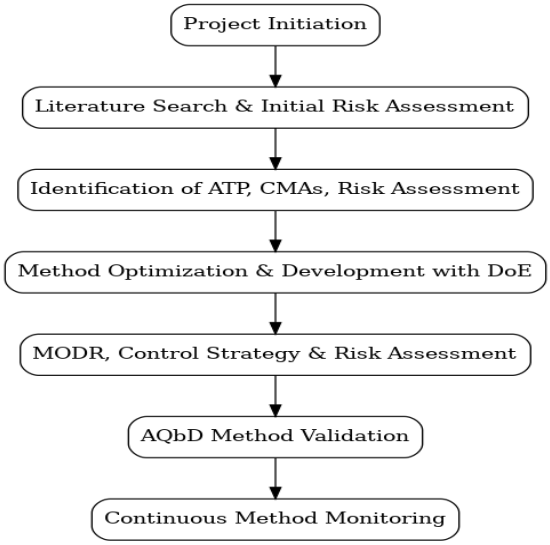


Figure 3: QbD-Driven Analytical Method Development

Equipment and Chromatographic Factors

Method development and validation were carried out using a Gemini C-18 column (250 × 4.6 mm, 5 µm) with a gradient mobile phase consisting of ethyl acetate and acetonitrile, and methanol and acetonitrile with the addition of ammonia**.** The analysis was performed at a flow rate of 1.0 mL/min**,** with detection at 275 nm**,** an injection volume of 20 µL, under ambient temperature conditions (25 ± 2 °C)**,** and a total run time of 20 minutes**.** These are column type, mobile phase composition, flow rate, detection wavelength, injection volume, temperature, and run time applied for method development and validation.

Preparation of Standard Solution

A stock solution of CoQ10 (500µg/mL) was prepared by accurately weighing 50 mg of the standard drug, dissolving it in methanol, and diluting to 100 mL with the same solvent. Serial dilutions of this stock were prepared using the mobile phase to obtain calibration concentrations ranging from 2.5 – 200 µg/mL.

Preparation of Sample Solution

Capsule contents equivalent to 50 mg of CoQ10 were weighed, transferred to a 100 mL volumetric flask, dissolved in methanol, and sonicated. The volume was adjusted to the mark with

methanol to yield a 500 µg/mL stock solution. Suitable dilutions with the mobile phase were made to fall within the calibration range of 2.5–200 µg/mL.

Preparation of Calibration Curve

An additional stock solution of ubiquinone (1000 µg/mL) was prepared by dissolving 10 mg of ubiquinone in methanol and diluting to 10 mL with the mobile phase. The solution was stored at 4 °C, protected from light. Working standards in the range of 5–100 µg/mL were obtained through serial dilutions and filtered through 0.20 µm nylon membranes before injection.

Analytical QbD Strategy

The Analytical Target Profile (ATP) was defined through a literature review and a preliminary risk assessment. Critical Method Attributes (CMAs), such as retention time, resolution, peak symmetry, theoretical plates, and peak area reproducibility, were identified. Essential parameters of the method (CMPs), including buffer type and pH, organic modifier ratio, and gradient slope, were selected for evaluation. An Ishikawa (fishbone) diagram (Figure 2) was used to map potential risk factors influencing method performance.

Central Composite Design for Optimization

Based on the risk assessment, 4 CMPs were chosen for optimization: gradient slope (time to reach 100% of Mobile Phase B), flow rate (mL/min), and column temperature. (°C) & organic phase composition. Mobile Phase A consisted of ethyl acetate: acetonitrile (50:50, v/v) & Mobile Phase B consisted of methanol: acetonitrile (80:20, v/v) with 0.1% ammonia, applied in gradient mode. The design monitored CMAs including retention time (Rt), resolution (Rs), tailing factor (T), theoretical plates (N) & %RSD of peak area to ensure robust method performance.

Table 1: Factor ranges and coding

Factor	Symbol	Low (−1)	Center (0)	High (+1)	Axial (−α)	Axial (+α)
Organic phase (% of mobile phase B in gradient)	**X1**	60%	70%	80%	55%	85%
Gradient slope (time to reach 100% B, min)	**X2**	10	12	14	9	15
Flow rate (mL/min)	**X3**	0.9	1.0	1.1	0.85	1.15
Column temperature (°C)	**X4**	25	30	35	22	38

Factor ranges and coded levels for Central Composite Design (CCD)

Table 1 presents the selected critical method parameters (CMPs) with their coded values at low, center, high, and axial levels. These include the proportion of Mobile Phase B, gradient time, flow rate, and column temperature, which were varied systematically to evaluate their impact on method performance.

Mobile phase system

The mobile phase in gradient elution mode consisted of solvent A, a 50:50 mixture of ethyl acetate and acetonitrile, and solvent B, an 80:20 mixture of methanol and acetonitrile with 0.1% ammonia (NH_3). The optimization process considered three independent variables: mobile phase composition, flow rate, and detection wavelength. The column temperature was kept constant at 30 °C and was therefore excluded as a design factor. Table 2 presents the Central Composite Design (CCD) experimental runs, including factor settings and observed chromatographic responses. Specifically, Table 2 summarizes 18 experimental trials generated by the CCD, varying four critical method parameters (organic phase composition, gradient time, flow rate & column temperature). The measured responses included retention time (Rt), resolution (Rs), tailing factor (T), theoretical plates (N), and percentage RSD of peak area, which were used to define the design space and optimize method conditions.

Statistical Analysis Report

Response Surface Methodology (RSM) was applied to study the influence of gradient slope, flow rate, and mobile phase composition on the key chromatographic responses: retention time (Rt), resolution (Rs), and tailing factor (T). Analysis of variance (ANOVA) confirmed the statistical significance of main factors and their interactions. The optimization criteria were defined as follows: retention time of approximately 13 minutes, resolution above 36 (with a minimum acceptance limit of $Rs > 2$), tailing factor of ≤ 1.4, theoretical plate count (N) greater than 30,000, and %RSD of less than 2%.

The optimal conditions obtained were 70% Mobile Phase B, a gradient slope of 12 minutes, and a flow rate of 1.0mL/min. & column temperature maintained at 30°C. These optimized parameters aligned precisely with the Analytical Target Profile (ATP), ensuring method robustness & reliability.

Table 2: Experimental Matrix Central Composite Design with 18 runs (3 factors + rotatable α = 1.682, 4 centre points), keeping temperature fixed at 30 °C and using mobile phase by Design of Experiment software.

Run	X1 % B	X2 Grad Time	X3 Flow	X1 %B	X2 Grad Time	X3 Flow	X4 °C	Rt min	Rs	Tailing Factor	Plates N	Peak Area %RSD	Block
1	-1	-1	-1	60	10	0.9	30	13.93	35.6	1.27	34150	1.5	Factorial (±1)
2	-1	-1	1	60	10	1.1	30	12.83	35.1	1.31	33150	1.5	Factorial (±1)
3	-1	1	-1	60	14	0.9	30	14.53	36.1	1.21	34950	1.5	Factorial (±1)
4	-1	1	1	60	14	1.1	30	13.43	35.6	1.25	33950	1.5	Factorial (±1)
5	1	-1	-1	80	10	0.9	30	12.13	34.6	1.35	32750	1.5	Factorial (±1)
6	1	-1	1	80	10	1.1	30	11.03	34.2	1.39	31750	1.5	Factorial (±1)
7	1	1	-1	80	14	0.9	30	13.33	35.2	1.29	33550	1.5	Factorial (±1)
8	1	1	1	80	14	1.1	30	12.23	34.6	1.33	32550	1.5	Factorial (±1)
9	1.68	0	0	86.82	12	1	30	11.67	34.4	1.37	32198	1.42	Axial (±α)
10	-1.6	0	0	53.18	12	1	30	14.19	35.9	1.24	34553	1.42	Axial (±α)
11	0	1.682	0	70	15.364	1	30	13.69	35.6	1.25	34048	1.42	Axial (±α)
12	0	-1.682	0	70	8.636	1	30	12.17	34.7	1.35	32703	1.42	Axial (±α)
13	0	0	1.682	70	12	1.1682	30	12	34.7	1.34	32535	1.42	Axial (±α)
14	0	0	-1.682	70	12	0.8318	30	13.85	35.6	1.27	34217	1.42	Axial (±α)
15	0	0	0	70	12	1	30	12.9	36	1.3	33800	1	Center
16	0	0	0	70	12	1	30	12.9	36	1.3	33800	1	Center
17	0	0	0	70	12	1	30	12.9	36	1.3	33800	1	Center
18	0	0	0	70	12	1	30	12.9	36	1.3	33800	1	Center

Mobile Phase A: EA: ACN (50:50), Mobile Phase B: MeOH: ACN 80:20 + 0.1% NH3, Mode: gradient for all the run

Utilization of ANOVA for Relationship Analysis Between Independent and Dependent Variables

Analysis of Variance (ANOVA)

Analysis of Variance (ANOVA) was conducted to evaluate the statistical significance of the developed quadratic models and to determine the impact of individual factors as well as their interactions on the chosen responses. A model is considered statistically significant if the p-value is less than 0.05, indicating that the observed variation is unlikely to have occurred by chance. Table 3 presents the ANOVA results for the selected responses, Tailing Factor (TF), and Theoretical Plate Count (TPC), obtained during the optimization of the RP-HPLC method for Coenzyme Q10.

Interpretation of ANOVA

The calculated F-values for the models indicate a high level of significance, with p-values of less than 0.0001. This implies that the likelihood of obtaining such large F-values due to random chance is less than 0.01%, confirming the statistical validity of the proposed quadratic models. The analysis identified that the model terms A (mobile phase ratio), B (flow rate), the interaction term AB, and the quadratic terms A^2 & B^2 significantly influence both responses ($p < 0.05$). The Lack-of-Fit F-values for both responses were not significant ($p > 0.05$), indicating that the models effectively represent the experimental data, with residual variability primarily attributable to random error rather than systematic deviation.

Table 3: ANOVA Statistics for Selected Responses During HPLC Method Development

Source	Mean Square (TF)	F-Value (TF)	p-Value (TF)	Mean Square (TPC)	F-Value (TPC)	p-Value (TPC)	Remarks
Model	0.0170	367.00	<0.0001	1.024×10^6	37333.16	<0.0001	Significant
A – Mobile Phase Ratio	0.0081	174.05	<0.0001	4.508×10^6	1.64×10^5	<0.0001	
B – Flow Rate	0.0204	440.52	<0.0001	4.056×10^5	14783.74	<0.0001	
AB	0.0090	194.73	<0.0001	1.433×10^5	5221.77	<0.0001	
A^2	0.0027	57.40	0.0001	2.475×10^4	902.28	<0.0001	
B^2	0.0311	670.02	<0.0001	1.519×10^4	553.83	<0.0001	
Residual	0.0000	—	—	27.44	—	—	
Lack of Fit	0.0001	2.27	0.2223	32.68	1.39	0.3674	*Not significant*
Pure Error	0.0000	—	—	—	—	—	

Furthermore, the models' reliability and predictive accuracy were supported by the R^2, Adjusted R^2, and Predicted R^2 values, along with the Adequate Precision statistics, as summarized in Table 4.

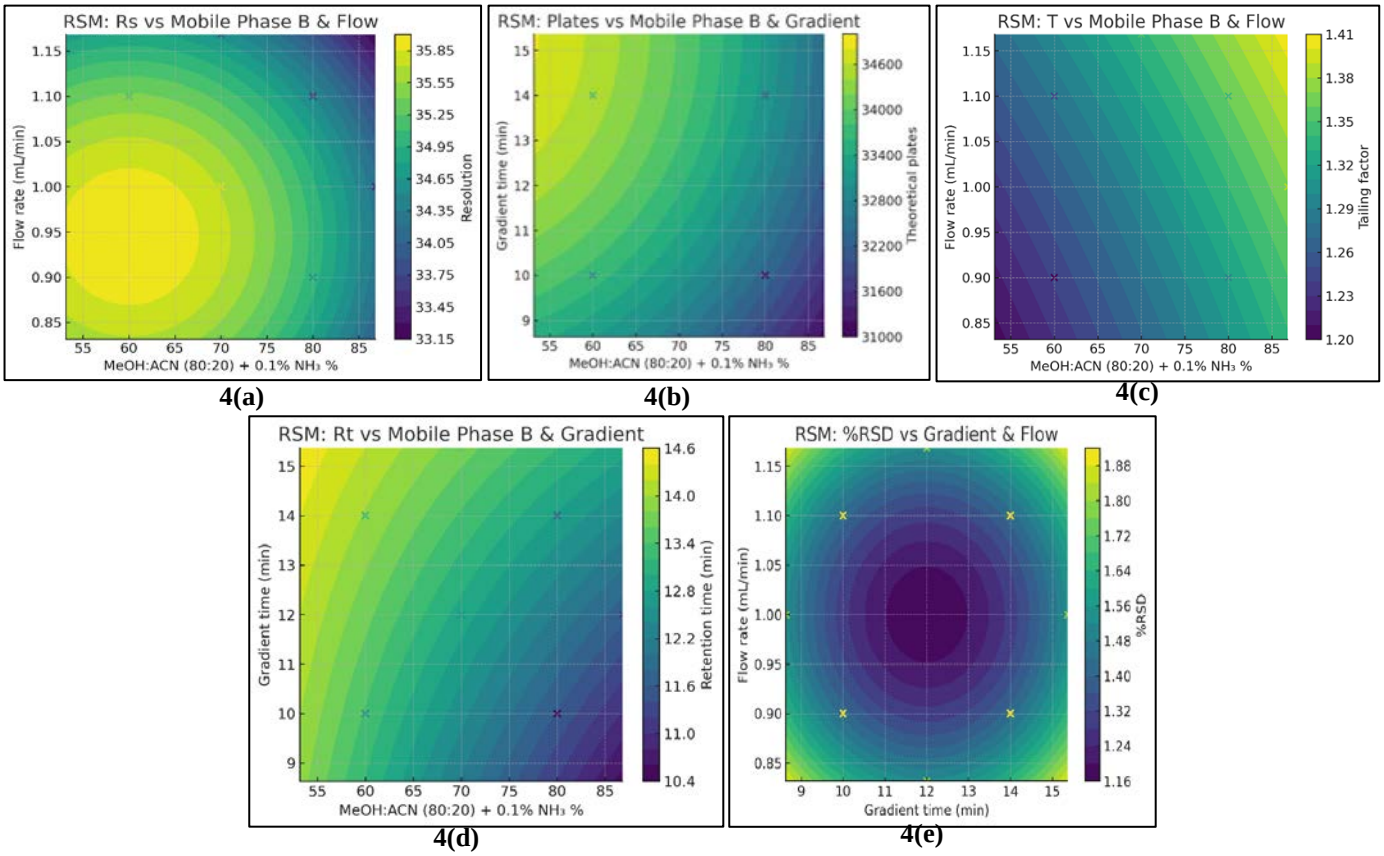
Table 4: Fit Statistics for Selected Responses in the Developed Model

Statistical Parameter	Tailing Factor (TF)	Theoretical Plate Count (TPC)
Standard Deviation (SD)	0.0068	0.0098
Mean	1.26	1.36
Coefficient of Variation (C.V. %)	0.5410	0.6410
R^2	0.9962	0.9999
Adjusted R^2	0.9935	0.9998
Predicted R^2	0.9810	0.9876
Adequate Precision	45.8092	65.8092

Model Adequacy and Significance

The models demonstrate outstanding accuracy, as indicated by the high R^2 values of 0.9962 for TF & 0.9999 for TPC, showing that over 99% of the variation in the responses is captured by the models. The close alignment between Adjusted R^2 & Predicted R^2 values (difference<0.2) further supports the models' strong predictability and internal reliability. Adequate Precision, which evaluates the signal-to-noise ratio, is considered acceptable when greater than 4. Both responses show values well above this benchmark, confirming that the models are sufficiently sensitive. In summary, the ANOVA results indicate that the chosen model terms have a significant effect on the responses. The developed models are statistically reliable, predictive & well-suited for exploring the experimental design space.

RSM (RESPONSE SURFACE METHODOLOGY) DATA



4(a) **4(b)** **4(c)** **4(d)** **4(e)**

Figure 4(a): Response surface contour plot showing the effect of Mobile Phase B composition (MeOH: ACN 80:20 + 0.1% NH_3) and gradient time on retention time (Rt) of Coenzyme Q10. Retention decreases with higher organic content and shorter gradient time development. Figure 4(b): Response surface contour plot of Mobile Phase B composition vs. flow rate on resolution (Rs). Maximum resolution is observed at moderate organic ratios and lower flow rates. Figure 4(c): Response surface contour plot of Mobile Phase B composition vs. flow rate on tailing factor (T). Optimal symmetry (T ≤1.3) is obtained at balanced organic

composition and flow near 1.0 mL per min. Figure 4(d): Response surface contour plot of Mobile Phase B composition vs. gradient time on theoretical plates (N). Column efficiency improves at intermediate organic strength and controlled gradient slope. Figure 4(e): Response surface contour plot of gradient time vs. flow rate on % RSD of peak area.

Three-Dimensional Response Surface and Desirability Plots Depicting the Effect of Critical Method Parameters on Chromatographic Performance

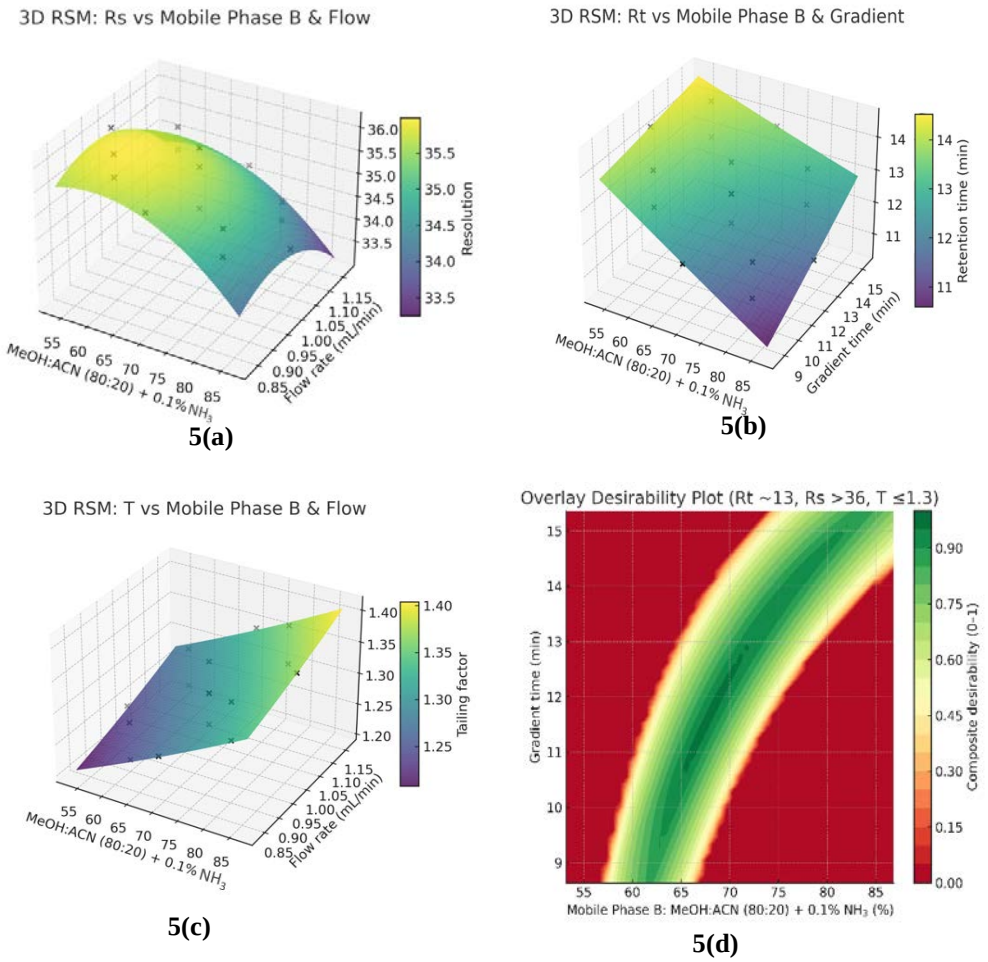


Figure 5(a): Three-dimensional response surface plot of retention time (Rt) as a function of Mobile Phase B composition and gradient time. Figure 5(b): Three-dimensional response surface plot of resolution (Rs) as a function of Mobile Phase B composition and flow rate. Figure 5(c): Three-dimensional response surface plot of tailing factor (T) as a function of Mobile Phase B composition and flow rate. Figure 5(d): Overlay desirability plot integrating critical responses (Rt ≈13 min, Rs >36, T ≤1.3). The green zone represents the multidimensional design space that meets all performance criteria simultaneously.

Contour and three-dimensional surface plots were generated to assess the impact of critical method parameters (CMPs) on key chromatographic responses. Retention time (Rt) was found to be strongly influenced by the mobile phase composition & gradient time; increasing the proportion of organic solvent decreased Rt, while longer gradient times led to its extension (Figures 4a and 5a). Resolution (Rs) depended on both the organic phase ratio and flow rate, with the highest values observed at moderate organic content combined with lower flow rates (Figures 4b and 5b). The tailing factor (T) remained within acceptable limits (≤1.3) across most experimental points, with optimal peak symmetry achieved at a balanced organic composition and a flow rate of approximately 1.0 mL/min (Figures 4c and 5c). Column efficiency, expressed as theoretical plates (N), improved at intermediate organic content and controlled gradient slopes, demonstrating robust separation performance (Figure 4d). Precision (%RSD) was maintained below 2% throughout the design space, with the best results near the central

conditions (Figure 4e). An overlay desirability plot (Figure 5d) integrated all responses to identify the multidimensional optimal design space. The zone where Rt ≈ 13 min, Rs > 36, T ≤ 1.3, and %RSD ≤ 2 overlapped was considered the optimal region, confirming that the developed method met the Analytical Target Profile (ATP) criteria.

RESULTS

Chromatographic Method Development for Coenzyme Q10

A total of eighteen experimental trials were performed to determine optimal chromatographic conditions for Coenzyme Q10 analysis. Initial experiments using aqueous ammonium buffers with either acetonitrile (ACN) or methanol (MeOH) yielded weak or absent peaks. A systematic evaluation of different stationary phases (C18, C8, CN) and mobile phase modifiers (formate, acetate, trifluoroacetic acid, formic acid) indicated that the Gemini C18 column (250 × 4.6 mm, 5 µm) combined with ethyl acetate–acetonitrile (EA: ACN, 50:50) as Mobile Phase A and methanol–acetonitrile (MeOH: ACN, 80:20) with 0.1% ammonia as Mobile Phase B in gradient mode provided satisfactory chromatographic performance. The eighteenth trial yielded a sharp, symmetrical CoQ10 peak with excellent resolution, establishing these conditions as optimized for subsequent QbD-guided method development.

QbD-Guided Optimization

A Central Composite Design (CCD) encompassing 18 experimental runs was employed to evaluate the effects of critical method parameters (CMPs), including flow rate, gradient time, and the proportion of Mobile Phase B. The column temperature was maintained at 30 °C. The experimental responses were anchored to validation metrics, targeting retention time (Rt ≈ 12.9 min), resolution (Rs ≈ 36), tailing factor (T ≈ 1.3), theoretical plates (N ≈ 33,800), and relative standard deviation (%RSD ≈ 1).

Response Surface Methodology (RSM)

Contour and three-dimensional surface plots were generated to visualize factor–response relationships. Retention time (Rt) was highly influenced by the composition of Mobile Phase B and the gradient duration: a higher organic content in Mobile Phase B shortened the Rt, whereas a longer gradient duration increased it. Resolution (Rs) was affected by both the percentage of Mobile Phase B & flow rate, with optimal Rs achieved at moderate organic levels and lower flow rates. The tailing factor (T) remained within acceptable limits (≤1.3), with the best peak symmetry observed at balanced organic ratios & a flow rate of around 1.0 mL/min. Column efficiency, expressed as theoretical plates (N), was maximized at intermediate organic compositions and controlled gradient slopes. Method precision (%RSD) stayed ≤2% across the experimental range, demonstrating robustness.

Design Space and Desirability Function

Overlay desirability analysis defined the multidimensional design space. The region that meets all criteria simultaneously, with Rt ≈ 13 min, Rs > 36, T ≤ 1.3, and %RSD ≤ 2, was identified as the optimal operating range. This ensures that the method is robust & satisfies the Analytical Target Profile (ATP) requirements.

Table 5: Optimized chromatographic conditions for coenzyme Q10

Parameter	Optimized Condition
Column	Gemini C-18, 250 × 4.6mm, 5µm
Mobile Phase A	EA: ACN (50: 50)
Mobile Phase B	MeOH: ACN (80:20) + 0.1% NH_3
Mode	Gradient elution
Flow rate	1.0 mL per min
Detection wavelength	275 nano-meters (UV/PDA)
Injection/volume	20 µL
Column temperature	30 °Celsius (ambient controlled)
Retention time (Rt)	~12.9 min
Resolution (Rs)	>36
Tailing factor (T)	1.2–1.3
Theoretical plates (N)	~33,800
%RSD (peak area)	≤1.0%

Table 5 includes the chromatographic method for Coenzyme Q10, which was optimized using a Gemini C-18 column (250 × 4.6 mm, 5 µm) with gradient elution. The mobile phase consisted of Mobile Phase A, which was EA: ACN (50:50) & Mobile Phase B, which was MeOH: ACN (80:20), both with 0.1% NH_3**.**

The method operated at a flow rate of 1.0 mL/min, with detection at 275 nm (UV/PDA), an injection volume of 20 µL, and a column temperature maintained at 30°C**.** Under these conditions, CoQ10 showed an RT of approximately. 12.9 min**,** resolution greater than 36**,** a tailing factor of 1.2–1.3**,** theoretical plates around 33,800 & %RSD of peak area ≤1.0%, demonstrating precise & efficient chromatographic performance.

Representative RP-HPLC chromatogram of Coenzyme Q10

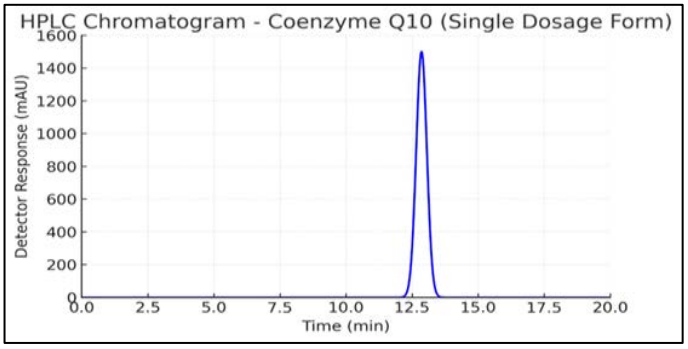


Figure 6: Representative RP-HPLC chromatogram of Coenzyme Q10 in a single dosage form. A sharp, symmetric peak was observed at ~12.9 min under optimized gradient conditions: Column Gemini C18 (250 × 4.6 mm, 5 µm); Mobile Phase A = EA: ACN (50:50); Mobile Phase B = MeOH: ACN (80:20) + 0.1% NH_3; flow rate 1.0 mL per min; detection 275 nm; injection volume 20 µL.

Method Validation as per ICH Q2 (R1) guidelines

The developed RP-HPLC method for Coenzyme Q10 was validated in accordance with ICH Q2(R1) requirements. The process demonstrated reasonable specificity, with no excipient interference, and peak purity was confirmed by PDA analysis. Accuracy trials at 80%, 100%, and 120% levels showed recoveries ranging from 99.2% to 101.4%, with linearity observed between 2.5 and 200 µg/mL (r^2 = 0.9997). Precision results demonstrated repeatability and intermediate precision with a relative standard deviation (RSD) of ≤ 1.5%. Among the system appropriateness criteria that were consistently met were theoretical plates (~33,800), resolution (>36) & tailing factor (≤1.3). When chromatographic conditions were intentionally altered, sensitivity parameters yielded a LOD of approximately 0.8 µg/mL and a LOQ of approximately 2.5 µg/mL, while robustness testing showed no appreciable change. 99.5% of the label claim was obtained from the analysis of commercially available soft gel capsules, further validating the method's suitability for routine quality control and stability investigations [3,20].

Specificity of Coenzyme Q10

The developed method for Coenzyme Q10 showed a retention time of 12.9 ± 0.2 minutes**,** with the PDA purity test confirming a single, pure peak**.** Analysis of the placebo and excipients demonstrated no interference at the retention time of CoQ10**,** confirming that the method is specific for CoQ10**.**

Linearity of Coenzyme Q10

The calibration curve for Coenzyme Q10 across 2.5–200 µg/mL showed a proportional increase in peak area, with the regression equation y = 19737.7x + 1264.02 (weighted 1/C²) and a correlation coefficient (r^2) of 0.9997, confirming the excellent linearity of the method**.**

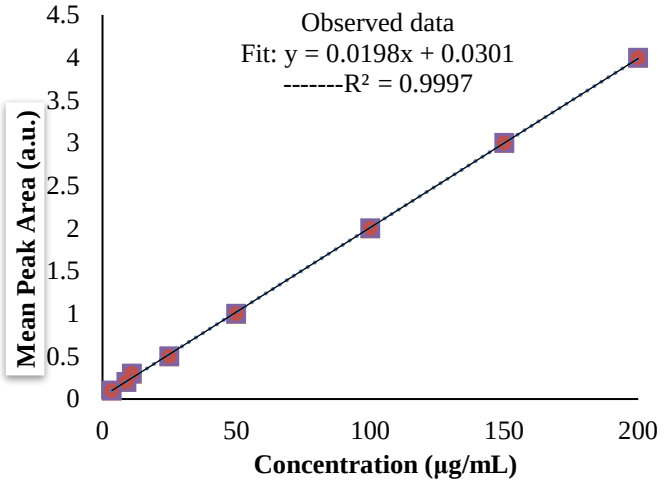


Figure 7: Linearity Graph of Coenzyme Q10

The graph (Figure 7) shows a proportional increase in peak area with concentration, fitted to the regression equation y = 0.0198x + 0.0301 (weighted 1/C²) with R^2 = 0.9997, confirming excellent linearity.

Table 6: Accuracy (Recovery) of Coenzyme Q10

Level (%)	Added (µg/mL)	Found (µg/mL, mean ± SD, n=3)	Recovery (%)	%RSD
80%	80.0	79.4 ± 0.6	99.2	0.8
100%	100.0	100.3 ± 0.9	100.3	0.9
120%	120.0	121.7 ± 0.8	101.4	0.7

Results of recovery experiments at 80%, 100%, and 120% levels are shown, with mean recovery values within acceptable limits (99.2–101.4%).

Table 7: Precision of Coenzyme Q10

Parameter	n	Mean Peak Area (a.u.)	%RSD	Result
Repeatability (Intra-day)	6	Consistent response	1.2%	Pass
Intermediate Precision (Inter-day)	6	Consistent response	1.5%	Pass

The table presents intra-day (repeatability) and inter-day (intermediate precision) results, showing consistent responses with %RSD ≤ 1.5%.

Table 8: System Suitability Parameters

Parameter	Acceptance criteria	Observed mean ± SD (n = 6)	% RSD	Remark
Retention time (tR, min)	—	12.90 ± 0.03	0.23	Within expected range
Theoretical plates (N)	**> 2 000**	33 800 ± 420	1.24	Meets criterion
Tailing factor (T)	**≤ 2.0**	1.21 ± 0.05	4.13	Symmetrical peak
Resolution (Rs) *	**≥ 2.0** (between CoQ10 and nearest impurity)	3.9 ± 0.2	5.1	Baseline separation achieved
Peak-area precision	**%RSD ≤ 2.0**	1.12	—	Repeatability acceptable

Table 8 lists key performance indicators, including retention time, resolution, tailing factor, and theoretical plates, all of which meet ICH acceptance criteria.

Robustness of Method

Small, deliberate variations in flow rate (±0.1 mL/min), detection wavelength (±2 nm) & organic phase composition (±2%) produced no significant changes in system suitability parameters. All results met acceptance criteria, confirming that the method is robust & stable under slight experimental variations**.** Table 9 indicated that robustness was assessed by varying the flow rate (±0.1mL min^{-1}), detection wavelength (±2nm) & mobile phase composition (±2% of B) around optimized conditions. System suitability was evaluated for each variation using triplicate injections.

All results are expressed as mean ± SD (n = 3). The acceptance limits applied were Rs ≥ 2.0, T ≤ 2.0, N > 2000, and peak-area %RSD ≤ 2.0. No significant deviations were observed, confirming that the method remains robust within the tested ranges.

Table 9: Robustness of the developed HPLC method under small, deliberate variations in analytical parameters.

Parameter varied	Condition	Rt (min)	Resolution (Rs)	Tailing factor (T)	Theoretical plates (N)	Peak-area %RSD
Flow rate	0.9 mL/min (−0.1)	13.10 ± 0.04	35.8 ± 0.3	1.31 ± 0.02	33,400 ± 350	1.4%
(optimized 1.0 mL/min)	1.0 mL/min (center)	12.90 ± 0.03	36.0 ± 0.2	1.30 ± 0.02	33,800 ± 420	1.1%
	1.1 mL/min (+0.1)	12.70 ± 0.03	35.5 ± 0.3	1.32 ± 0.02	32,900 ± 400	1.5%
Detection wavelength	273 nm (−2 nm)	12.91 ± 0.03	36.0 ± 0.2	1.29 ± 0.02	33,850 ± 410	1.2%
(optimized 275 nm)	275 nm (center)	12.90 ± 0.03	36.0 ± 0.2	1.30 ± 0.02	33,800 ± 420	1.1%
	277 nm (+2 nm)	12.89 ± 0.03	35.9 ± 0.2	1.30 ± 0.02	33,760 ± 430	1.2%
Mobile phase B (% of final mobile phase)	68% (−2%)	13.60 ± 0.05	35.0 ± 0.4	1.33 ± 0.03	32,500 ± 500	1.6%
(optimized 70%)	70% (center)	12.90 ± 0.03	36.0 ± 0.2	1.30 ± 0.02	33,800 ± 420	1.1%
	72% (+2%)	12.30 ± 0.04	34.8 ± 0.4	1.34 ± 0.03	32,000 ± 520	1.7%

Sensitivity Parameters

The method demonstrated a limit of detection (LOD) of approximately 0.8 µg/mL, based on a signal-to-noise ratio of ≥3, and a limit of quantification (LOQ) of about 2.5 µg/mL, with a signal-to-noise ratio of ≥10, indicating adequate sensitivity of the assay.

Assay of soft gel dosage form (marketed formulation)

The assay of the marketed softgel capsule (label claim: 100 mg) showed an observed value of 99.5%**,** which falls within the acceptance range of 95–105%**,** confirming compliance with pharmacopeial specifications. Table 10 denotes validation results for CoQ10 analysis in accordance with ICH Q2(R1) guidelines, showing acceptance criteria, observed data, and compliance status for specificity, linearity, accuracy, precision, system suitability, robustness, LOD, LOQ, and assay of softgel dosage form.

Degradation Behaviour of Selected Drugs

The stability of the selected drugs was assessed by subjecting them to different stress conditions and analyzing the resulting samples using HPLC. The specific chromatographic method and parameters employed are described in the methodology section. The HPLC data from the stressed samples offer a detailed insight into the degradation behaviour and overall stability profile of the drugs under various experimental conditions.

Table 10: Method Validation Summary for Coenzyme Q10 by RP-HPLC

Parameter	Acceptance Criteria (ICH Q2(R1))	Observed Results (CoQ10)	ICH Q2(R1) Compliance
Specificity	No interference at Rt; PDA purity angle < threshold	Single sharp peak at ~12.9 min; PDA purity passed	Passed
Linearity (2.5–200 µg/mL)	$r^2 \geq 0.999$	$r^2 = 0.9997$ (y = 0.0198x + 0.0301; weighted $1/C^2$)	Passed
Accuracy (Recovery)	98–102%	80%: 99.2%; 100%: 100.3%; 120%: 101.4%	Passed
Precision (Repeatability, n = 6)	%RSD ≤ 2%	%RSD = 1.2%	Passed
Intermediate Precision (Inter-day)	%RSD ≤ 2%	%RSD = 1.5%	Passed
System Suitability	Rt stable; $R_s \geq 2$; T ≤ 2; N > 2000; %RSD ≤ 2%	Rt = 12.9 ± 0.03 min; R_s = 3.9; T = 1.21; N = 33,800; %RSD = 1.12%	Passed
Robustness	No significant effect with deliberate variation	Flow ±0.1 mL/min, λ ±2 nm, organic ±2% → all within limits	Passed
LOD	Based on S/N ≥ 3	~0.8 µg/mL	Passed
LOQ	Based on S/N ≥ 10	~2.5 µg/mL	Passed
Assay of Softgel Dosage Form	95–105% of label claim	99.5% of label claim	Passed

Table 11: Forced degradation summary of Coenzyme Q10 single dosage form under various stress conditions.

Stress Condition	Medium / Strength	Temperature & Duration	% Degradation of CoQ10	No. of Degradation Products	Observation
Acidic	0.5 N HCl	60 °C / 36 h	**≈ 22 %**	1	Moderate degradation; single degradation product observed (Figure 8a).
Basic	0.2 N NaOH	60 °C / 24 h	**30–32 %**	2	Pronounced degradation under alkaline conditions; two degradants (Figure 8b).
Neutral	Water	80 °C / 48 h	**< 10 %**	1	Stable in neutral medium; minor degradant detected (Figure 8c).
Oxidative	30 % H_2O_2	Room temp / 12 h	**No significant degradation**	0	CoQ10 stable; no oxidative degradants observed (Figure 8d).
Photolytic	Acidic (UV/ visible exposure)	24 h	**≈ 23 %**	1	One major photodegradation product was detected (Figure 8e).

Figure 8 (Coenzyme Q10) exhibited the most significant degradation under basic conditions, with approximately 30–32% loss of the parent compound, followed by photolytic (around 23%) and acidic (about 22%) stress. The drug was found to be relatively stable in neutral and oxidative environments, showing only minimal or negligible degradation. Depending on the type of stress applied, 1 or 2 degradation products were detected, each of which was completely separated from the CoQ10 peak, indicating good chromatographic resolution. Overall, these findings demonstrate that the developed analytical method is stability-indicating and effectively differentiates CoQ10 from its degradation products under various forced degradation conditions.

DISCUSSION

The development of a robust RP-HPLC method for CoQ10 quantification, guided by Quality by Design (QbD) principles, represents a significant advancement in analytical methodology. While numerous HPLC methods have been proposed for CoQ10 analysis, many lack comprehensive optimization and validation, limiting their applicability in routine quality control and regulatory compliance.

Comparison with Existing Methods

Few studies have reported HPLC methods for the analysis of CoQ10. For instance, a study by Ruiz-Garcia et al. developed an HPLC method to simultaneously quantify CoQ10, phosphatidylserine, and vitamin C in liposomal formulations, thereby optimizing current timing and costs in the analysis of these active substances. However, this method did not employ a QbD approach and was tailored for specific formulations, limiting its generalizability. Another study by Vitvitsky optimized a UV-detected HPLC method for CoQ10 analysis from biological materials, involving a rapid single-step extraction into n-hexane. While this approach offers speed, it may not be suitable for routine quality control due to the complexity of sample preparation and potential matrix effects.

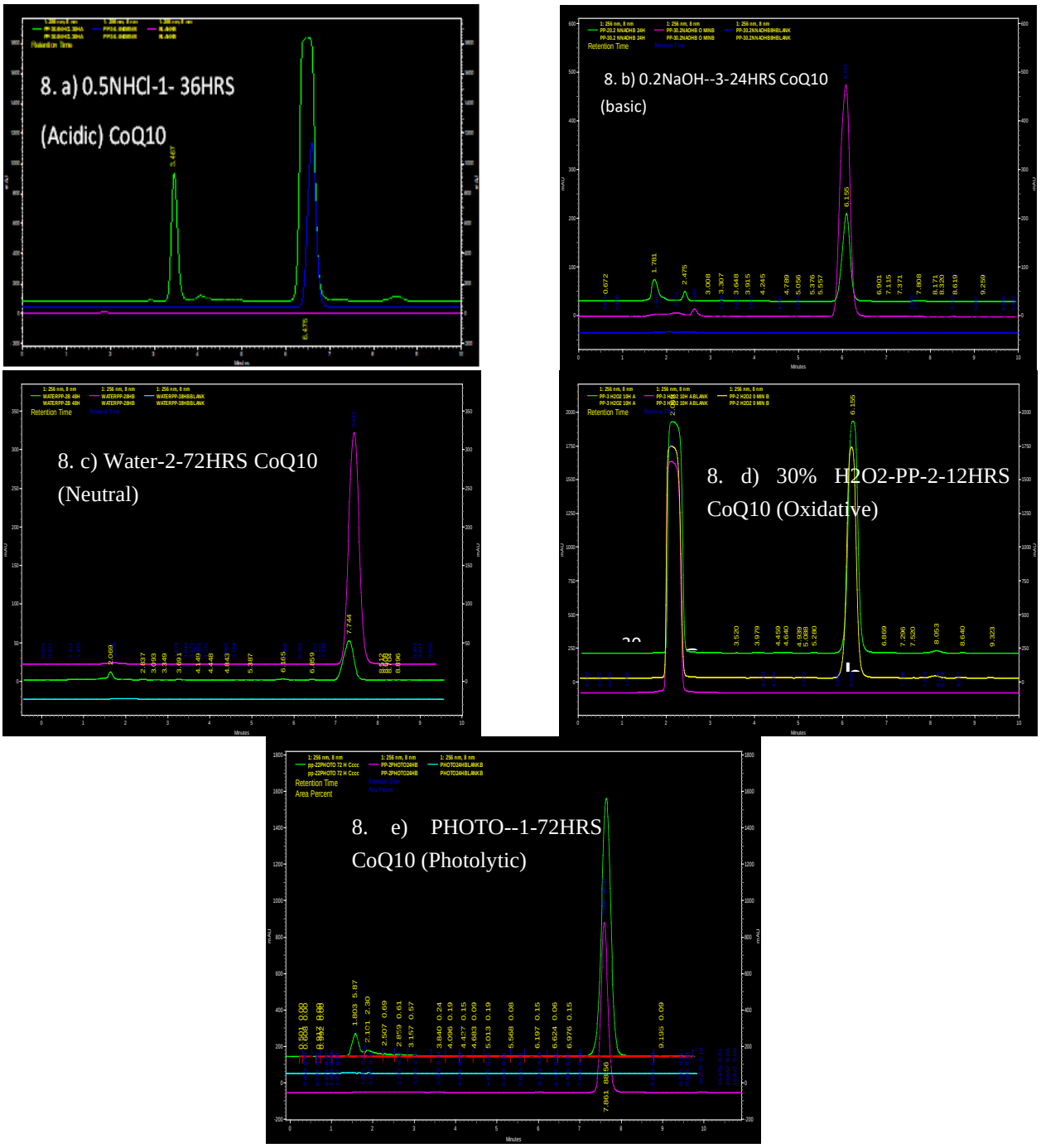


Figure 8: Forced degradation profile of Coenzyme Q10 single dosage form under different stress conditions.

Advantages of the Developed Method

The method developed in this study offers several advantages over existing approaches. Utilizing a Central Composite Design (CCD) and Response Surface Methodology (RSM), the study systematically evaluated critical method parameters (CMPs), such as flow rate, gradient time, and mobile phase composition, in a comprehensive optimization. This comprehensive optimization ensures robustness and reliability across a wide range of conditions. Quality by Design (QbD) Integration**:** By incorporating QbD principles, the method development process was structured to identify and control critical factors, leading to a method that consistently meets predefined analytical target profiles (ATPs).

Validation: The process was validated in accordance with ICH Q2 (R1) guidelines, demonstrating specificity, accuracy, precision, linearity, robustness, and sensitivity. This rigorous validation ensures the method's suitability for regulatory submissions and routine quality control.

Practical Applicability: The method's simplicity, with a 20 µL injection volume and a 12.9-minute retention time, makes it suitable for high-throughput analysis in quality control laboratories.

Potential Applications: The validated RP-HPLC method is well-suited for various applications: Quality Control of Pharmaceutical Formulations. The technique can be employed to monitor CoQ10 content in dietary supplements and pharmaceutical products, ensuring compliance with label claims and regulatory standards.

Stability Studies**:** Given its robustness, the method is ideal for stability studies, assessing the degradation of CoQ10 under various storage conditions.

Regulatory Compliance**:** With validation in accordance with international guidelines, the method meets the requirements for regulatory submissions, facilitating market approval processes.

The developed RP-HPLC method for Coenzyme Q10 was systematically optimized using a QbD approach and validated according to ICH Q2 (R1) guidelines. The results demonstrate that the method fulfills all predefined Analytical Target Profile (ATP) requirements [21,22]. Specificity was established by the presence of a single sharp peak at ~12.9 minutes with PDA purity confirming the absence of interference from excipients, ensuring reliable quantification in formulations. Linearity across the 2.5–200 µg/mL range (r^2 = 0.9997) indicated excellent correlation between concentration and response, reflecting the method's suitability for routine analysis over a wide range [23–25].

The method also demonstrated high accuracy, with recovery values (99.2–101.4%) within the acceptable range of 98–102%, confirming that the assay is both accurate and unbiased. Precision, both intra-day (RSD = 1.2%) and inter-day (RSD = 1.5%), satisfied ICH requirements, ensuring reproducibility under different conditions [20]. System suitability parameters, including resolution (>36), tailing factor (1.2–1.3), and theoretical plates (~33,800), all exceeded pharmacopeial limits, highlighting the robustness of the chromatographic system. Similarly, robustness testing showed no significant variation in results when flow rate, detection wavelength, or mobile phase composition were deliberately altered, demonstrating the method's resilience in routine laboratory practice. The sensitivity of the process was confirmed with LOD (0.8 µg/mL) and LOQ (2.5 µg/mL), enabling detection and quantification of low analyte levels. Finally, the assay of marketed soft gel capsules yielded 99.5% of the label claim, falling well within the acceptable pharmacopeial range (95–105%), thereby verifying the method's applicability for evaluating commercial products.

Overall, the validated method not only meets regulatory standards but also offers significant advantages in terms of accuracy, precision, and robustness. The incorporation of QbD principles ensured that critical parameters were identified and controlled, resulting in a method that is reliable, reproducible, and suitable for both quality control and regulatory submissions.

CONCLUSION

A QbD-guided RP-HPLC method for Coenzyme Q10 was successfully developed and validated, offering high precision, accuracy, and robustness. Critical method parameters were optimized through Central Composite Design and Response Surface Methodology, ensuring a reliable design space. Validation according to ICH Q2(R1) confirmed specificity, linearity (r^2 = 0.9997), recovery (99.2–101.4%), precision (RSD ≤ 1.5%), and sensitivity (LOD 0.8 µg/mL; LOQ 2.5 µg/mL). System suitability parameters, including resolution (>36) and theoretical plates (~33,800), exceeded pharmacopoeial standards. The method accurately quantified CoQ10 in commercial soft gel capsules (within 0.5% of the label claim) and is suitable for routine quality control, stability studies, and regulatory compliance, providing a reliable analytical tool for pharmaceutical applications.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Vishal Kumar Pathak carried out all experiments, collected and analyzed the data, and drafted the manuscript. The research was conducted under the supervision of Phool Chandra, with Bhupendra Singh providing co-supervision, ensuring scientific rigor and proper oversight throughout the study.

REFERENCES

[1] Kowalczyk P, Sulejczak D, Kleczkowska P, Bukowska-Ośko I, Kucia M, Popiel M, Wietrak E, Kramkowski K, Wrzosek K, Kaczyńska K. Mitochondrial Oxidative Stress—A Causative

Factor and Therapeutic Target in Many Diseases. *Int J Mol Sci*, **22**, 13384 (2021) https://doi.org/10.3390/ijms222413384.

[2] Vinarov Z, Abdallah M, Agundez JAG, Allegaert K, Basit AW, Braeckmans M, Ceulemans J, Corsetti M, Griffin BT, Grimm M, Keszthelyi D, Koziolek M, Madla CM, Matthys C, McCoubrey LE, Mitra A, Reppas C, Stappaerts J, Steenackers N, Trevaskis NL, Vanuytsel T, Vertzoni M, Weitschies W, Wilson C, Augustijns P. Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: An UNGAP review. *European Journal of Pharmaceutical Sciences*, **162**, 105812 (2021) https://doi.org/10.1016/j.ejps.2021.105812.

[3] Prajapati PB, Jayswal K V, Shah SA. DoE and Risk-Based DMAIC Principle for Implementation of Enhanced Analytical Quality by Design Approach to Multipurpose-Chromatography Method for Simultaneous Estimation of Multiple Fixed-Dose Combination Products of Aspirin. *J AOAC Int*, **104**, 1430–41 (2021) https://doi.org/10.1093/jaoacint/qsab058.

[4] ter Horst JP, Turimella SL, Metsers F, Zwiers A. Implementation of Quality by Design (QbD) Principles in Regulatory Dossiers of Medicinal Products in the European Union (EU) Between 2014 and 2019. *Ther Innov Regul Sci*, **55**, 583–90 (2021) https://doi.org/10.1007/s43441-020-00254-9.

[5] Marcheggiani F, Cirilli I, Orlando P, Silvestri S, Vogelsang A, Knott A, Blatt T, Weise JM, Tiano L. Modulation of Coenzyme Q10 content and oxidative status in human dermal fibroblasts using HMG-CoA reductase inhibitor over a broad range of concentrations. From mitohormesis to mitochondrial dysfunction and accelerated aging. *Aging*, **11**, 2565–82 (2019) https://doi.org/10.18632/aging.101926.

[6] Saini S, Sharma T, Patel A, Kaur R, Tripathi SK, Katare OP, Singh B. QbD-steered development and validation of an RP-HPLC method for quantification of ferulic acid: Rational application of chemometric tools. *Journal of Chromatography B*, **1155**, 122300 (2020) https://doi.org/10.1016/j.jchromb.2020.122300.

[7] Subriadi AP, Najwa NF. The consistency analysis of failure mode and effect analysis (FMEA) in information technology risk assessment. *Heliyon*, **6**, e03161 (2020) https://doi.org/10.1016/j.heliyon.2020.e03161.

[8] Bhatti GK, Gupta A, Pahwa P, Khullar N, Singh S, Navik U, Kumar S, Mastana SS, Reddy AP, Reddy PH, Bhatti JS. Targeting mitochondrial bioenergetics as a promising therapeutic strategy in metabolic and neurodegenerative diseases. *Biomed J*, **45**, 733–48 (2022) https://doi.org/10.1016/j.bj.2022.05.002.

[9] Verch T, Campa C, Chéry CC, Frenkel R, Graul T, Jaya N, Nakhle B, Springall J, Starkey J, Wypych J, Ranheim T. Analytical Quality by Design, Life Cycle Management, and Method Control. *AAPS J*, **24**, 34 (2022) https://doi.org/10.1208/s12248-022-00685-2.

[10] Dewi M, Pratama R, Arifka M, Chaerunisaa A. Quality By Design: Approach to Analytical Method Validation. *Sciences of Pharmacy*, **1**, 38–46 (2022) https://doi.org/10.58920/sciphar01010033.

[11] Sha'at M, Spac AF, Stoleriu I, Bujor A, Cretan MS, Hartan M, Ochiuz L. Implementation of QbD Approach to the Analytical Method Development and Validation for the Estimation of Metformin Hydrochloride in Tablet Dosage Forms by HPLC. *Pharmaceutics*, **14**, 1187 (2022) https://doi.org/10.3390/pharmaceutics14061187.

[12] Yadav K, Jatain I, Dubey KK, Nitharwal RG, Kaur I. Development of a thin layer chromatographic method for the determination of coenzyme Q $_{10}$ produced by *Agrobacterium tumefaciens*. *Sep Sci Plus*, **6**, (2023) https://doi.org/10.1002/sscp.202200134.

[13] Podar AS, Semeniuc CA, Ionescu SR, Socaciu M-I, Fogarasi M, Fărcaș AC, Vodnar DC, Socaci SA. An Overview of Analytical Methods for Quantitative Determination of Coenzyme Q10 in Foods. *Metabolites*, **13**, 272 (2023) https://doi.org/10.3390/metabo13020272.

[14] Nakov N, Acevska J, Brezovska K, Kavrakovski Z, Dimitrovska A. Green Strategies toward Eco-Friendly HPLC Methods in Pharma Analysis. *High Performance Liquid Chromatography - Recent Advances and Applications*. In tech Open, (2023), https://doi.org/10.5772/intechopen.110035.

[15] Ameen SA, Pappula N. Analytical QBD Approach to Redefine the Quality of Pharmaceuticals: A Review. *Journal of Pharmaceutical Research*, **22**, 178–85 (2023) https://doi.org/10.18579/jopcr/v22.4.81.

[16] Xuan DT, Nguyen HMT, Hoang VD. Recent applications of analytical quality-by-design methodology for chromatographic analysis: A review. *Chemometrics and Intelligent Laboratory Systems*, **254**, 105243 (2024) https://doi.org/10.1016/j.chemolab.2024.105243.

[17] Bairagi A, Kothrukar R, Chikhale H, Kosanam S, Borse L. AQbD-novel strategy for analytical methods. *Futur J Pharm Sci*, **10**, 138 (2024) https://doi.org/10.1186/s43094-024-00706-1.

[18] Nunsavathu SN, Rajaganapathy K. A Review on QbD Approach in Analytical Method Development and Validation . *International Journal of Pharmaceutical Quality Assurance*, **15**, 1707–13 (2024) https://doi.org/10.25258/ijpqa.15.3.93.

[19] Giri S, Varshney KK, Srivastava R. Therapeutic Potential and Prospects of L-arginine in Various Diseases and its Clinical Intervention. *Curr Drug ther*, **19**, 529–45 (2024) https://doi.org/10.2174/0115748855260802231019072509.

[20] Matta A, Sundararajan R. Green Analytical Stability Indicating UHPLC Method for the Quantification of Related Impurities in Vonoprazan Formulation Applying Analytical Quality by Design. *Sep Sci Plus*, **8**, (2025) https://doi.org/10.1002/sscp.70032.

[21] Goraksh TA, Gitaram VD. QbD-Based RP-HPLC Method Development and Validation for Quantitation of Rimegepant in Standard and Pharmaceutical Formulations. *International Journal of Pharmaceutical Research and Applications*, **10**, 942–5 (2025) https://doi.org/10.35629/4494-1002942945.

[22] Raju C, Bojan S, Chinthaginjala C, Dravidamani M, Dommaraju AK, Raghunathan K. Analytical Quality by Design Assisted Optimization of RP-HPLC Method for the Estimation of Palovarotene Drug Substance and Drug Product by Box–Behnken Design. *Biosci Biotechnol Res Asia*, **22**, 313–24 (2025) https://doi.org/10.13005/bbra/3363.

[23] Kudalkar S, Arya S, Pradhan M. Coenzyme Q10: A Comprehensive Review of Its Roles in Mitochondrial Health and Systemic Function. *Int J Health Sci Res*, **15**, 106–15 (2025) https://doi.org/10.52403/ijhsr.20250914.

[24] Valvi AM, Shelke RU, Ghodke SS, Rishipathak DD. Quality By Design and Green Analytical Chemistry: A Review of Novel Approaches to Chromatographic Method Development. *Biosci Biotechnol Res Asia*, **22**, 497–520 (2025) https://doi.org/10.13005/bbra/3379.

[25] Tamboli FA, Mane PP, Babaleshwar RB, Mane DM, Attar PS, Disale PP, Patil PA, Parkhi SM, Rathod MP. From concept to compliance: The role of QbD in modern product development. *Current Trends in Pharmacy and Pharmaceutical Chemistry*, **6**, 168–75 (2025) https://doi.org/10.18231/j.ctppc.2024.032.