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Original Article

Implementation of Analytical Quality by Design Methodology to Develop a UV-spectrometric Technique for Arteether Quantification

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ABSTRACT

Aim/Background: Analytical Quality by Design refers to applying the Quality by Design idea to the development of analytical procedures. The present study emphasizes the AQbD concept of developing and validating a spectrophotometric technique for detecting α, β-arteether per the ICH Q8 (R^2) requirements for the first time. **Materials and Methods:** Sample preparation, sample pH, and wavelength were all integrated into the Ishikawa diagram, and essential parameters were obtained. The ratio of ethanolic PBS 6.8 and HCl was taken as factors. At the same time, the drug molecule's absorbance was identified as a critical factor that was further analyzed using a simple mixture design of experiments methodology for method robustness and optimization. The novel, durable, precise, and accurate UV-spectrophotometric method, α, β-arteether, was developed using the Quality by Design principle. By adjusting the HCl concentration (for acid degradation) and ethanolic PBS 6.8, the highest absorption of α, β-arteether was achieved by heating at 50°C for 30 min. **Results and Discussion:** The percent RSD less than 2, R^2> 0.99 was recorded for the concentration range of 2–20 µg/mL at λ_{max} 254 nm. The developed method's LOD and LOQ were within acceptable limits. The presented approach might be used at the industrial level as a rapid, precise, accurate, and cost-effective quality control method for a frequent and simultaneous estimate of α, β-arteether.

Keywords: α, β-arteether, Analytical method development, Accuracy, Acid degradation, ICH, Malaria, Quality by Design (QbD), UV-spectrometry.

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INTRODUCTION

The pharmaceutical industry has intensely focused on product safety, quality, and efficacy. By increasing productivity and product quality, QbD solutions will reduce the product's failure risk. In recent years, the QbD technique has been effectively applied to manufacture generic/local formulations. The USFDA has issued particular QbD recommendations for pharmaceutical formulations with the immediate and prolonged release. Analytical Quality by design approach is represented by Analytical Target profile, Critical Quality Attributes, Risk Assessment, Method optimization and development utilizing DoE, and developed method validation, achieving the desired product quality.[1] QbD is a technique of product development that includes assessing the influence of a variety of input elements (such as process settings and materials) on the final product (drug molecule or active pharmaceutical ingredient). Consequently, the QbD approach defines acceptable input parameter ranges within which the quality of the final output may be guaranteed.[2] Analytical methods necessitate a thorough understanding of how analytical technique attributes and operating conditions influence analytical performance. AQbD depends on the method used, amount and type of reagent, and instrumental parameters.[3] The AQbD technique may be used to design a reliable and cost-effective analytical way that can be utilized at any point in a product's lifetime. The foremost step in the AQbD process is setting the Analytical Target Profile (ATP). The goal of the analytical method development process is determined by ATP, which is a measure of method performance. ICH Q2(R^1), the International Conference on Harmonization's recommendations for validating analytical processes, has provided several method parameters for method development.[2] Thus, using the ICH recommendations Q2 as a reference, a QbD-based UV-spectrometric approach may be created (R^1).[3]

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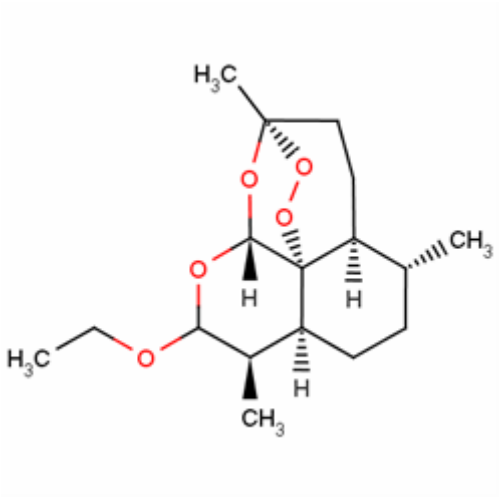

Figure 1: Chemical structure of α,β-Arteether.

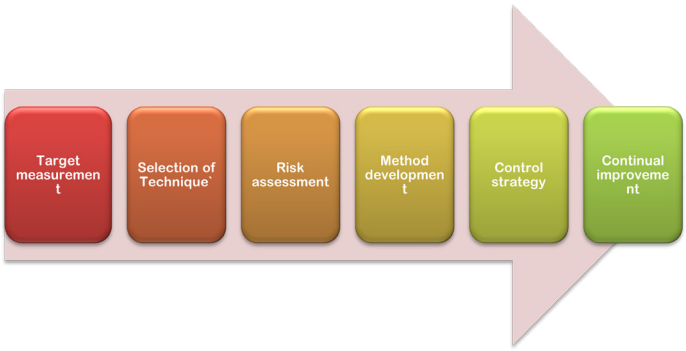

Figure 2: Quality by design approach to analytical method development.

Artemisinin is a sesquiterpene lactone with an endoperoxide bridge that is required for anti-malarial action and is a common property of this family of medicines. Due to solubility concerns with the parent medicine artemisinin, the carbonyl group was reduced to produce dihydroartemisinin and its derivatives, such as the oil-soluble artemether, arteether, and the water-soluble artesunate. Arteether is a semi-synthetic ethyl ether derivative of dihydroartemisinin, a dihydro derivative of qinghaosu (artemisinin) isolated from *Artemisia annua*, a plant noted for its anti-malarial qualities in Traditional Chinese Medicine and one of the most promising prospects for the treatment of malaria.[4]

Gametocytocidal properties of arteether have been documented.[4] Arteether has also been shown to minimize the development of hemozoin. Arteether has now established itself as a cornerstone in treating malaria, and its protection against the development of resistance is a critical component in the battle against the disease.

Although numerous techniques such as HPTLC, HPLC for arteether measurement, and simultaneous estimation methods employing HPLC/MS have been described earlier, a thorough review of the literature indicated that many others have not. A simple approach for routine α, β-arteether estimation is urgently needed.

The study objective was to develop a simple, rapid, robust, adaptable, and cost-effective UV-spectrometric technique of α, β-arteether Figure 1 utilizing the AQbD approach. Moreover, none of the reported methods of α,β-arteether supported ensuring the reliability of data obtained. Hence, lack of reliability and robustness were the two critical aspects upon which the authors attempted to develop a new simple UV-spectroscopic method using the quality by design approach. AQbD has been applied to the development of UV-spectrophotometric analytical way by keeping the amount of ethanol, phosphate buffer, and HCl used while absorbance maxima response parameters.[5] The suggested and validated model was then verified using the ICH Q2 criteria (R^1).[3] Thus, the study employs the QbD technique to create innovative, simple, sensitive, precise, and accurate methods for estimating α, β- arteether in pure form and pharmaceutical formulations and evaluate the proposed plans for reliability and industry acceptability using ICH principles.

MATERIALS AND METHODS

SHIMADZU UV-1700 double beam UV-vis spectrophotometer with a 1 cm complete set of oval quartz cells was employed in this experiment. The slit widths of the absorption and emission monochromators were both set to 10 nm. All of the instruments were calibrated and checked before the start of the experiment.

Materials

Brooks Private Limited Baddi provided a free sample of arteether pure drug. Absolute alcohol was purchased from Hong Yang Chemical Corporation, China while buffer capsules pH 4.0 ± 0.05 and pH 7 was purchased from Merck Specialities Pvt. Ltd., Mumbai, India. All the chemicals and reagents used were of analytical grade. MATCH (MANKIND) and KAPITHER-150 (GODRAMS LIFELINE) were two injectable formulations purchased from the local market containing 150 mg/2 mL of α,β- arteether. UV-vis spectrophotometer of Shimadzu UV-1700 double beam Shimadzu Corporation was used during the experiment.

Standard stock solution preparation

10mg of arteether was weighed accurately and dissolved in ethanolic phosphate buffer solution pH 6.8 (50mL), making the stock solution of 1000 µg/mL.

Pipette 0.4 mL to 1.8 mL from the aforementioned 1000 µg/mL stock solution into 10 mL volumetric flasks and add 2.0 mL of 5M HCl solution to each. The solutions were maintained in a water bath at 50±2°C for 30 min for acid degradation. Finally, ethanolic PBS 6.8 was used to make a volume of up to 10 mL, resulting in a drug concentration of 2 to 20 µg/mL. Using ethanolic PBS 6.8 and HCl (8:2) as a blank, the absorbance was measured at 254 nm. A calibration curve was produced by constructing a graph between

absorbance and corresponding concentrations at this wavelength maxima.

The analytical method development for arteether was planned as per ICH Q8 (R^2) guidelines with implementing the QbD approach. Figure 2 describes analytical method development steps using the quality by design approach.

Design of method

The most crucial step in any research project is to design the method, which entails first researching and understanding the quality method development requirements and then carrying out the experiment while keeping in mind the critical method variables that must be controlled for the method to be rugged and robust.[6]

The next step is to determine if the experiment's goal satisfies the conditions for understanding the technique's Analytical Target Profile (ATP). Spectrophotometric or chromatographic methods may determine the amount of ATP in any molecule.[7]

Development of Method

Once the ATP has been established, the researcher must choose the best approach and circumstances to satisfy the ATP's criteria.[8]

Assessment of Risk

Understanding the influence of significant input factors on the technique's performance characteristics is critical for developing a robust and rugged analytical approach. In method development, risk assessment may be done using a fishbone diagram and an FMEA, which are used to specify which factors can be managed to reduce risk.[9] Risk identification and analysis are the most important parts of risk assessment methodology, according to ICHQ9 standards. The trouble was identified using an Ishikawa fishbone diagram, which depicted all conceivable aspects that may affect the technique development.[10] The Critical Analyte Attribute (CAA) and variables involved in method development were identified as the first phase, and an Ishikawa fishbone diagram depicting the link between them was drawn (Figure 3). Following the study of the Ishikawa fishbone diagram, several technique factors such as sample/instrument scanning speed, solvent kinds, sampling interval, sample integrity, and so on were investigated.

Important key parameters were also exposed to an optimum optimization design to establish the best number of variables for obtaining the predefined analytical goal profile.[11]

Experiment Design (ED)

After deciding on the variables, such as the quantity of HCl to be used for acid degradation of arteether and the pH of the ethanolic phosphate buffer, a design of experiment was created to guarantee that the most information was gathered while limiting the number of tests. Surrogate measurements of attributes such as accuracy or precision may be assessed depending on the technique. Design-Expert software version 13.0.5.0 was used to determine the relationship between the independent and dependent variables. All of the experiments in the optimization studies were carried out in triplicate. The QbD-based treatment of an analytical method's robustness necessitates the evaluation of all characteristics (factors) that most significantly impact selectivity (results), both alone and in combination.[12] Various approaches may be used to calculate effects and interactions, equation coefficients, and statistical significance of coefficients that have been obtained. ANOVA based on the Student's *t*-test may be used to calculate the value of coefficients.[13] Simple mixture design was used to determine the stock solution and subsequent dilutions to be prepared, yielding 13 experimental runs for three components, as shown in Table 2.

A statement expressing the dependency of a response variable on the independent variables is referred to as the model. Empirical or theoretical mathematical models are both possible.[14] This factor-response connection may be described using an empirical model. It's usually, but not always, a collection of polynomials of a certain order. One answer, arteether absorbance, was chosen for analysis.[14] By entering the data into the Design-Expert program and displaying the link between the independent method factors and dependent variables or responses, a suitable mathematical model in the form of a polynomial equation (Equation 1) was developed. $Y= 0 + 1A + 2B + 3AB + 4A^2+ 5B^2$..... 1st equation

Where Y denotes the measured response, 0 represents the intercept, 1 and 2 indicate the first-order parameters of the two chosen variables, A and B, three means the coefficients of an A and B correlation term, and 4 and 5 denote the coefficients of quadratic of selected factors, and 4 and 5 represent the coefficients of quadratic of selected aspects. Equation 1 is a linear second-order model that arises from the quadratic terms. The positive and negative signs of each coefficient were used to determine synergistic and antagonistic effects from the polynomial equations.[15]

Method Design Output

A set of method conditions will have been established and described that are expected to meet the ATP. ANOVA should be used to properly examine the significance of each model using the Lack of Fit and Goodness of Fit statistics before selecting a model. This will look at the *f* value, *p*-value, accuracy value, and R^2 adjusted and forecast. Different polynomial equations were created with significant *p* values less than 0.5.[16] Also, with the help of software, plots like Normal plots, Predicted vs. Actual actions, and Residual vs. Predicted properties. Residual vs. Run schemes were created to analyze the behavior or effect of the ratio of ethanol, phosphate buffer, and HCl used as independent

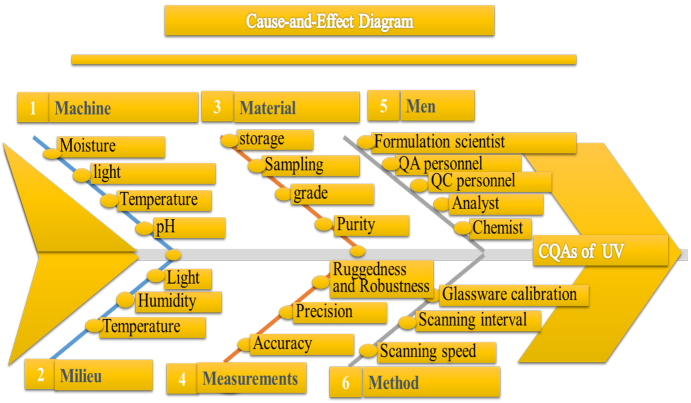

Figure 3: Ishikawa fishbone diagram for finding the potential factors that affect the method development.

variables on the dependent variables or responses (absorbance 254 nm). Model graphs will demonstrate how the answer will likely evolve at various amounts of variables at different times using a predicted response equation with individual coefficients. With the help of 3-D response surface plots, we can understand the system's behavior by presenting the independent factors' contribution. The contour plots display slices of the response surface. A normalized story represents a response plotted between normalized factor levels (N) and a response variable.[17] They are only shown when the component levels are not central, i.e., when the middle class is not the mean of the high and low levels. 2D contour and 3D response surface plots were used to demonstrate the relationship between the selected independent method variables and response variables. Following the development of the design space, a minimum of three confirmatory experimental runs should be undertaken within the design space's designated range for verification.[18]

Validation of ED

The point prediction feature was studied to acquire the value of the answers at specific values of method variables in the design space for further optimization of the method for analyzing the applicability or functioning of the generated model on a lab scale. The optimal value of replies was compared to the responses obtained by experimenting on a lab scale with identical procedure variables. This comparison confirmed the proposed polynomial model-based predictions in an analytical lab under real-world conditions.[19]

Conditions for Spectrophotometry

A standard solution of arteether was scanned in the spectrum mode from 400 nm to 200 nm to determine the analytical wavelength. The maximum wavelength of arteether, 254 nm, was chosen for method development from the spectrum.

Stock and Working Solution preparation

Ethanolic PBS was utilized as a solvent for spectrophotometric techniques. 10 mg of arteether was dissolved in a 10 mL volumetric flask, diluted, and then disbanded in ethanolic PBS 6.8 to attain a concentration of 1000 µg/mL for the stock solution. Working solution (50 µg/mL) was utilized for the initial spectrum scan in the spectrophotometric technique, and further different concentrations for linearity were prepared from standard solutions.

Linearity Analyses

0.4 to 1.8 mL of stock standard solution (1000 µg/mL) were added to a 10 mL volumetric flask, along with 2 mL of 5M HCl. Each was given an HCl solution, which was then maintained in a water bath at 50±2°C for 30 min to allow the acid to decompose, yielding α, β- unsaturated decline [8-methyl-5-(2-propanyl) decalin-4-ene3-one]. Finally, each solvent was used to make a volume of up to 10 mL, resulting in a drug concentration of 2 to 20 µg/mL. Using ethanolic PBS 6.8 and HCl (8:2) as a blank, the absorbance was measured at 254 nm. The calibration curve was plotted as drug concentration vs. absorbance at this wavelength maximum. The y-intercept, slope, and correlation coefficient of the regression equation are presented.

Method Validation

Validation is one of the most critical aspects of developing a reliable, robust, and repeatable UV-spectrophotometric analytical technique that the industry will accept. When evaluated using chemometrics, many validation factors, including ruggedness, robustness, and System Suitability Test (SST), were shown to provide effective and valid findings. It's critical to determine how robust or rugged a new approach is when it's optimized.[13] However, the terms robustness/strong and ruggedness/sound for describing analytical techniques are generally misinterpreted. According to the ICH Q2(R^1) guideline, the improved UV-spectrophotometric technique was verified for specificity, accuracy, repeatability, linearity, precision, intermediate precision, robustness, and system appropriateness.[3] All of the tests were done in triplicate.

Precision, Linearity, robustness, accuracy, the Limit of Detection (LOD), and the Limit of Quantification (LOQ) were used to validate the development of the UV method.

Linearity

Linearity refers to an analytical technique's capacity to provide test findings proportional to the concentration of analyte in samples in a particular range. In a UV-vis spectrophotometer, the samples were scanned against ethanolic PBS 6.8 and HCl (8:2) as blanks, as well as different aliquots of the stock solution (1000 µg/mL) in

Table 1: ATP for UV spectrophotometric method development of arteether.

Sl. No.	ATP parameter	Objective	Explanation
1.	Sample	Arteether	Quantitative assessment of a pharmacological molecule in a pharmaceutical dosage form utilizing a well-established analytical technique.
2.	Methodologies	UV spectrophotometric method	The UV-spectrophotometric method is a simple way to assess drug molecule.
3.	Instrument requirement	UV spectrophotometer	UV-spectrophotometer with high accuracy and precision.
4.	The nature of the analyte	Liquid (Solution)	In the case of the liquid analyst (solution), uniform miscibility exists.
5.	Stock solution	Dilution of arteether in ethanolic PBS6.8	For proper dilution, arteether in ethanolic PBS6.8 solution should have 100% miscibility with the necessary solvent.
6.	Sample preparation	Stock solution was used for sample preparation	As per the SOP, different concentrations of solution containing pharmaceuticals were generated by manually weighing the required excipients and drug molecules.
7.	Acid degradation	5M HCl was used for acid degradation	HCl was added in appropriate amounts to make the desired dilutions, and then these were kept on a water bath at 50±2°C for 30 min for acid degradation to get α, β- unsaturated decalone [8-methyl-5-(2-propanyl) decalin-4-ene3-one].
8.	Method Utilized	Purity estimation of arteether	The developed approach has been used to estimate the purity of arteether in a variety of pharmaceutical formulations.

the range from 2-10 μg/mL. The absorbance was measured at a maximum wavelength of 254 nm.

Precision

The accuracy (intra-day and inter-day) of the developed method were determined using percent relative standard deviation (percent RSD). The study utilized approx. Three times day for intra-day accuracy and for inter-day precision (separate day). The % RSD was calculated using values of concentration for intra-day and inter-day precision six times each.

Accuracy

Excipients utilized in the dosage form were evaluated for interference with the suggested approach, and recovery tests were done using the conventional addition method.[2] The quantity of standard recovered was calculated as a mean recovery with percent relative standard deviation upper and lower limits. To evaluate the accuracy of the analytical approach, three dilutions of market formulation with the same concentration were spiked with various concentrations of standard drug solutions, i.e. 80 percent, 100 percent, and 120 percent. The absorbance of several solutions was determined, and the findings were plotted on a calibration curve. Concentration and accuracy values were calculated.

Robustness and Ruggedness

The capacity of an analytical approach to survive small but deliberate changes in method parameters is measured by its robustness. Analyses at different wavelengths and deliberate small changes in the concentration of solvent used were used to prove the resilience of the proposed method.[1] The absorbance was measured, and the percentage of RSD was calculated. The method's robustness may be described by the Relative Robustness value generated in an intra-laboratory experiment, while the method's ruggedness is defined by the Relative Ruggedness value established in inter-laboratory studies. The higher the relative robustness or relative roughness score, the more robust or rough the approach will be.

Detection and quantification limits (UV-spectroscopy)

The detection as well as quantification limits were calculated using a method based on the calibration plot's standard deviation (s) and slope (S).LOD = 3.3s/S and LOQ = 10s/S

RESULTS AND DISCUSSION

Determination of wavelength

Figure 5 shows the spectrum of arteether in ethanolic phosphate buffer (pH 6.8), α, β-Arteether has no UV absorption above

Table 2: **Experimental design matrix obtained through mixture design.**

Run	A: amount of ethanol	B: Amount of HCl	C: PBS	Absorbance 254 nm
1	6	2	2	0.922
2	5	2	3	0.848
3	5	1	4	0.595
4	5.51081	1.43437	3.05482	0.588
5	5	1.54236	3.45764	0.442
6	6	1	3	0.864
7	6	1.81241	2.18759	0.702
8	6	1	3	0.862
9	5.37796	1	3.62204	0.806
10	6	1.81241	2.18759	0.702
11	5	2	3	0.848
12	6	1.81241	2.18759	0.702
13	5	1	4	0.579

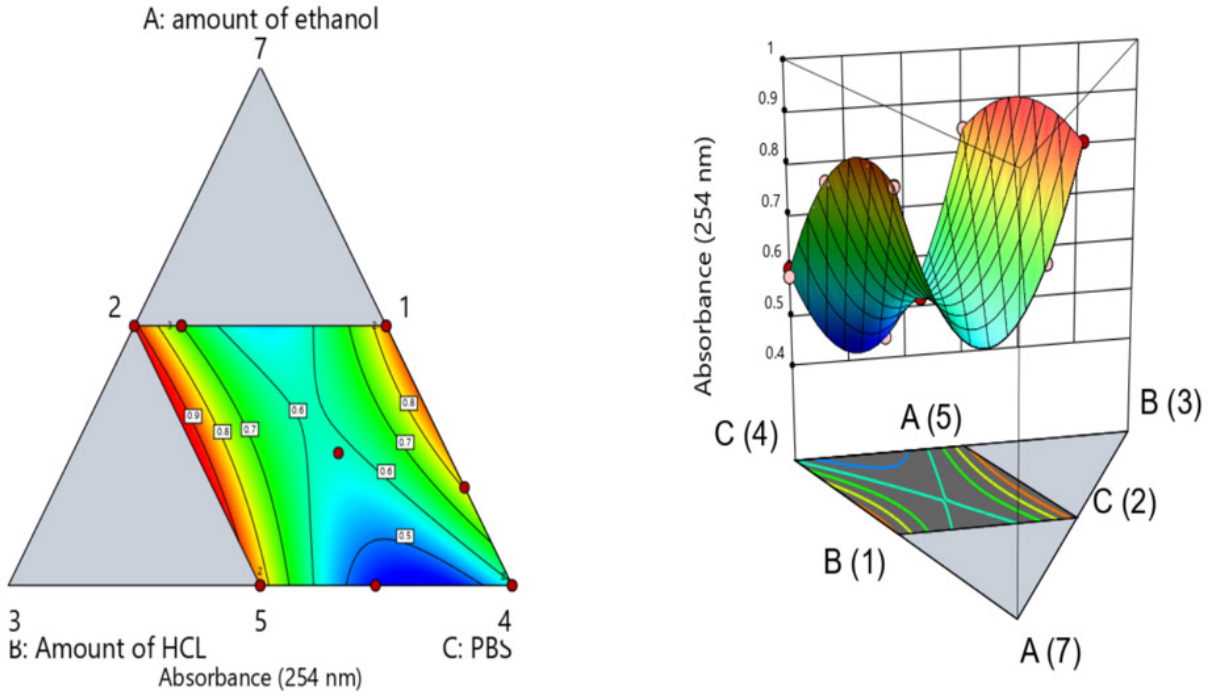

Figure 4: (a) 2D contour plot (b) 3D Response surface plot demonstrating the correlation between the specified variables (scanning speed, sample interval, and response).

220 nm, hence it was degraded using 5M HCl, resulting in a chromophoric group that can absorb at 254±2 nm. The observed findings matched the stated value, verifying the identity and purity of the medicine obtained.

Experimental design

Analytical Target Profile (ATP) was developed as a need for the UV-spectrophotometric analytical method development QbD technique by defining the quality ascribes of the proposed UV-spectrophotometric method.[19] Table 1 lists the potential ATP for arteether's current UV-spectrophotometric technique development.

The aim of developing a novel UV-spectrophotometric approach was to allow for rapid and straightforward drug analysis in comparison to previous sophisticated analytical methods. The

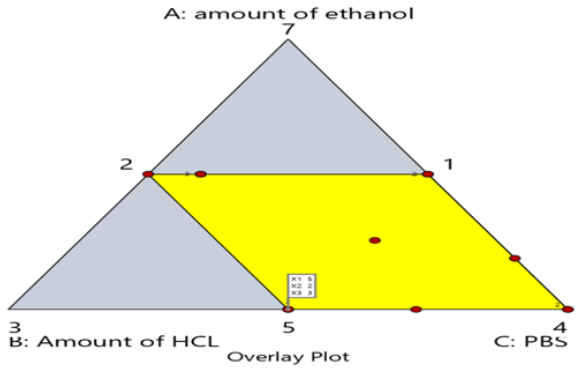

Figure 5: Overlay plot of the experimental area's design space (dark yellow).

Table 3: Precision of α, β-arteether (4µg/mL) in various solvents.

	Intraday	Interday
Absorbance at λ_{max}	0.242	0.245
	0.246	0.249
	0.247	0.247
Calculated concentration (µg/mL)	4.012	3.964
	4.061	4.029
	4.029	3.996
Mean ±SD; RSD	4.034±0.024; 0.612	3.996±0.032; 0.809

Table 4: Validation parameters for developming UV method.

Validation parameters	Values (Ethanolic PBS 6.8)
Absorption maxima (nm)	254
Linearity range	4-20 µg/mL
Standard regression equation	Y = 0.0014x + 0.0162
Correlation coefficient (R^2)	0.9969
Accuracy	100.14±0.678; 0.677
Precision	Intraday (0.536) Interday (0.553)
Robustness	1: 9 ratio (0.512) 2:8 ratio (0.539) 3: 7 ratio (0.315)
LOD	0.394
LOQ	1.263

absorbance of attire was chosen as the Critical Analyte Attribute (CAA) to achieve the predetermined goal profile.[6]

The Ishikawa fishbone diagram (Figure 3) was created to demonstrate the correlation between the numerous process factors and essential analytical attributes,[16] with absorbance at 254 nm chosen as the response. When the quantity of phosphate buffer pH 6.8 is raised, the absorbance increases, however when the amount of 5N HCl is increased, the medication degrades, resulting in a drop in absorbance.

Based on the results of the research, absorbance at 254 nm was identified as the most important risk variables based on severity, which may be managed using the DoE technique. The remaining moderate and less effective risk variables were set to a constant value since they had comparably less or moderate influence on the risk factors chosen.[20]

The mixture design, was used to optimize crucial analytical variables. As responses or dependent variables, absorbance at 254 nm was used as shown in Table 2.

Statistical optimization data analysis based on QbD

After displaying the mathematical model using software, the experimental data was analyzed using the multiple linear regression analysis approach. A second order quadratic model was built with the use of a coded equation that anticipated the link between the provided parameters and response.[21]

Responses are equated in terms of coded variables

The correlation between the independent and dependent variables was predicted at different levels using equations in the form of coded factors. The lowest and highest levels were distinguished, with -1 (low level) and +1 (high level) respectively (higher levels).

The equation of selected responses is shown as Equation 3.

Absorbance at 254nm = 0.153176 * A + 3.41772 * B + 0.58681 * C + -3.45762 * AB + 1.97128 * AC + -4.6167 * BC + -1.66614 * ABC……. Equation 1.

Where, A is the amount of ethanol, B-Amount of HCl while C is amount of PBS.

Analysis of Variance was used to conduct a statistical Analysis of the selected Variables (ANOVA). A significant model with a *p*-value less than 0.05 and the suitability of the model were identified using the lack of fit, R^2, and modified R^2 values. The disparity between anticipated and exploratory data information points was not explained by the change judged by the limitates, according to a model with a significant 'Lack of Fit.' In ANOVA, R^2 values around 1 showed how well the predicted model matched the experimental model, and the value should be near 1.[12] The results of different ANOVA studies of selected responses, such as absorption at 254 nm, using various criteria such as *p*-value, lack of fit, and R^2 value.

The value obtained for model F is 1886.88 which indicates the significant model and this magnitude has a 0.01% probability of arising due to noise. The ANOVA model also shows that model terms with *p*-values are significant as the value is less than

0.0500. The *F*-value of 0.14 for the Lack of Fit indicates that it is not significant in comparison to the pure error. There's a 72.39 percent likelihood that a big Lack of Fit *F*-value is caused by noise. The lack of fit not significant indicates that the model is perfectly validated. The Adjusted R^2 of 0.9989 is quite close to the Predicted R^2 of 0.9707; that is, the difference is less than 0.2. The signal-to-noise ratio is measured by Adeq Precision.[13] It is preferable to have a ratio of more than four. The signal-to-noise ratio of 138.316 suggests a good signal.

To demonstrate the correlation between variables and response, 2D contour plots and 3D response surface plots were constructed using the Design Expert application, as shown in Figure 4 (a) and (b). The absorbance starts to decline at moderate and low values of both the specified variables, namely scanning speed and sample interval, according to the analysis of the aforementioned plots.[14] In order to maximize selected essential aspects (sample interval and scanning speed) in proportion to the desired response, it is critical to pick the design space from the experimental region (absorbance at 254 nm).[20] Figure 5 illustrates an overlay plot with the experimental area in grey and the design space in dark yellow. The light yellow zone denotes the region where several variables may be changed.

Experimental model validation

The validation of experimental design is also an essential criterion in the risk assessment and practical application of any analytical technique development based on the QbD and QRM principles. When the predicted value was compared to the experimental value, the development model was found to be 96 percent similar to the experimental value, confirming that the mathematical model can be accepted for defining the interrelationship of selected variables to obtain the desired response within the identified design space.[15]

Linearity

The regression equations for the findings were determined using the least squares approach. Within the concentration range of 2-10 µg/mL, the Beer's Lambert law graphs (n=6) were linear. Linear equation R^2 values vary from 0.9996 to 0.9999 (p<0.001), showing a good degree of linearity in the chosen solvent system. The calibration curve yielded the following equation: y = mx + c.

Precision

The intraday and interday precision percent RSD values vary from 0.186 to 0.612 and 0.809 to 0.970, (n=6) respectively. In terms of linearity, accuracy, and precision, the spectrophotometric analysis technique of, α, β-arteether in diverse solvent systems was shown to be fairly excellent. The precision results (Table 3) revealed appropriate sample stability and procedure dependability, with a percentage RSD of <2%.[16]

Accuracy

The percent accuracy of known concentrations (2, 4, 8µg mL^{-1}) of α, β-arteether in different solvent systems was found to be between 99.01 and 100.26 utilising the aforementioned analytical approach. The measured values are very comparable to the actual ones. The percent RSD values were less than 2%, suggesting that the approach was accurate.

Robustness

The novel method's resilience was validated using three duplicate trials with three different solvent ratios and three different wavelengths. The developed technique was shown to be robust, with a percent RSD of 2%.

LOD and LOQ

The calculations were done using conventional curve formulae. LOD is 3.3 times the standard deviation of the Y-axis intercepts, whereas LOQ is 10 times the standard deviation. Table 4 shows LOD and LOQ data, and it was proven that the suggested approach was sensitive and specific.

Hence, the above results demonstrate the acceptability of UV-spectrophotometric method of analysis of arteether in variety of solvents with respect to linearity, accuracy and precision.

CONCLUSION

The present research work highlights the development and optimization of simple, repeatable, sensible, and cost-effective analytical UV-spectrophotometric method of arteether by Quality by Design approach. Following the construction of the ATP and CAAs, the CMVs were chosen using a risk assessment analysis based on the Ishikawa fish bone diagram. The risk surface approach was then used to optimise the chosen model utilising mixture design as the optimization design, offering a thorough grasp of the specific response factor connection as well as the interaction between them.

The developed analytical technique was validated further using ICH Q2 validation criteria such as linearity, accuracy, precision, robustness, repeatability, LOD, and LOQ. The findings revealed that the procedure was unique, linear, accurate, and exact. Furthermore, the observed values of LOQ and LOQ indicated that the created model was more sensitive.

With the implementation of QbD approach the developed method can be used at the large scale with more accuracy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AQbD: Analytical Quality by Design; **ATP:** Analytical Target Profile; **ANOVA:** Analysis of Variance; **CAA:** Critical Analyte Attribute; **ED:** Experiment Design; **SST:** System Suitability Test; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **PBS:** Phosphate Buffer Solution.

SUMMARY

AQbD is the most precarious step in formulation development as it detects the risk at its earliest stage. The article highlights the development of novel and precise UV-spectroscopy method using quality by design approach. Simultanously, adoption of AQbD offered both qualitative and quantitative data. As a result, an analyst utilising this methodology might confidently choose numerous working circumstances inside the operational space rather than a single condition for everyday usage of the method. This unique approach also combines a learning process with a rigorous risk assessment.

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